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What are the new technologies that are having the greatest impact on your area of ion channel research?

Trevor Smart. The greatest impact on ion channel research is really coming from refinements to existing technologies. These include cell biology and imaging techniques, which are revealing ion channel movements (trafficking) in real time and targeting to particular parts of cell membranes (for example, synapses); the application of X-RAY CRYSTALLOGRAPHY, which allows greater resolution of the atomic structures of crystallized channels; the innovative design of ion channel mutants, such as reporter mutations, to assist in the interpretation of channel function using electrophysiology; and the careful use of knock-in animals, in which the effect of subtle ion channel mutations can be assessed on an organism's phenotype (behaviour).

John Peters. Although not strictly a new technology, the ability to generate knock-in animals with transmitter-gated ion channels, which are subtly altered regarding their pharmacological modulation (rather than the gross abrogation of function obtained with knock-outs), is an exciting area that potentially links molecular structure and cellular and systems physiology, through to behaviour and the elucidation of targets for therapeutic intervention. The best example, to my knowledge, is the work of Hanns Möhler and Uwe Rudolph, and also researchers at Merck, Sharp and Dohme (Harlow, UK), which has enabled aspects of the behavioural repertoire of classical benzodiazepine receptor ligands (including sedative, amnestic and anxiolytic actions) to be linked to specific isoforms of the GABA (γ-aminobutyric acid) receptor $GABA_A$ that are differentially expressed in the central nervous system (CNS)[1]. Combined with a knowledge of the regional distribution of the mutated subunits, it is possible to ascribe specific behavioural actions to particular neuronal circuits. Of equal importance, these studies identify particular $GABA_A$-receptor isoforms as selective targets for drug action.

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Frances Ashcroft is the Royal Society GlaxoSmithKline Research Professor at the University Laboratory of Physiology, Oxford, UK, and a Fellow of Trinity College, Oxford. She holds a B.A. degree, a Ph.D. and an Sc.D. from Cambridge University, UK, and was elected a Fellow of the Royal Society of London in 1999. Her research focuses on ATP-sensitive potassium channels and their role in insulin secretion in both health and disease. She is interested in how channel function relates to structure, and in particular how nucleotides regulate channel activity. The ultimate goal is to elucidate how a rise in the concentration of blood glucose stimulates the release of insulin from pancreatic β-cells, what goes wrong with this process in type 2 diabetes, and how drugs used to treat this condition exert their beneficial effects. She has written a textbook, *Ion Channels and Disease*, and is Director of OXION, a training and research programme on the integrative physiology of ion channels, funded by the Wellcome Trust. She is also actively involved in promoting the public understanding of science, and her popular science book *Life at the Extremes* became a bestseller.

Chris Miller. X-ray crystallography — an old technology at long last finding an application in ion channel research.

Michael Sanguinetti. From our personal experience, the most important advance has been the determination of the three-dimensional structure of K^+ channels by the MacKinnon group at Rockefeller University[2–4] (FIGS 1,2). This has allowed more directed structure/function studies using site-directed mutagenesis and has also enabled modelling of drug–channel interactions.

David Clapham. The high-resolution structures of ion channels resolved by Rod MacKinnon and colleagues have had by far the greatest impact[2–4]. Two key areas of ion channel function that lacked a detailed understanding were selectivity and gating; MacKinnon has contributed greatly to this understanding by defining the mechanism of K^+ and Cl^- selectivity at atomic resolution and providing structural insight into the mechanisms for gating (opening and closing) of the channel. Although not a new technology, MacKinnon has had great success in crystallizing these difficult membrane proteins (BOX 1). (Membrane proteins, by virtue of their presence in the lipid bilayer, are difficult to purify and lag about 100-fold behind soluble cytoplasmic proteins in the number of structures that have been solved.)

Dennis Dougherty. Crystallography of bacterial channels has been valuable. Since 1998, crystal structures of several prototypical ion channels have appeared, primarily due to the efforts of Rod MacKinnon and Doug Rees. As noted by several contributors to this report, MacKinnon's structures have been quite influential, and three structures from the Rees group[5–7] have also greatly expanded our understanding of channel structure. The two most recent structures from these labs — K_VAP, a voltage-gated K^+ channel from the bacterial organism *Aeropyrum pernix*, from MacKinnon[4], and the small-conductance mechanosensitive (MscS) channel from Rees[5] — are both voltage-sensitive channels. Although the two differ in significant ways, it may be that the fundamental voltage-sensing mechanisms are similar.

These structures have provided an immensely useful framework for efforts to unravel the mechanisms of action of a wide range of important channels. However, it must be remembered that in all cases the crystal structures are of bacterial channels. It remains to be determined to what extent these structures are informative about the key players in the mammalian CNS. So in many ways the value of the bacterial ion channel structures is in guiding future experimental efforts on the real targets — the mammalian channels. The bacterial channels are very valuable, but they are by no means the end of the story.

Unnatural amino-acid mutagenesis has also been very useful. To some extent this is self-serving, as this is a major component of my own research. However, it is important to perform experimental, functional tests of the degree to which a crystal structure of a model membrane protein is relevant to the desired mammalian target. It is essential that the functional probe is

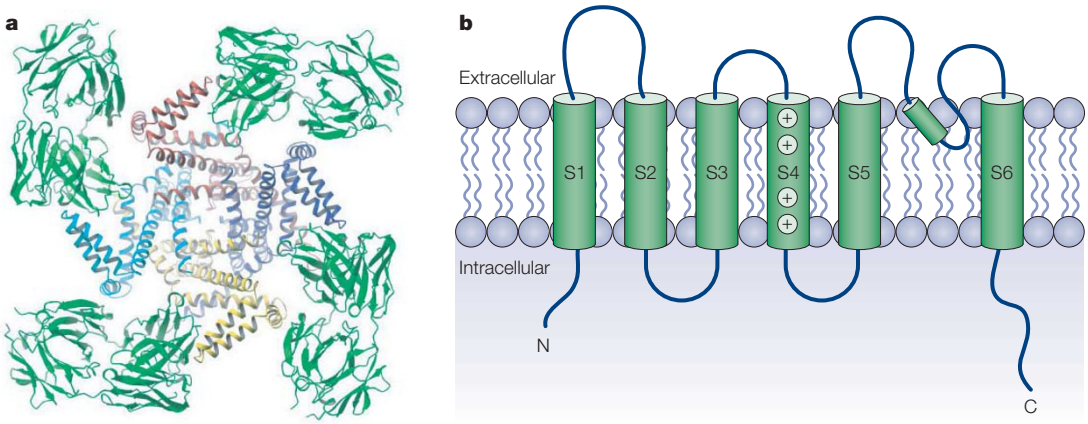


Figure 1 | **Structure of an assembled potassium channel. a** | A plan view of the voltage-dependent potassium channel (K_VAP), isolated from *Aeropyrum pernix*[4]. The tetrameric ion channel is viewed from the inside of the cell looking towards the extracellular side. The α-helical structures represent the S1–S6 elements, including the channel P domain. Surrounding the channel are the antigen-binding fragments (Fabs; coloured green) that were used to encourage crystallization of the ion channel as a tetramer by forcing the protein to adopt less dynamic structural conformations (see also BOX 1). **b** | The classical (pre-crystallization) transmembrane view of a single voltage-gated ion channel subunit. This diagram is based largely on hydropathy plot analyses. A complete channel would comprise four of these subunits. Crystallization of ion channels now indicates that some revision is necessary regarding the mechanism by which membrane voltage causes channel activation (FIG. 2). Part **a** reprinted by permission from *Nature* (REF. 4) © (2003) Macmillan Magazines Ltd. Figure prepared by Trevor Smart.

of a precision comparable to that of the X-ray crystallographic structure, and unnatural amino-acid mutagenesis is perfect in that regard. This methodology is used by several groups to study soluble proteins; our group, in collaboration with Henry Lester, has focused on using unnatural amino acids to probe ion channels[8].

Sarah Lummis. X-ray crystallography (atomic resolution) structure determination is currently having the greatest impact on my area of ion channel research (structure/function relationships in ligand-gated ion channels). Unfortunately, there are no such structures available at present for any Cys-loop receptors, which include nicotinic acetylcholine (nACh), $GABA_A$, glycine and 5-hydroxytryptamine $(5\text{-HT})_3$ receptors. However, the publication of the structure of the acetylcholine-binding protein (AChBP) has had a dramatic impact on the field[9]. It has allowed researchers to identify critical binding-site and intersubunit residues of the nACh receptor, and, combined with previous electron microscopy structural data, has allowed identification of the probable mechanism of movement at the binding site that could trigger channel opening. The most recent combination of electron microscopy data with known sequence and structural information[10] has now indicated a possible mechanism by which the binding-site conformational change could result in channel opening; this provides for some exciting and testable hypotheses, which will allow yet more clarification in this area.

Francisco Bezanilla. Spectroscopic techniques, including ELECTRON PARAMAGNETIC RESONANCE (EPR), nuclear magnetic resonance (NMR) spectroscopy and fluorescence, in addition to expression, purification and reconstitution of bacterial channels for spectroscopy and crystallization.

Senyon Choe. X-ray crystallography and the discovery of bacterial equivalents of ion-selective channels have made the greatest impact on my area of ion channel research. I believe that NMR spectroscopy and X-ray crystallography will soon start to have a greater impact than before in determining channel structures of eukaryotic origin. There are two main reasons for this. First, NMR technology has evolved to the stage of being useful for large-sized ion channels, providing novel information regarding the dynamics of ion channel conformations. This crucial information will complement the structural knowledge obtained from X-ray crystallography and optical spectroscopic methods in a very powerful way. Transitions between METASTABLE CONFORMATIONAL states are probably hallmarks of ion channel functionality. Spectroscopic technology that allows the detailed conformational changes of individual atoms to be followed on the timescale over which ion channels operate will therefore be instrumental in completing our understanding of the structural mechanisms of channel functionality. Second, technical breakthroughs will be made for the production of sufficient quantities of eukaryotic ion channels for structural studies. This will enable the knowledge gained from bacterial channels to be verified, tested and improved, so that we can directly address the outstanding biological questions in neuroscience, such as the effects of ligand–channel and protein–protein interactions on channel regulation, from new structural perspectives.

Dale Benos. Mass spectrometry and microarray analysis are having an important impact. With the more sensitive spectrometers and associated proteomic databases now available, low-abundance proteins either associated with, or comprising part of, an ion channel complex can now be identified. Microarray analysis has

made possible the identification of ion channel genes that are either up- or downregulated under a specific set of physiological conditions or disease states. For example, using microarray analysis our laboratory has identified a myriad of ion channels, aquaporins and transporters with mRNA levels that differ between normal brain and the highly malignant glioblastoma multiforme brain tumour[11]. We and others have used mass spectrometry/proteomics to analyse protein components in biological fluids and to identify or confirm the presence of particular ion channel subunits on a gel.

Kenneth Chien. Genomic databases to find new channel co-regulators have been useful, in addition to new assay systems to elucidate their intersection with defined signalling pathways in cells and intact organisms. For example, recent studies have uncovered a family of voltage-gated K^+ (K_V)-channel-interacting protein (KChIP) genes. Specific tissue-restricted isoforms play a key role in quantitatively regulating the TRANSIENT OUTWARD K^+ CURRENT (I_{to}) in the heart, and the loss of the regulator increases susceptibility to cardiac ARRHYTHMIAS[12]. The KChIPs are dynamically regulated

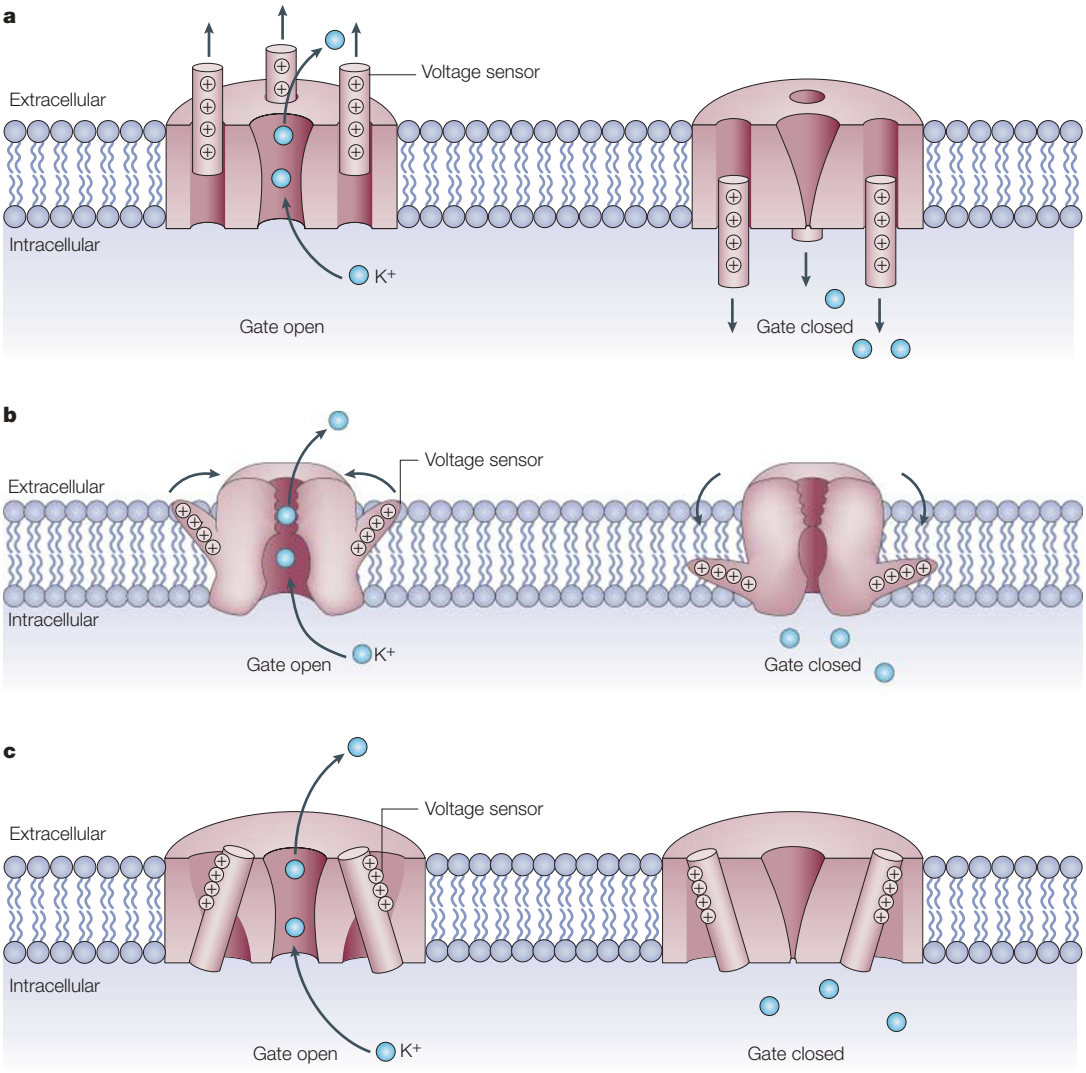


Figure 2 | **Gating of voltage-dependent potassium channels. a** | The conventional model for channel gating. This relies on the charged S4 segments moving, in relation to the channel protein, across the membrane in a manner similar to a peg in a hole. Either this motion, or a rotational movement, was thought to be sufficient to cause channel opening. **b** | A new 'paddle model' for channel gating. Following the publication of the new structure[4] (FIG. 1), it is now proposed that the gating charge is carried by paddles, composed of an α-helical hairpin formed by S3 and S4, on the outside of the channel that pivot against the membrane like levers, directly causing channel activation. This new interpretation of the channel structure, plus insights as to how it operates, must be considered with regard to the nature of the isolated protein, which has been forced to adopt a particular conformation by antibody binding and represents a snapshot view of a fixed structure. How this pertains to the dynamic state(s) of ion channels in the fluid cell membrane awaits further study. **c** | The transporter-like model. This recent model[106] suggests that the charged residues on S4 do not translocate across the membrane (as advocated by the other two models), but simply pivot along their longitudinal axis, transporting the gating charge from an extracellular to an intracellularly connected water crevice, and coupling to the opening and closing of the ion channel. Part **a** modified with permission from *Nature* (REF. 4) © (2003) Macmillan Magazines Ltd. Part **b** modified with permission from *Nature* (REF. 107) © (2003) Macmillan Magazines Ltd. Part **c** modified with permission from *Nature* (REF. 108) © (2004) Macmillan Magazines Ltd. Figure prepared by Trevor Smart.

by cardiac signalling pathways during acquired forms of the disease, suggesting a potential link between inherited and acquired diseases. The question arises as to whether there will be additional classes of channel accessory proteins and cytoplasmic modulators that are expressed in specific subsets of excitable cell lineages, which might allow the design of therapeutic strategies that modulate a given current instead of interacting directly with the channel itself. Databases could be informative in uncovering putative candidates, and new technology to perform PATCH CLAMPING on a chip in an array format will allow a higher-throughput evaluation of such candidates (BOX 2). A host of new technologies, ranging from somatic gene transfer of the regulator itself to RNA INTERFERENCE (RNAi) strategies, could reveal the *in vivo* relevance of these findings.

Walter Stühmer. Technologies that have had the greatest impact have been molecular biology and patch-clamp recording. Molecular biology has allowed the identification and manipulation of the channels themselves, in addition to enabling the production of transgenic mice with modifications of the genes encoding them. Patch-clamp recording has allowed ion channels to be characterized in great detail. I consider that the future

Box 1 | **The fine art of ion-channel crystallization**

The fine structural detail of proteins at the atomic level can be analysed by X-ray crystallography of the protein constrained in an ordered state (that is, as a crystal structure). Although the process of protein crystallization has been applied successfully to many soluble proteins, it has been far harder to achieve for membrane proteins. But why is it so problematic? There are two main issues: first, as receptors and channels are present in the membrane at relatively low concentrations, it is often difficult to obtain sufficient quantities of purified protein for crystallization; and second, the purification process can cause a membrane protein to become structurally unstable, and the detergents used for membrane extraction can hinder crystallization.

Increasing membrane protein availability

It is no coincidence that systems in which channels are expressed at high density, such as the nicotinic acetylcholine receptor in the electric organ of *Torpedo* electric rays, have yielded the most information to date on channel structure. If natural sources of high-density ion channels are unavailable, recombinant proteins can be overproduced by transfecting heterologous expression systems with vectors that incorporate powerful promoters. Methods range from using yeast and bacterial systems, such as *Pichia pastoris* and *Escherichia coli*, to insect-cell-based systems, such as Sf9 cells infected with baculoviruses[101–103]. However, vital post-translational modifications and the correct folding of the protein cannot always be guaranteed in some heterologous systems. This has led to investigations into the use of mammalian cells as vehicles for high protein expression, particularly using viral infection, so that the cell's protein synthetic machinery can be commandeered into producing the ion channel of interest[104].

Solving the protein-purification problem

If sufficient protein can be produced and correctly processed in a suitable cell type, the issue of purification becomes key.

The antibody approach. Although antibodies can aid purification, membrane proteins must be removed from their supportive lipid environment for complete isolation. Membrane proteins are naturally amphipathic, with largely non-polar surfaces that contact the membrane lipid phase, and polar surfaces that face the aqueous environment and the polar groups of the lipid bilayer; these properties mean that their extraction and solubilization usually requires the use of detergents. The protein–detergent complex isolated after purification is a flexible structure, which can prevent the protein from assembling into the rigid lattice-like arrays that are required for crystallization. To overcome this problem, the polar surfaces of the isolated proteins can be increased or accentuated by antibody binding. Native antibodies are considered unsuitable because of their own inherent structural flexibility; however, antigen-binding fragments (Fabs) can produce a more rigid structure by providing a protein scaffold. This approach has been successful for several proteins[105], and was recently used by MacKinnon and colleagues in their crystallization of bacterial potassium channels[4].

The lipidic cubic phase approach. As an alternative, a molecular scaffold or matrix can be used as a 'seeding site' for crystalline protein growth. In this regard, recent work on 'lipidic cubic phases' shows promise[22]. The phase is constructed by combining the membrane protein and its solubilizing detergent with lipids in water. The polymorphism of the lipids in solution produces a micellar phase and a structured bicontinuous phase; the latter has several components that are conducive to crystallization, including a lipid matrix that supports the membrane protein (plus the detergent) in a 'pseudo bilayer' without any disruption to the lipid structure itself; a matrix that allows crystal formation from seed sites; and aqueous channels that penetrate deep into the phase, allowing more protein to flow into the structure to 'feed' the crystal seeding sites. These characteristics were adequate to allow the growth of bacteriorhodopsin crystals[22].

Interpreting static images of ion channels

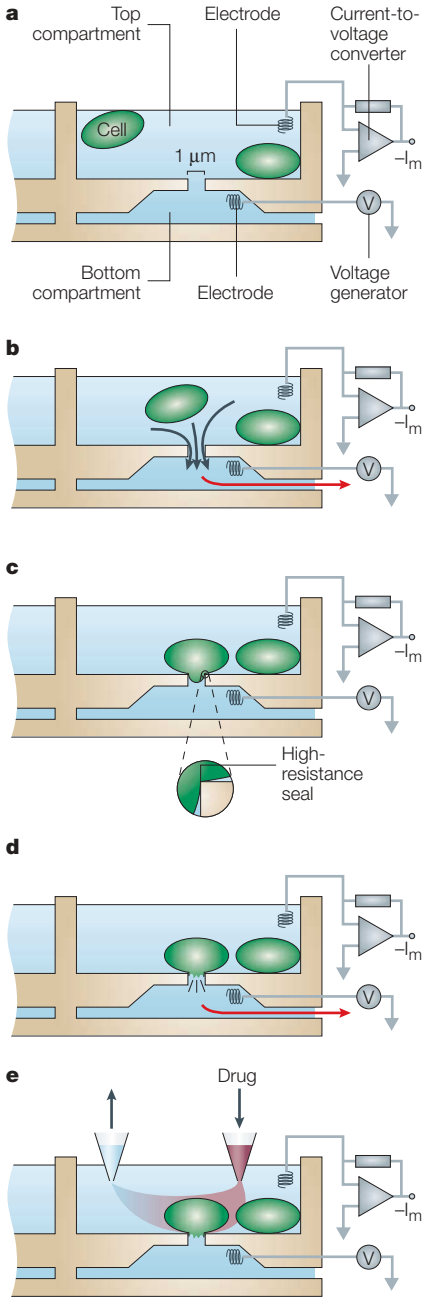
Even if crystallization is achieved, we must consider the particular state in which the once highly dynamic protein has been captured. In the cell membrane, such proteins will exist in several open, shut, desensitized (ligand-gated) or inactivated (voltage-gated) states; however, crystallization might require the protein to be forced into a specific or unusual conformation (possibly by antibody binding). The isolated protein will also be devoid (most probably) of intracellular anchoring proteins, kinases and other regulatory features that could influence its structure. Essentially, the crystal is just one static image of a dynamic protein. Nevertheless, with careful investigation, such images are likely to be informative about fundamental questions about the operation of ion channels and their regulation by drugs. *Trevor Smart*

technologies that will impact ion channel research will be: electrophysiological, high-throughput screening machines using patch-clamping techniques; gene arrays to identify ion channels and interacting partners; and humanized monoclonal antibodies. The latter have great potential to be used in diagnostics and therapeutics, given that ion channels naturally have an extracellular epitope and that monoclonal antibodies can distinguish between similar but distinct ion channels (see question 12).

Richard Lewis. With recent technological advances, several parallel patch-clamp systems are now commercially

Box 2 | **The challenge of developing higher-throughput patch clamps**

The ionic current through ion channels can be recorded by connecting an electrode inside the cell to another outside. An accurate measurement requires accessing the inside of the cell without introducing extra pathways through which the current can leak. The preferred method for small cells is patch clamping. This technique consists of approaching the tip of a very clean pipette (diameter ~1 m) to a cell, making contact and obtaining a high-resistance seal (gigaseal) by applying gentle suction between the glass and the cell surface. Next, by applying greater suction or a large voltage, it is possible to break the membrane and thereby make direct electrical contact between the cell interior and the contents of the pipette. Different voltages can then be applied to the pipette, and the currents measured represent the current through the cell membrane, which includes the current carried by the ion channels present.



An ultra-low-throughput technique

Although patch clamping is the central tool for studying ion channels at present, it is time consuming and requires a highly trained experimenter. There is a need to develop more convenient and higher-throughput methodology to enable many cells to be studied simultaneously.

Achieving high-throughput patch clamping

It is first necessary to automate the patching procedure, and then scale it up to many cells in parallel. Several academic and industrial laboratories have approached this challenge by developing a 'patch-on-a-chip' concept using various polymers, silicon or glass as substrates[17–20]. The concept is summarized in the figure, which shows one of possibly hundreds of unit cells built on a chip. Each cell has a top and bottom compartment that communicate through a hole of ~1 m diameter. The compartments are connected to a network of microfluidics, allowing solutions to be exchanged independently. Reversible electrodes are built into both compartments and connected to a current-to-voltage converter and a voltage generator (see figure, panel **a**). The cells are deposited by a robotic arm into the top compartment, and the electrical resistance between the compartments is monitored continuously. Suction or electric fields are then applied to direct a cell to the hole (panel **b**). When the cell blocks the hole, the resistance rises, signalling the control system to decrease the pressure until a high-resistance seal is formed (>1 gigaohm; panel **c**). It is then possible to record the currents from one to several individual ion channels in this area. If the whole-cell current is sought, the membrane in the hole must be broken by applying negative pressure in the bottom compartment (panel **d**), or a large voltage pulse between the compartments. Whole-cell membrane current (I_m) recording is done by applying different voltage waveforms (V). At any time, drugs can be applied to the top compartment, while the electrical recording continues (panel **e**). The main hurdle of this technique is the reliability of obtaining gigaseals, a process that is still not understood. Of the several substrates being tested (see above), silicon is particularly attractive, because it would be possible to include the required patch-clamp electronics (using very large-scale integration (VLSI)) and microfluidics control systems on a single chip. Alternatively, the complete system can be assembled with a mixture of different substrates using microfabrication techniques. A multiple-cell system with microfluidics and electronics in one device would result in a disposable chip that could be used to simultaneously study hundreds of compounds and conditions in cells expressing the ion channel of interest.

Francisco Bezanilla and Chris Miller

available (for example, IonWorks HT system, Molecular Devices; PatchXpress, Axon Instruments; see Further Information for company websites). High-throughput patch-clamp analysis can greatly accelerate ion channel drug-lead discovery. The technique controls cell membrane potential to provide a functional measure of ion channel activity. Using this approach, which traditionally has been accomplished by a researcher working on a single cell at a time, ensures that the effects of a large number of compounds can be determined on ion channels maintained at physiological or pathological states. With this technique, use-dependent inhibitors (ligands that prefer to bind to the channel state found in diseased rather than normal tissue) and selective inhibitors of pathological states can be identified early in a screening programme. Parallel and automated oocyte electrophysiology is also now available, such as the eight-channel OpusXpress from Axon Instruments. This approach is more useful for examining compounds that act at the extracellular face of ion channels, especially for those channels that are difficult to express in mammalian cells (for example, the voltage-sensitive Na^+ channel $Na_V1.8$).

Birgit Liss. Microarray techniques and real-time quantitative polymerase chain reactions (PCR) have had a great impact on my area of ion channel research. The latter technique enables the detection of quantitative differences in ion channel subunit expression, and allows microarray data validation at the single-cell level. In addition, the cell-specific delivery of RNAi/SMALL INTERFERING RNA (siRNA) technology might provide promising tools.

Alan Verkman. Our drug discovery work in the ion channel field involves the identification of inhibitors of the cystic fibrosis transmembrane conductance regulator (CFTR) protein and activators of mutant CFTRs that cause cystic fibrosis[13,14]. Bright GREEN FLUORESCENT PROTEIN (GFP) mutants with strong halide-sensitive fluorescence have been key for efficient high-throughput screening in a kinetic cell-based assay to identify inhibitors and activators of CFTR-mediated halide fluxes[15,16].

2 What tools and technologies for ion channel research would you most like to be available in the future?

Dennis Dougherty. Higher-throughput electrophysiology (see question 6). This tool seems likely to become available in industrial labs very soon, although it may be longer before academic labs obtain it.

Michel Lazdunski. More of the classical physiological models, such as electrophysiological recording adapted to mice (for knockouts), in addition to high-throughput 'electrophysiological' techniques for pharmacology.

Kenneth Chien. I think we will find new uses for channels in the future, as the technology for assaying their function becomes more widespread and user-friendly for non-electrophysiology-based labs; that is, if the functional assay can be reduced to a level that can be used easily by molecular biologists and physician scientists, similar to GFP-based systems now. I then believe we will use them as 'electrophysiological fingerprints' to identify specific cardiovascular lineages and neuronal cell lineages. Specific combinatorial groupings of the channels may afford a new way to build in specificity for channels that are widely expressed.

The ability to perform higher-throughput, single-cell recordings on a variety of primary cardiovascular-derived cell lineages could be powerful for drug discovery. Most of the higher-throughput screening for small molecules is being done in surrogate cell systems (for example, human embryonic kidney 293 cells), in which the channel of interest is stably expressed, rather than using the native cell of interest (such as cardiac cells and neuronal cells). There is likely to be a unique cell-type-specific complex of proteins that regulates the function of key channels in excitable cells, but this specificity may be lost in the primary screen, thereby biasing against finding new classes of lead compounds that might have the desired activity.

David Clapham. More crystal structures of ion channels at 2–3-Å resolution would be very useful. The selectivity filters of Na^+ and Ca^{2+} channels have not been solved, and more data are needed on all the conformations of voltage-gated ion channels in the multiple configurations of their gating states. In addition, high-throughput patch-clamp arrays with multi-well recording at gigaohm or higher seal resistance would be valuable. For academic labs, 16 wells would be most useful. For the pharmaceutical industry, 96 wells or above would be helpful. The main hurdles to obtaining reliable high-resistance seals are the identification and mass production of ideal coatings that enable cell-membrane seal formation with the substrate. Intelligent software that incorporates years of electrophysiological training by humans in recognizing artefacts is also essential, in addition to more accessible technologies for identifying proteins that associate with ion channels, such as simple membrane preparations that work for mass

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Dale Benos, Professor and Chair, received his B.A. degree in Biology in 1972 from Case Western Reserve University, Cleveland, Ohio, USA. He earned his Ph.D. degree at Duke University in Durham, North Carolina, in the Department of Physiology and Pharmacology in 1976. After two further years of postdoctoral study at Duke, he moved to the Department of Physiology and Biophysics and the Laboratory of Human Reproduction and Reproductive Biology at Harvard Medical School, Boston, Massachusetts, where he was Assistant and then Associate Professor, and an Andrew W. Mellon Scholar. He joined The University of Alabama at Birmingham, Alabama, in 1985. In 1996, Benos became Chair of the Department of Physiology and Biophysics. His main area of research is in ion channels and transporters, especially their role in diseases such as genetic hypertension, cystic fibrosis, HIV-induced dementia and brain tumours.

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Francisco Bezanilla received a B.S. in Biology and an M.S. and Ph.D. in Biophysics from the Catholic University, Santiago, Chile. Following a visiting postdoctoral fellowship at the Laboratory of Biophysics of the National Institute of Neurological and Communicative Disorders (now the National Institute of Neurological Disorders and Stroke; NINDS) at the National Institutes of Health (NIH) in Bethesda, Maryland, USA, with Kenneth Stewart Cole and Robert Taylor, Bezanilla undertook postdoctoral research with Paul Horowicz at the University of Rochester, Rochester, New York. He returned to Chile in 1972 to the University of Chile in Santiago, where he was Assistant Professor and then Professor in the Department of Biology, and made yearly visits to the Marine Biological Laboratory in Woods Hole, Massachusetts, USA, where he described the properties of gating currents of the sodium channel with Clay Armstrong. Bezanilla joined the Department of Physiology at the University of California, Los Angeles, as Professor of Neuroscience in 1977. He received the K. S. Cole award in 1990, was named Susumu Hagiwara Professor of Neuroscience in 1995, Fellow of the Biophysical Society in 1999, and was elected to the Latin American Academy of Sciences in 2002. Professor Bezanilla's main area of research is the structure/function of voltage-dependent ion channels and adaptation of the nervous system to the environment. His laboratory uses a combination of biophysical, physiological, optical and molecular biology techniques to search for the molecular basis of voltage sensor operation.

spectrometry (this would require more successful mass spectrometric analysis in the presence of detergents).

Michael Sanguinetti. High-capacity voltage-clamp instrumentation will revolutionize the way in which drugs are screened for ion channel activity. At present, the greatest rate-limiting factor with regard to data collection in electrophysiology is the voltage-clamp technique, which can be applied to only a single cell at a time. In addition, the technique is difficult to learn and perform, limiting the number of labs that routinely use it to quantify the biophysical properties of ion channels. Highly automated and relatively large-capacity voltage-clamp instrumentation will be available before the end of 2004, and will both increase the output of an experimenter by one or two orders of magnitude, and simplify procedures by automating tasks such as obtaining gigaohm seals, rupturing membrane patches, exchanging fluids in the experimental chamber and so on. In addition, these new instruments will be even better at automated data collection and analysis.

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Kenneth Chien received a B.A. in Biology from Harvard College, Boston, Massachusetts, USA, and a Ph.D. and M.D. from Temple University, Philadelphia, Pennsylvania. He completed his internship and residency in internal medicine at Parkland Memorial Hospital, Dallas, Texas, and subsequently undertook a clinical and research Cardiology Fellowship at the University of Texas Southwestern Medical Center in Dallas. In 1988, Chien moved to the University of California, San Diego (UCSD) to initiate a programme in molecular cardiology, one of the first of its kind in the United States. In 1992, he became Co-Director of the UCSD Cardiovascular Center, and in 1998 he was appointed Director of the National Heart, Lung and Blood Institute (NHLBI) USCD/Salk Institute Program in Molecular Medicine. Chien is currently Director of the USCD Institute of Molecular Medicine, which was established in 2000 with the aim of encouraging interactive, interdisciplinary educational and research opportunities in the field of molecular medicine. He is the recipient of numerous awards, including the 1995 Pasarow Foundation Award (co-recipients, A. Knudsen and S. Prusiner) and the 1995 Walter B. Cannon Award of the Amercian Physiological Society, and is the American Heart Association's Endowed Chair in Cardiovascular Research. Chien has been a pioneer in applying the techniques of molecular biology to studying cardiovascular disease. His laboratory uses a combination of genetically engineered mouse models, miniaturized physiological techniques and functional genomic strategies to identify molecular pathways for complex human cardiac diseases.

Francisco Bezanilla. High-throughput, multiple patch clamping will be particularly useful, and several companies are developing such technology at the moment, including Sophion Bioscience, Aviva Biosciences Corporation and Nanion Technologies. Although this technique is still in development, there are at least three different groups working to achieve gigaohm seals in different types of substrate (silicon, glass or resin) with high reliability[17–20,116]. One of these systems is already on the market (PatchXpress 7000A, Axon Instruments).

In addition, I would like to see single-molecule fluorescence and real-time spectroscopic techniques, such as second harmonic generation, infrared and Raman spectroscopy.

Trevor Smart. There is a need to improve imaging tools to enable better real-time imaging of ion channels, in order to understand how discrete domains (such as ligand-binding sites and channel gates) of ion channels interact (for example, optical interaction techniques, such as FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)). Tools are also required to ensure the easier crystallization of ion channels and receptors for analysis of their three-dimensional structures. In this way, we would be able to consider mapping ligand-binding sites to different states of the channel (open, shut, desensitized and so on) for the first time; this would provide clear molecular templates for the binding of drugs to individual ion channels, which will aid in the design of new ligands. There is also a need for a channel database that is capable of allowing easier cross-referencing of structure/function studies, particularly when these involve mutations of ion channels.

Sarah Lummis. I would like tools to specifically label individual amino-acid residues without disrupting the protein structure. This would allow subsequent identification of their movement and/or interactions during channel activation and DESENSITIZATION. At present, it is possible to follow the movement of certain amino acids, but this is commonly achieved by mutagenesis (for example, to cysteine) followed by labelling (for example, with a fluorescent residue)[21]. Although this has provided useful information, a non-disruptive and more flexible approach would be much more informative. I believe that such ligands could be designed by interested chemists, but unfortunately there are relatively few chemists that are concerned with basic research in this area.

Richard Lewis. Although high-throughput patch clamping and fluorescent dyes are available, present

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Senyon Choe gained B.S. and M.S. degrees in Biology from Seoul National University, Korea, and did his thesis on channel-forming proteins and channels in Robert Stroud's laboratory at the University of California, San Francisco, USA, graduating with a Ph.D. degree in Biophysics and Medical Physics from the University of California, Berkeley, in 1987. After a stint at Cornell University, Ithaca, New York, he did postdoctoral research in David Eisenberg's laboratory at the University of California, Los Angeles, between 1988 and 1993, studying structures of the channel-forming diphtheria toxin and synthetic coiled-coil peptides. He joined the Salk Institute, La Jolla, California, in 1993 as the first member of the Structural Biology Laboratory. His group uses a combination of X-ray crystallography and nuclear magnetic resonance spectroscopy to analyse the structure and conformational dynamics of eukaryotic ion channels, and also to enable drug discovery for neurological disorders. Choe's laboratory provided the first atomic details of the tetramerization domain of eukaryotic voltage-gated (K_V) potassium channels, which led to the proposal of the lateral opening for the cytoplasmic vestibule of the channels. Subsequently, Choe's group discovered that eukaryotic K_V channels are zinc-bound, which provides an essential molecular determinant for channel assembly. More recently, the group determined the structure of the ubiquitous cytoplasmic regulatory K^+-transport, nucleotide-binding (KTN) domain of bacterial transporters, which led to the proposal of a novel structural mechanism for regulating K^+ flux through changes in its oligomeric state. He was awarded a Klingenstein Foundation Fellowship in 1996, and was elected a Fellow of the American Association for the Advancement of Sciences in 1999.

platforms have limitations, either due to the cost of hardware or range of assay formats available, which limits their use, particularly by biotechnology companies and mid-sized pharmaceutical companies. Advances in nanotechnologies and dye chemistries (such as an expanded range of Na^+-selective dyes) will broaden the use of fluorescence-based technologies in ion channel drug discovery. In addition, overcoming difficulties in expressing certain ion channels in mammalian cells (for example, $Na_V1.9$) by identifying new co-factors or chaperones will help broaden the implementation of these technologies.

Alan Verkman. Na^+- and K^+-sensitive GFP mutants would be very useful for cell-based, primary high-throughput screening. Direct measurement of intracellular ion concentrations in a primary screen can provide a sensitive and specific read-out of ion transport to complement membrane-potential-based read-outs. Efficient electrophysiological analysis of compounds for secondary screening is also needed, including improvements in automated oocyte two-electrode voltage-clamp and mammalian-cell patch-clamp methods. Also, automated Ussing chamber/short-circuit current measurements offer a relatively technically simple approach to collecting electrophysiological data on ion channel regulation. Various techniques can be readily exploited to focus on specific aspects of ion channel regulation and gating, such as ion substitution, apical/basolateral membrane permeabilization and specific agonists/inhibitors.

Jamie Vandenberg. Improvements in tools/technologies to facilitate purification (and crystallization) of membrane proteins would be useful. This could include, for example, developments in lipidic cubic phases for the solubilization of membrane proteins. Lipidic cubic phases are thermodynamically stable lattice-like structures composed of a lipid component with three-dimensional periodicity pervaded by an intercommunicating aqueous channel system[22]. In addition, it would be useful to develop integrated models of cellular electrophysiology; that is, models that include details of all the ELECTROGENIC PROCESSES in a cell, as well as the pathways that modify them, including, for example, metabolic pathways and signalling pathways. Ultimately, such models will also include the signalling pathways that regulate gene transcription, intracellular trafficking and intercellular communication, followed by the development of whole-organ models, such as those being developed by Peter Hunter and colleagues at the University of Auckland, New Zealand (see, for example, REF. 23). Finally, the discovery of a model organism that has a cardiac electrical phenotype more similar to that of humans would be valuable (the mouse is a very poor model for human ventricular REPOLARIZATION).

Birgit Liss. Reliable RNA amplification techniques to generate sufficient mRNA or cDNA for microarray-based expression profiling from limited amounts of tissue would be useful. Ideally, the nucleic acids need to be amplified in an unbiased manner; that is, without disturbing the relative abundance of each transcript. At present, PCR-based amplification methods and antisense RNA amplification methods (such as T7 polymerase) are available. The former technique is easy to perform, but exponentially amplifies DNA molecules with different efficiencies. The latter technique, however, should amplify nucleic acids in a linear way. Recently, some kits have become available that allow RNA/cDNA amplification, although it is still not a routine tool. As microarray techniques and single-cell mRNA isolation (using patch-clamp pipettes or laser microdissection) are now routine techniques, it is the amplification procedure that remains the bottleneck for this type of experiment. Robust RNAi protocols for brain slice cultures would also be valuable, which might allow electrophysiological patch-clamp analysis before and after gene silencing.

Alan North. More of the above technologies (see question 1); refined microscopy, including variations of surface microscopy and ATOMIC FORCE MICROSCOPY; and channels on chips for high-throughput screening.

Frances Ashcroft. On my wish list are a fluorescent probe for quick, easy and accurate measurement of intracellular nucleotides (ATP, ADP) to enable correlations between cell metabolism and ATP-sensitive K^+ (K_{ATP})-channel activity; better fluorophore couples for FRET that do not show 'bleedthrough', which are small enough not to disturb channel function and which can easily be used to label proteins in intact cells; a simple means of measuring mitochondrial metabolism in single cells; selective ligands for different types of INWARDLY RECTIFYING K^+ CHANNEL; a good annotated database of ion channel structure, function, expression and so on; and an expression system for the large-scale production of eukaryotic membrane proteins (to enable structural studies).

3 How close are we to being able to integrate understanding of the cellular physiology or biophysics of ion channels with their role in disease states?

Chris Miller. We are not that close in most cases. Physiology is fraught with complexity, and it is the rare case for which the function of a specific, molecularly defined ion channel can be traced in detail to a physiological response.

Irwin Levitan. With rare exceptions, not close at all. I believe that the molecular biophysics approach that dominated ion channel research during the 1990s, although exceptionally productive, came at the expense of cellular physiological approaches. The structural advances of the past several years, as spectacular as they have been, have also shifted the focus in a more reductionist and non-physiological direction. There is a lot of catching up to do, particularly in relating particular ion channel proteins to specific membrane currents, before we can hope to understand the pathophysiology of ion channel diseases. Unfortunately, there is minimal interaction between the molecular biophysicists and cellular physiologists, so this problem is not being addressed effectively.

Trevor Smart. We are still some way from a clear understanding. One of the largest gaps in ion channel research is the link(s) between channel function and mutations (natural or engineered) and the behaviour of an organism. In simpler organisms, there are some promising links, but in higher-order species further clarification of the relationship between channel function, signalling pathways, the behaviour of networks of cells (such as neurons) and eventual behaviour is required. There are many promising indicators, but much more basic research is necessary to investigate how ion channels affect single-cell and network function. One example serves to illustrate this point: although the $GABA_A$ receptor is the major inhibitory receptor in the brain, linking mutations in this receptor to CNS diseases has proved difficult, despite empirical observations that many drug classes (for example, the benzodiazepines and selected general anaesthetics) target this receptor to good therapeutic effect[24]. However, it seems more promising that some forms of human startle disease (hyperekplexia) can be associated (although not exclusively) with mutations in the glycine receptor α_1-subunit[25].

Alan North. We are not yet very close, because details of the functional roles of channels in individual cells are not yet understood except in direct signalling (electrical terms). By this, I mean electrical interactions that result simply from flow of current (and which can all be modelled with cables, branching cables, tapering cables, resistors or capacitors), and which exert their effects on electrically excitable molecules, such as voltage-gated Ca^{2+} (Ca_V), Na_V and K_V channels — that is, classical synaptic integration. Electrical signals from channels spread quickly and affect a large area, whereas chemical signals from channels are very localized. We must concentrate, therefore, on understanding the microdomain — that is, effects of changes in ion concentration on neighbouring molecules within the complex rather than on cytoplasmic concentrations. This is already happening (for example, Ca^{2+}-channel-to-K^+-channel signalling, and Ca^{2+} channel to EXOCYTOSIS). A good example is the work of Neil Marrion, which shows that the Ca^{2+} entering through N-type Ca^{2+} channels rapidly activates large-conductance Ca^{2+}-activated K^+ channels, whereas the Ca^{2+} entering through L-type Ca^{2+} channels more slowly activates small-conductance Ca^{2+}-activated K^+ channels[26].

Frances Ashcroft. In many cases, a long way off! For example, despite the fact that the *CFTR* gene was cloned some years ago, our understanding of precisely how it causes cystic fibrosis is still limited. The correlation between genotype and phenotype is also unclear for mutations in many other ion channel genes. It is often not possible to correlate the severity of a disease with the functional effect of a given mutation, and the same mutation may cause different effects in different patients[27]. Clearly, genetic background plays a significant role; thus, the identification of other genes that influence disease susceptibility/severity is important.

Alan Verkman. We are much closer than a decade ago, but not very close. For example, although much is known about CFTR biophysics and cell function 14 years after its identification, the mechanism by which CFTR causes cystic fibrosis, the most common hereditary disease in Caucasians, remains unknown. Cystic fibrosis subjects suffer from repeated lung infections and deteriorating lung function, as well as problems outside of the lung, such as malabsorption resulting from pancreatic insufficiency. There are many hypotheses but little direct evidence about how defective CFTR causes lung defects, such as abnormal functioning of airway submucosal glands, altered airway-surface liquid composition or rheology, and an intrinsic hyperinflammatory response[28,29]. The complexities of mammalian physiology and compensatory changes in disease remain major challenges in linking protein dysfunction to clinical disease.

Francisco Bezanilla. We know what a particular mutation can do in the operation of an ion channel. However, the clinical aspects are normally much more complex, and they cannot be explained directly by the effects observed with the channel in isolation. To get the complete picture will require studying the physiology of the cell *in vivo* with the mutation present and the development of more comprehensive models of channel operation, including their interactions with the rest of the cell. Examples are hyperkalemic periodic paralysis and paramyotonia congenita, which have been traced to mutations in the Na_V channel in skeletal muscle fibres (see, for example, REF. 30). In one case of hyperkalemic periodic paralysis, the mutation produced a larger overlap of activation and inactivation, generating an inward current that depolarized the fibre[31]. However, how the actual episode of paralysis is evoked will require a detailed study of all the ionic currents, ideally in muscle fibres from biopsies, to assess

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David Clapham earned his Bachelor of Electrical Engineering degree at the Georgia Institute of Technology, Atlanta, Georgia, USA, and his M.D. and Ph.D. degrees from Emory University School of Medicine in Atlanta. He completed his residency in internal medicine at Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts. Clapham was a senior Fulbright Fellow during his postdoctoral training with Erwin Neher at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany. With Diomedes Logothetis and Eva Neer, he discovered the role of the Gβγ subunits as an independent G-protein activator in signal transduction. He defined the G-protein-gated inward-rectifier potassium channel (GIRK) heteromer as the target of Gβγ, and showed that this molecular mechanism mediates neuronal slowing of heart rate. He received the American Heart Association Basic Science Prize and the K. S. Cole Award from the Biophysical Society in 1996. Clapham returned to Harvard Medical School in 1997 to become Director of Cardiovascular Research at the Children's Hospital. He was elected to the American Academy of Arts and Sciences in 1999, and is an investigator of the Howard Hughes Medical Institute. His research efforts are focused on new Ca^{2+}-permeant ion channels, transient receptor potential (TRP) channels, and the high-resolution structure of bacterial channels.

the roles of all the ionic conductances and their interactions in producing the final DEPOLARIZATION under the appropriate stimulus.

Sarah Lummis. In the ligand-gated ion channel field, a number of specific mutations that relate to disease states have been identified, and characterization of these has shown that they are responsible for the underlying physiological state. Perhaps one of the most interesting of these is autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), for which there are a variety of mutations that can cause the disease, and there is still some controversy about the integration of the underlying physiology with its subsequent cellular effect. Daniel Bertrand and colleagues, for example, suggest, following analysis of a number of mutants associated with ADNFLE, that a gain of function of the receptors (that is, they are more efficient) might be responsible for initiating the changes that ultimately result in epileptic seizures[32]. However, Bruce Cohen and co-workers suggest that the ADNFLE mutations reduce the Ca^{2+} dependence of the acetylcholine-induced response, which could trigger seizures by increasing nACh-receptor-mediated glutamate release during bouts of synchronous repetitive brain activity[33]. For many disease states, however, I believe we are far from this level of understanding, although the sequencing of the human genome and advances in microarrays and proteomics (see question 9) will, in my opinion, significantly advance our understanding of these relationships.

David Clapham. We have quite a bit of information on single-gene channelopathies, but this only scratches the surface. Further progress will depend on a more complex analysis of human genetics and more extensive analyses of physiological end points in transgenic mouse models. There is a real need for improved tests for alterations in nervous system function in knockout models.

Michel Lazdunski. We are already there; such integration will now be very rapid for a number of human diseases. Progress is always more rapid when animal models are available.

John Peters. At least in specific instances, very close indeed. Numerous channelopathies that are associated with specific disease states support this (see question 14).

Kenneth Chien. We are getting closer, but one of the barriers is that a more interdisciplinary approach will be required in the future that spans computational, structural and clinical expertise.

Dale Benos. With time, more and more ion channel associations with human disease are being elucidated. Dysregulation of channel function and/or modulatory pathways, such as second messengers, biochemical changes, ionic milieu and so on, are in many cases directly responsible for the pathophysiology associated with a particular disease. For example, Liddle's syndrome is known to result from mutations in the carboxyl terminus of either the β- or γ-subunit of the epithelial Na^+ channel (ENaC), leading to a hyperactive reabsorptive Na^+ channel in the distal nephron and collecting duct[34]. So, understanding these alterations in function will clearly lead to an understanding of the disease itself.

Michael Sanguinetti. In many cases, we are already at this point. Channelopathies have been well characterized, and mouse models exist for some. Long-QT syndrome (LQTS), a disorder of ventricular repolarization that predisposes affected individuals to lethal cardiac arrhythmia, is a good example (FIG. 3a). A few years ago, LQTS was recognized as a rare syndrome that could be either inherited (TABLE 1) or acquired (for example, as an unwanted side effect of some common medications (FIG. 3b)). Molecular genetic discoveries by Mark Keating's laboratory showed that inherited LQTS was due to gain-of-function mutations in the cardiac Na^+ channel or loss-of-function mutations in the α- or β-subunits of two different DELAYED-RECTIFIER K^+ CHANNELS[35]. These discoveries defined LQTS as a heterogeneous disorder, and indicated that different treatment strategies would probably be required to prevent arrhythmias. For example, gain-of-function mutations in the Na^+ channel could be treated with Na^+-channel-blocking agents, whereas LQTS due to loss-of-function mutations in K^+ channels could be treated with a K^+-channel activator. The most effective drugs for these purposes have not yet been developed, in part because of economics (small market), but we now have the basic understanding of the disease to enable us to apply rational drug design to the discovery of new drugs.

Drug-induced LQTS is almost always the result of blocking the human ether-a-go-go-related gene (HERG) K^+ channel as an unwanted side effect. The structural basis of the binding site has been partially

defined[36]. When this knowledge is combined with QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP (QSAR) data, it will be possible to perform *in silico* (virtual) screening of compounds for their ability to bind to the channel. This approach will vastly accelerate the identification of drugs that are devoid of HERG-channel-blocking activity.

Jamie Vandenberg. In many 'monogenetic' diseases, such as the LQTSs, the causal relationship between abnormal ion channel activity and the disease state is well established. However, despite this, we are still unable to predict the clinical course of the disease. For example, why might one family member die in childhood, but another affected member survive well into adulthood? Indeed, why do almost all affected individuals survive for many years and, in some cases, tens of years, apparently symptom free? There are likely to be two main reasons for this: first, despite being a monogenetic disorder, the clinical outcome is almost certainly

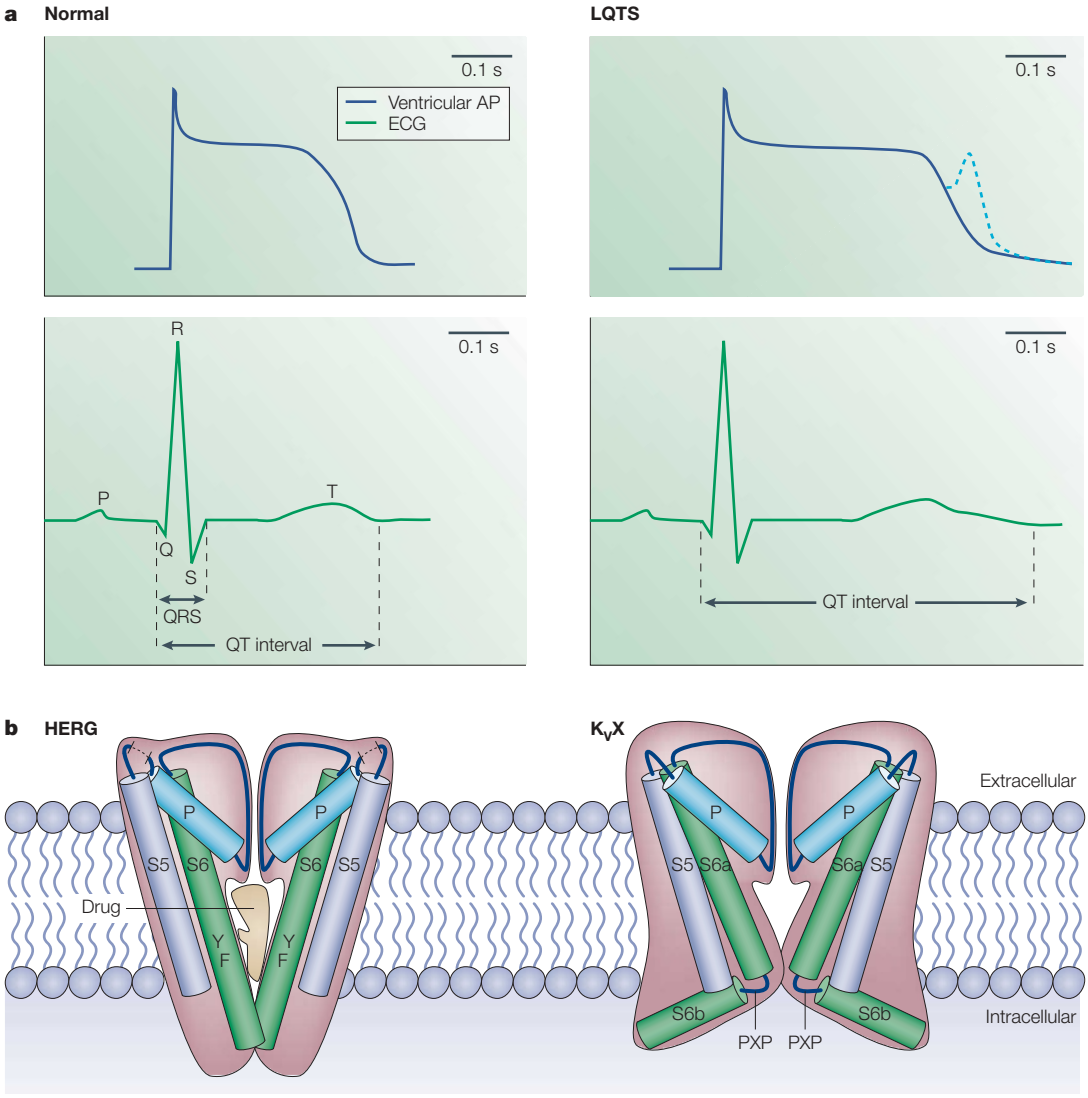

Figure 3 | **Long-QT syndrome is an example of a well-characterized channelopathy. a** | Ventricular action potential (AP; top panel) and ELECTROCARDIOGRAM (ECG; bottom panel) recorded from a normal patient and a patient with long-QT syndrome (LQTS). The P-wave corresponds to atrial depolarization, the QRS complex to ventricular depolarization and the T-wave to ventricular repolarization. The QT interval is measured from the start of the QRS complex to the end of the T wave. The QT interval is normally <0.44 s. In LQTS, abnormalities in currents during the plateau phase of the AP (decreased repolarizing or increased depolarizing currents) lead to prolongation of the ventricular AP and hence the QT interval, as well as increasing the tendency for early after-depolarizations (dotted line), which markedly increases the risk of arrhythmias. **b** | Unintended blockage of human ether-a-go-go-related gene (HERG) channels is a common cause of acquired LQTS. HERG channels contain two aromatic residues (Y652 and F656) in the S6 helices (green), which line the pore cavity and can therefore form cation–π interactions with multiple drugs. HERG also has a larger cavity than other K_V channels, and so can accommodate a wide size range of drugs. The smaller cavity in other K_V channels (K_VX in the diagram) is due to the presence of a Pro-X-Pro (PXP) motif in the S6 helix, which causes a kink and thereby reduces the size of the pore cavity. The long extracellular linker between the S5 helix (blue) and pore helix (P, turquoise) in the HERG channel is represented with a dashed line. A database of drugs associated with acquired LQTS can be found at http://www.qtdrugs.org/. Figure prepared by Jamie Vandenberg.

Table 1 | **Mutations in several ion channels can cause inherited LQTS**

Ion channel	Function	Swiss-Prot reference
K_VLQT1	α-subunit of the slow component of the delayed rectifier K^+ current, I_{Ks}	P51787
HERG	α-subunit of the rapid component of the delayed rectifier K^+ current, I_{Kr}	Q12809
minK	β-subunit of the slow component of the delayed rectifier K^+ current, I_{Ks}	P15382
mirP1	β-subunit that may associate with multiple α-subunits, including HERG	Q9Y656
SCN5a	α-subunit of the cardiac sodium channel, I_{Na}	Q14524

The table outlines ion channels encoded by genes associated with LQTS. A frequently updated database of LQTS-associated mutations can be found at http://www.ssi.dk/graphics/html/lqtsdb/lqtsdb.htm. HERG, human ether-a-go-go-related gene; LQTS, long-QT syndrome.

modified by compensatory responses (at the gene-transcription level), which will vary from person to person; and second, although the abnormal ion channel greatly increases the risk of sudden death, the lethal arrhythmia still has to be triggered (usually a response to an environmental stress). We therefore need more information with respect to which genes have altered transcription patterns in response to 'knocking out' individual ion channels, as well as a better understanding of the link between environmental stresses and the regulation of cellular electrophysiology. The first goal will require long-term studies of alterations in gene expression and the identification of modifier gene influences (such as an analysis of QUANTITATIVE TRAIT LOCI in several generations of model organisms). The second will require the development of better models, which incorporate ion channel activity, metabolic pathways, signalling pathways (both intracellular and intercellular) and intercellular communication.

Walter Stühmer. Diabetes affects a large number of people, and the incidence is rising mainly due to greater carbohydrate consumption in an affluent society. The high incidence, together with the fact that the disease is manageable, has generated intense research into the various processes affected in diabetes. One particular type of K^+ channel, which depends on intracellular ATP, was found to play a pivotal role in determining insulin secretion[37]. The modulation of this channel by drugs, such as sulphonylureas, has therefore been intensively investigated for use in treating diabetes, and we consequently have a broad understanding of the disease[38].

Another group of diseases for which the link to ion channels is understood at a biophysical level is the muscular dystrophies. This is due to the fact that many of these diseases are genetically linked, and the loci of many of them have been traced to ion channels[27,39].

For many other diseases, our knowledge is in its infancy and the field of 'channelopathies' is just emerging. The awareness that ion channels, in addition to being excellent subjects for applying biophysics, are excellent diagnostic and therapeutic targets, is relatively new. However, there is great potential to increase our understanding, which I feel is underestimated by clinicians at present. Within this context, it has to be taken into account that a significant fraction of drugs used at present act directly or indirectly on ion channels.

4

As we enter a post-structural era in voltage-gated ion channel research, what big questions remain at the structure/function interface?

David Clapham. The most pressing need is to understand the various mechanisms of channel gating, particularly voltage gating and ligand gating. The mechanisms of selectivity for K^+ and Cl^- channels are largely solved, but we still need high-resolution structures of the pores of Na^+ and Ca^{2+} channels.

Jamie Vandenberg. There is a need to understand the dynamics of intramolecular motions in voltage-gated ion channels. This will come through a combination of mutagenesis experiments (combined with amino-acid-side-chain modifications), molecular dynamics simulations and imaging techniques, such as NMR spectroscopy, EPR spectroscopy and FRET. We also need to determine the structural basis of subtype specificity; for example, in terms of differences in rates of voltage-sensor motion and range of voltage sensitivity.

Senyon Choe. Aside from fundamental physicochemical questions, such as ion selectivity, elucidating the regulatory mechanisms unique to each eukaryotic ion channel is a key question to follow up. The answers to these questions will enable us to apply structural information directly to the biology of these channels, particularly the regulation of individual channel functionality. For obvious reasons the regulatory mechanisms will be diverse, but there should be a common thread linking to the transmembrane domain of the channels, because these mechanisms will all ultimately regulate the selectivity filter of the channels (the 'business end' of the ionic pathway), which is built on common physical principles governing charged ions. I think some consolidated views on the fundamental mechanics will emerge from a number of related channel structures to explain how, in general, controlling signals — whether they are voltage, small molecules or cytoplasmic proteins — exert their effects, and whether they use common mechanisms of control. What fundamental questions will arise out of these studies is difficult to predict; but in general, I would suspect questions about protein–protein interactions inside the cell, the mechanisms that control their interactions at the atomic and molecular levels and, finally, the mechanisms of controlling the intra- and intercellular localization of the channels, are likely to occupy us for some time.

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Dennis Dougherty received a B.S./M.S. in 1974 from Bucknell University, Lewisburg, Pennsylvania, USA, and a Ph.D. from Princeton University, Princeton, New Jersey, in 1978, followed by a year of postdoctoral study at Yale University, New Haven, Connecticut. In 1979, he joined the faculty at the California Institute of Technology (Caltech), Pasadena, California, where he is now George Grant Hoag Professor of Chemistry. Professor Dougherty is perhaps best known for his discovery of the cation–π interaction, a novel but potent non-covalent binding interaction. The fundamental nature of this interaction has since been established through extensive theoretical studies by the Dougherty group. Dougherty also established the prevalence of the cation–π interaction in biological systems, and it is now recognized to be important in a wide range of biological processes, including neurotransmission, steroid biosynthesis, drug–receptor interactions and structural biology in general. More recently, he has addressed molecular neurobiology, developing an *in vivo* nonsense suppression method for unnatural amino-acid incorporation into proteins expressed in living cells, in collaboration with Professor Henry Lester at Caltech. This powerful new tool enables chemical-scale studies of the full range of ion channels and neuroreceptors that have central roles in synaptic transmission. On another front, the Dougherty group has recently begun extensive experimental and computational studies of the bacterial mechanosensitive channels MscL and MscS, building on the crystal structures of these channels recently reported by the Rees group at Caltech.

Alan North. The era is not yet 'post-structural'! We need many more structures. The issue is that the crystal structures of membrane proteins are not the same when they have been removed from the lipid environment, and that associated proteins and other features of the cellular environment have profound effects on channel function. Although structures of single proteins are fine, we were only able to understand fully, for example, the ribosome or electron transfer in intermediary metabolism when we had structures of large (functioning) complexes.

Irwin Levitan. In my view, it is premature to conclude that we are entering a 'post-structural' era. Only a handful of structures have been solved, and virtually all of these by a single laboratory (the MacKinnon laboratory). Although this laboratory is led by a superbly productive and, indeed, visionary investigator, we should not lose sight of the fact that reproducibility is a central hallmark of scientific advances. I do not question any of the structures, but there simply has not been time for this critical test to be applied. The biggest question right now is to what extent the published structures are 'real'. For example, the recent structure of a K_V channel was achieved only by using a monoclonal antibody to force the protein into what the authors concede is an artificial conformation[4].

Francisco Bezanilla. The crystal structure of K_VAP, which has recently been published[4,40], does not represent the structure of the channel in the native membrane. This is because, although in the crystal the pore seems to be in the open conformation, the voltage sensor is in the internal face of the bilayer, a position that is consistent with the closed conformation. In addition, the authors found that the antigen-binding fragments (Fabs) that were attached to the voltage sensor and used to crystallize the channel do not interact from the inside with the functional channel reconstituted in bilayers, even though in the crystal the sensor is in the inside. This was recognized by the authors, who modified the structure by docking to the pore region another crystal structure they had solved of the first four transmembrane segments to propose a new model of channel activation. However, with the new model, it is very difficult to account for more than 20 experimental observations previously made in eukaryotic voltage-dependent channels (see, for example, REFS 41,42,117). Among these is the observation that the linker between segments one and two is extracellular, whereas in the model it is buried in the bilayer. In addition, there is a large discrepancy in the distances measured using tethered blockers or resonance energy transfer (FRET and lanthanide-based resonance energy transfer (LRET)) between two equivalent residues in segment four of two subunits, which are about half the value of the distances proposed in the new model.

The outcome of this is that several investigators are performing a series of new biophysical experiments to test the old or new models, and others are constructing new models by modifying the crystal structure in a different manner than done by the authors of the K_VAP papers[42]. It is hoped that new crystal structures will be obtained in the near future by using a different set of Fab fragments or a different technique to immobilize the obviously floppy structure of the voltage sensor. In addition, there are other bacterial voltage-dependent channels, such as NaChBac (a Na^+ channel from *Bacillus halodurans*), the structures of which may be solved in the near future. In other words, the big questions of the structure/function operation of the voltage sensor are still as open as they were before, except that now we have rough structural guidelines provided by the crystal structures.

Michael Sanguinetti. X-ray crystallography will be extremely useful in telling us about the static structures of channels captured in specific states, but may not be as successful in defining the nature of intermediate states or how channels gate in response to changes in voltage or binding of ligands. The crystal structures of several ion channels that were recently published by the MacKinnon laboratory were a 'giant leap for mankind' in the eyes of ion channel biologists[2–4]. The insights into the inner workings of, first, the selectivity filter and, second, the voltage sensor, were not predictable on the basis of existing biophysical data. KcsA (the K^+ channel from *Streptomyces lividans*) provided an unprecedented picture of the closed state[2], and MthK (a K^+ channel from *Methanobacterium thermoautotrophicum*) provided a view of the open state of K_V channels[3]. Most recently, the structure of K_VAP astonished us with how the voltage sensor is likely to move in response to changes in membrane voltage[4]. Nonetheless, the structures leave a most perplexing problem unanswered: how is voltage-sensor movement coupled to activation (opening) of the channel?

Dennis Dougherty. It remains to be determined the extent to which static images of bacterial analogues of mammalian channels will lead naturally to insights relevant to the drug discovery process. The challenge now is to develop chemical-scale methods that evaluate the structures and tell us to what extent they truly reveal the nature of mammalian channels. Along with the unnatural amino-acid methodology mentioned above (see question 1), other established tools include the introduction of chemical probes by cysteine modification, FRET and EPR studies (for example, Eduardo Perozo's work[43]).

In addition, the structures are spawning a huge effort in computer simulation of channels — building homology models of the mammalian channels based on the bacterial structures. However, modelling without precise experimental testing of the models is a sterile exercise. Only a close interaction of theory and experiment can hope to produce information of relevance to the drug discovery process. So, the challenge for structure/function studies is to produce high-precision insights that stringently test the predictions of structural models. It will always be essential to understand function, not just structure, and so there is a strong future for structure/function studies.

Trevor Smart. There are many unanswered questions. The lack of knowledge regarding the three-dimensional structures of the vast majority of channels means we cannot precisely locate ligand-binding sites, nor completely, or even initially, understand how different domains of an ion channel interact when the channel is activated. We need to be able to translate the linear amino-acid sequence of a channel into a dynamic, three-dimensional functioning protein. To date, we are closest to achieving this for the muscle nACh receptor and some bacterial K^+ channels[2,3,7,10,44–46]. This has been possible because the nACh receptor can be found in very high density in the electric organs of fish such as the *Torpedo* electric ray, making these organs an ideal source of material for studying the structure of this channel with electron microscopy. Similarly, the ability to obtain large quantities of purified bacterial K^+ channels has facilitated crystallization of channel proteins. With this approach in particular we need to be able to discern how signals are transduced through the channel proteins, thereby aiding our interpretation of channel mutations that can either affect ligand binding directly, or affect the response to a ligand via allosteric effects. At present, being able to determine a binding site from mutation analysis alone is extremely difficult and fraught with problems.

Richard Lewis. Although ion channel crystal structures have been produced, only AChBP gives a useful view of a part of an ion channel with relevance to a drug target (the nACh receptor)[9]. High-resolution crystal structures of ion channels involved in disease states, or closely homologous to those involved in disease states, are required before significant structure–activity relationships (SARs) can be established for therapeutically relevant classes of molecules. Of particular importance will be crystal structures of mammalian voltage-sensitive Na^+ and Ca^{2+} channels, hopefully obtained with and without bound ligands. These structures will allow the rationalization of existing extensive SARs for many important drug classes, including local anaesthetics, antiepileptics, antiarrhythmics and antihypertensives. There is the very real potential that such insights will allow the development of new molecules with improved selectivity. Developing an understanding of the structural basis for use-dependent activity at key ion channel targets, most likely from a combination of crystal structures and computational approaches, will allow further advances in ion channel therapeutics.

Walter Stühmer. As has occurred in other fields, the clinical aspects are likely to increasingly take centre stage, and structure/function relationship issues will probably recede into the background, becoming interesting in a more academic sense. However, extensive and detailed knowledge gained from biophysical and physiological studies at the single-molecule level will be helpful in identifying second-messenger pathways that are involved in channel modulation and signal transduction. These secondary pathways could become interesting targets in channelopathies.

Kenneth Chien. From a clinical standpoint, drugs targeted directly against channels themselves have been disappointing, and we now know that many of these drugs can have serious side effects. It would be helpful to find a way to modulate channel function without directly interacting with the channel itself, perhaps by affecting an intersecting signalling pathway or by affecting the channel co-regulator. A good example of the former is provided by TRP channels, which are activated

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Michel Lazdunski did his undergraduate degree in chemical engineering, and then completed a Ph.D. in Physical Chemistry and a Doctorat ès Sciences in Biochemistry. His first research activities in biological sciences were in enzymology. He then gradually started specializing in ion transport systems, and most specifically the molecular analysis of ion channels, at a time when it was not even known that these channels were proteins. He first developed tools (toxins in particular) to analyse the properties of voltage-gated Na^+ (Na_v) channels, then worked with Ca_v channels, a number of of K^+ channels, the cystic fibrosis transmembrane conductance regulator (CFTR) protein, epithelial Na^+ channels, acid-sensitive ion channels and peptide-gated ion channels, always linking molecular studies with developmental biology, pharmacology, physiology and pathophysiology. At present, he has a more specific interest in the implications of ionic channels in epilepsy, ischaemia, pain and behavioural disorders. He was previously Director of the Centre de Biochimie at the Centre Nationale de la Recherche Scientifique (CNRS) in Nice, France, and is now Professor at the Institut Universitaire de France and Director of the Institut de Pharmacologie Moleculaire et Cellulaire, CNRS, Sophia Antipolis, France. He has received several national and international awards, including the Gold Medal of the CNRS. He is a member of several Academies, including the French Academy of Sciences and the Academia Europea.

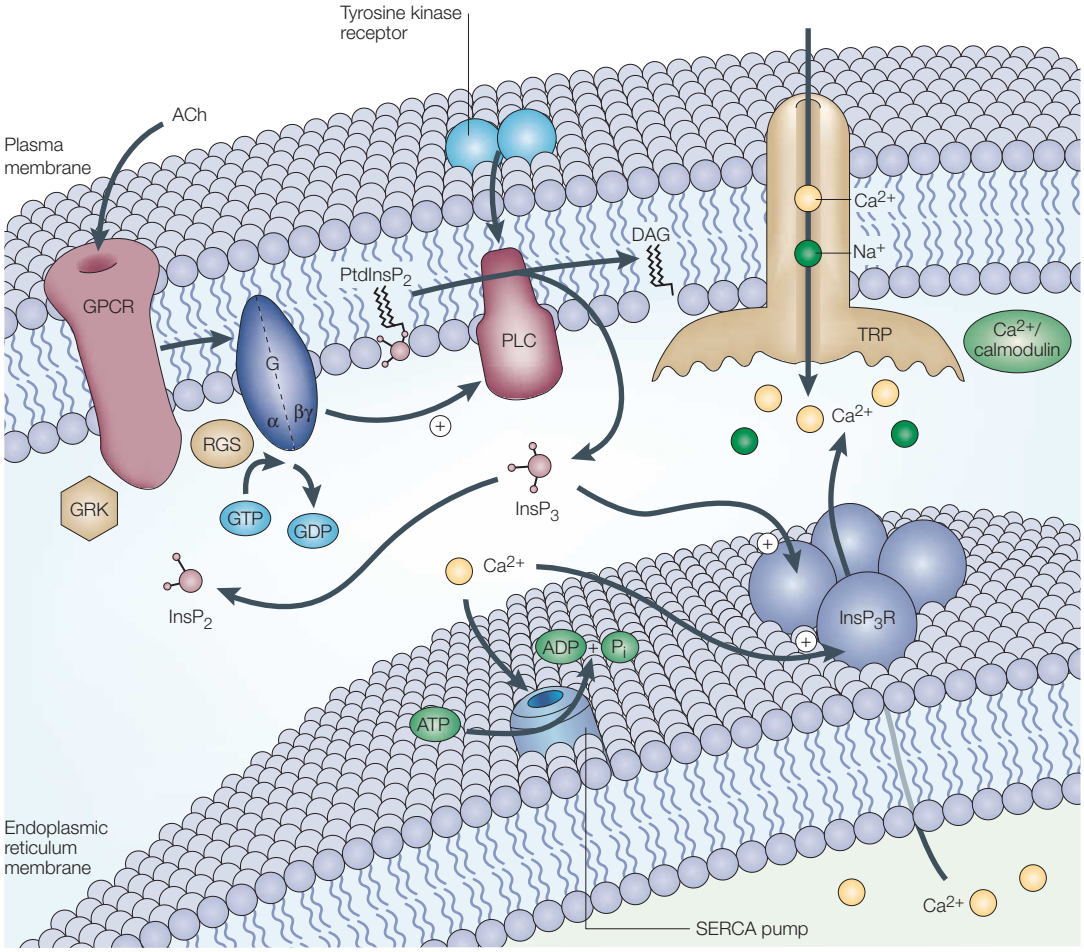

Figure 4 | **Ion channels present a multitude of opportunities for points of intervention by pharmaceutical agents.** As an example, transient receptor potential (TRP) channels are activated primarily by signal transduction pathways. A G-protein-coupled receptor (GPCR; for example, the muscarinic M1 acetylcholine receptor) catalyses G-protein nucleotide exchange to form active Gα and Gβγ subunits, in turn activating phospholipase C (PLC-β). Alternatively, tyrosine kinase (TK) receptors activate PLC-γ. PLC hydrolyses an abundant membrane component, phosphatidylinositol-4,5-bisphosphate ($PtdInsP_2$), into soluble and lipophilic messengers. Diacylglycerol (DAG), one product of $PtdInsP_2$ hydrolysis, remains in the membrane. These elements seem to be common to a number of TRP channel activation pathways. Soluble inositol-1,4,5-trisphosphate ($InsP_3$) activates the $InsP_3$ receptor ($InsP_3R$) on the endoplasmic reticulum to release intracellular Ca^{2+}. Ca^{2+} modulates many TRP channels, but $InsP_3$ itself does not. Interest in these channels is relatively new, and therefore effective small-molecule inhibitors or toxins are not generally available. The signal transduction pathway components are common targets of pharmaceutical agents, but are thus more pleiotropic. As for other ion channels, it is likely that small molecules can be found to inhibit gating via interaction with the pore, or with components of the protein that regulate pore access from the intracellular surface. Modified, with permission, from *Nature Reviews Neuroscience* (REF. 109) © (2003) Macmillan Magazines Ltd. Figure prepared by David Clapham. ACh, acetylcholine; GRK, GPCR kinase; RGS, regulator of G-protein signalling; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase.

primarily by signal transduction pathways[47] (FIG. 4), and an example of the latter is provided by KChIP2 regulation of the $K_V4.2/4.3$-channel proteins[12].

Michel Lazdunski. I am not particularly impressed by the elucidation of the three-dimensional structures of some channels coming more than 35 years after the first X-ray structure of an enzyme in complex with its substrates or inhibitors. I do believe that these X-ray structures will be very useful for further understanding channel biophysics at the atomic level. However, I do not believe that they will be very useful for the discovery of new drugs. Even if we had the X-ray structure of the Na_V channel in hand, we would probably not have discovered the antiepileptic or type I antiarrhythmic drugs that we now have, and we would certainly not have discovered the pyrethroids, which are such potent insecticides. I do not believe that the X-ray structure of L-type Ca^{2+} channels would have led us to discover dihydropyridines, diltiazem (Cardizem) or verapamil (Calan, Isoptin), which are so useful in the treatment of cardiovascular diseases. Important efforts have to be made in devising a pharmacology directed towards the interacting surfaces of ion channels and their partner proteins (which can drastically modify their activity), or towards the interacting proteins themselves.

5 How useful will molecules derived from natural sources prove to be in probing ion channel structure/function?

Alan Verkman. Probably very useful, as nature has evolved high-affinity toxins and other modulators of ion channels.

Francisco Bezanilla. They have been extremely useful in probing the structure of the channel and it is expected that many more will become available.

Alan North. Known molecules from natural sources will continue to be useful because of their high affinity, high selectivity, their amenability to labelling and so on. Furthermore, new molecules will be discovered as new areas of the earth's biodiversity are opened up (for example, China).

Walter Stühmer. Molecules derived from natural sources are likely to maintain their importance given the large reservoir available, particularly from marine and tropical organisms. These compounds will also be interesting as lead compounds. However, as drugs, their lower selectivity but better accessibility has to be balanced against monoclonal antibodies.

Kenneth Chien. These will be useful if they are specific, selective and, of course, have few side effects *in vivo*. They could be useful in providing chemical leads to then perform more rational drug design (FIG. 5).

Dale Benos. I think natural molecules have been, and will continue to be, extremely useful. Most natural compounds, be they inhibitors or activators of ion channels, are highly specific and act with high affinity. There are already several wonderful examples of venom components being successfully used to explore the intricacies of ion channel structure; for instance, the large family of neurotoxin peptides (the α-K^+-toxins) found in scorpion venom, typified by charybdotoxin.

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Irwin Levitan received his B.Sc., M.Sc. and Ph.D. degrees in Biochemistry from McGill University in Montreal, Canada. After undertaking postdoctoral research at the University of Gothenburg in Sweden and the University of California, San Diego, California, USA, he was a Group Leader at the Friedrich Miescher Institute in Basel, Switzerland, where he began investigations of the regulation of neuronal excitability in the marine mollusc *Aplysia*. He continued these studies after moving to the Department of Biochemistry at Brandeis University, Waltham, Massachusetts, USA, where he was Professor of Biochemistry and founding Director of the Volen Center for Complex Systems. While at Brandeis, he began investigating the modulation of ion channel proteins, using a combination of molecular and biophysical approaches. More recently, he has been pursuing the concept that ion channels do not function on their own in the plasma membrane, but rather exist as part of a multi-protein regulatory complex that includes not only the ion channel protein itself, but also one or more signalling proteins that participate in the modulation of channel properties. In 2000, Levitan moved to the University of Pennsylvania, Philadelphia, Pennsylvania, where he is the David J. Mahoney Professor and Chair of the Department of Neuroscience, and Director of the campus-wide Mahoney Institute of Neurological Sciences.

Jamie Vandenberg. Natural compounds have been, and will continue to be, very useful. Scorpion toxins, for example, have a much higher specificity for individual ion channels compared with most pharmaceutical compounds, because they bind to subtype-specific epitopes (in this respect they are analogous to antibodies). They are therefore very useful tools for probing the structural basis of the differences between homologous ion channels.

John Peters. Historical precedent, provided by examples such as tetrodotoxin, α-bungarotoxin, (+)-tubocurarine and many others, suggests that they will continue to be of considerable value as selective ligands that help to discriminate between ion channel subtypes with differing subunit compositions. The α-conotoxins from marine snails of the *Conus* genus provide an excellent example of venoms that distinguish between nACh-receptor subtypes[48].

Frances Ashcroft. It seems unlikely that we have exhausted the range of toxins present in the natural world that target ion channels, and new ones are likely to be as valuable for studying ion channels as those we have at present. Many bind with high affinity and specificity, and can be used to biochemically purify channel proteins, map the structure of the binding site in mutant cycle analysis and so on. Novel agents that discriminate between different K^+-channel subtypes, or that interact with β-subunits, would be especially helpful.

Sarah Lummis. Toxins from natural organisms have proved to be extremely important in our understanding of many ion channels (for example, α-bungarotoxin in allowing the purification and, ultimately, the cloning of nACh-receptor subunits[49]), although they have been less popular in recent years, perhaps because of the perception that molecular biological approaches are more at the cutting edge of science. However, given the very high specificity of many such compounds, and the present need to probe the structure of ion channels in increasingly fine detail, I believe naturally occurring compounds will experience a revival.

Richard Lewis. Natural products that target ion channels will continue to be pivotal in understanding their structure/function relationships and physiological roles. For example, peptide toxins are often highly selective for a specific ion channel or class of ion channels, allowing their role to be cleanly dissected experimentally in *in vitro* and *in vivo* models of normal and disease states. It is often through the use of natural products that the roles of specific ion channels are first appreciated. Natural products continue to be a key source of novel chemistry and structural templates from which new therapeutics can be designed and developed. For example, Elan Pharmaceuticals expects to file a New Drug Application (NDA) for ziconotide (Prialt; a first-in-class N-type Ca^{2+}-channel inhibitor, ω-MVIIA, for pain) around the second quarter of 2004.

Michel Lazdunski. Toxins have been invaluable for understanding ion channels at a molecular level. They have been essential for identifying ion channels at a time when we did not even know whether channels were proteins; purifying ion channels (for example, tetrodotoxin, saxitoxin, scorpion toxins, sea anemone toxins, veratridine, batrachotoxin, pyrethroids, grayanotoxins, brevetoxins and ciguatoxin for voltage-sensitive

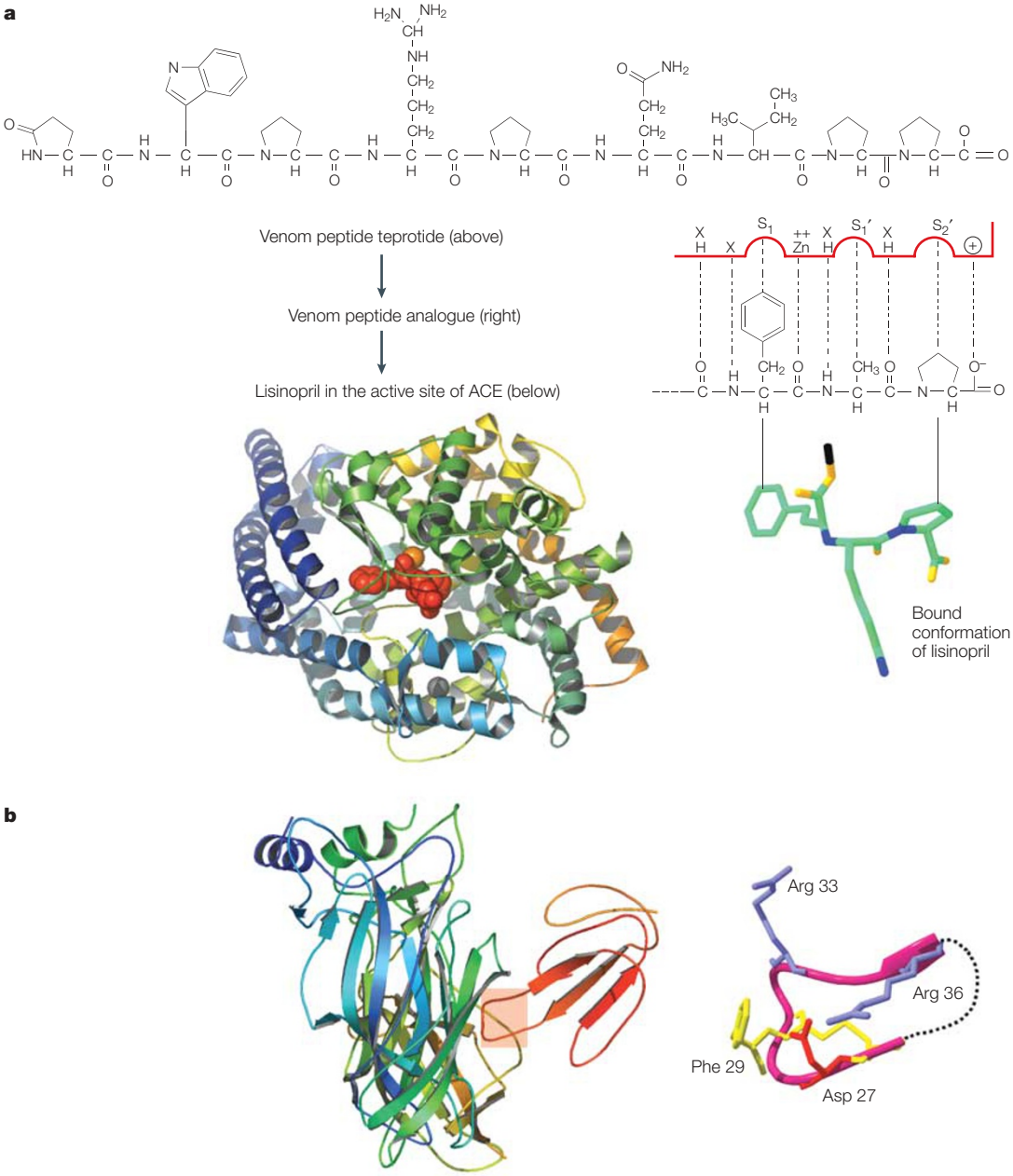

Figure 5 | **Natural products, including venom peptides[110], can be sources of specific chemical modulators. a** | The venom peptide teprotide, a long-acting angiotensin-converting enzyme (ACE) inhibitor, initiated research that led to the development of captopril and subsequently lisinopril. These drugs were designed to bind to a 'classical model' of the ACE active site (underlined in red) in the same manner as predicted for the venom peptide analogue, and to be orally active. The recently obtained crystal structure of the ACE–lisinopril complex provides the first high-resolution template that could allow the design of improved ACE inhibitors for the treatment of cardiovascular diseases[111,112]. A space-filling model of lisinopril (red) co-crystallized in the active site of ACE is shown on the left, with its bound conformation highlighted in expanded view on the right, confirming the Phe/S_1 and Pro/S_2' interactions originally predicted by the classical model. **b** | An experimentally derived model of a snake toxin (α-cobratoxin; shown in red) docked onto the α7 nicotinic acetylcholine receptor[113]. Interestingly, the key binding residues (shown in expanded view on the right) are clustered in loop 2 of the snake toxin (shaded portion on the left). By engineering a structural clip (dashed line) that works in synergy with the crucial disulphide bond (light yellow) at the tip of loop 2, it might be possible to achieve this conformation in a smaller peptidomimetic. However, agonists rather than antagonists are typically needed for the treatment of a diverse range of neurological diseases. Whether this approach can be extended to the development of subtype-specific, orally active agonists remains to be seen. Figure prepared by Sebastien Dutertre (Institute for Molecular Bioscience, Queensland) and Richard Lewis.

Na^+ channels; and mast-cell degranulating (MCD) peptide, dendrotoxins and apamin for K^+ channels); localizing channels at a time when it was not possible to use immunological techniques or *in situ* hybridization; establishing the diversity of channels within a family (that is, distinguishing the different types of Na_V channel (tetrodotoxin-sensitive and tetrodotoxin-resistant) and K^+ channel (Ca^{2+}-activated K^+ channels sensitive to apamin or to charybdotoxin)); and understanding the physiological role of channels. A very specific toxin is at least as good as a classical knockout, because there is no compensation due to the expression of other genes.

In addition, the physiological and pharmacological analysis of toxin effects provided essential information on channelopathies well before human ion channel mutations had been identified and well before the word 'channelopathies' had been invented. In my opinion, toxins will continue to be essential tools, if not *the* essential tools, in ion channel studies. Many venom peptides will turn out to be used as drugs, and they will certainly be used as 'leads' for future drug discovery.

Trevor Smart. Natural products will continue to be valuable in probing channel function. They often have high selectivity and potency, derived in part through being honed over long periods of evolutionary time by selective pressures. They have often been important for aiding the purification of receptor/channel proteins and have provided templates for the design of synthetic ligands. The fields of K^+ and Ca^{2+} channels have benefited considerably from the use of a battery of snake and spider toxins, some of which have influenced channel classification before cloning. They have also been extremely useful in native cells, in combination with electrophysiology, for deducing which types of channel might be present and their likely function. The latter is a very incisive method and is currently the only method for determining the functional consequences of channel activation.

There are now several centres devoted to producing receptor-specific and subtype-selective ligands based on rational synthetic chemistry. These approaches have been markedly successful in elucidating the roles of receptors and channels, as evidenced by their use, for example, in studies of metabotropic glutamate receptors and $GABA_B$ receptors.

David Clapham. The toxins from poisonous animals have been very useful in understanding structure/function: antibodies that block function are lacking; other products, such as potential therapeutics from plants, are poorly studied. By far the most amenable pharmaceutical agents for production, delivery and absorption are small molecules.

Natural products are often difficult to isolate and are frequently impossible to synthesize from scratch. In addition, as they usually evolved as agents in biological warfare to fend off predators or decrease competition for space, food or reproduction, they are frequently toxic. Finally, in order to survive, humans have developed very efficient immune or other protective strategies to combat such agents. Nature has not put a great deal of effort into developing molecules that cure diseases.

Nonetheless, there are several examples of useful plant molecules that became important early therapeutics, such as quinine (cinchona bark) for malaria, atropine (belladonna or deadly nightshade plant) for the control of secretions and increasing heart rate, and digitalis (foxglove plant) for congestive heart failure. There are surely more of these substances of which we are not aware or have not sufficiently exploited.

Michael Sanguinetti. Toxins isolated from venomous animals have always played an important role in probing gross aspects of channel structure and in helping to understand channel gating. However, I do not think natural toxins will be any more important than synthetic drugs. The potential structural diversity in synthetic compounds exceeds that of natural compounds. In addition, the search for novel natural compounds is slow, unorganized and relatively random. By contrast, combinatorial chemistry methods provide the opportunity to systematically provide biologists with an almost endless array of compounds, including peptides.

Chris Miller. Natural molecules will become less important in ion channel research, I think, than previously, as the bar has been raised recently. 'Structure/function studies' in the ion channel field no longer means, 'we study function and wish we had a structure'. Natural channel-altering toxins from the venoms of snakes, spiders, scorpions, jellyfish and dinoflagellates were used in the past as 'probes' of channel structures in the physical vicinity of the binding site, or of the electrostatic environment there. It required a lot of work, and some luck too, to obtain quite a low-resolution picture of a very localized place on the channel. But now, with

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Richard Lewis gained a B.Sc. degree in Chemistry and Zoology before completing a Ph.D. on the isolation and pharmacology of ciguatoxins at The University of Queensland, Brisbane, Australia. He joined the Department of Primary Industries just north of Brisbane in 1986 to continue research into the origins, chemistry, pharmacology and detection of ciguatoxins, a family of polyether toxins that accumulate in fish and act on sodium channels to cause ciguatera. In 1994, he returned to The University of Queensland to initiate research into the discovery and characterization of venom peptides from Australian cone snails. During 1994–2000, he led a team that discovered ω-conotoxin CVID (AM336, which is being developed by Amrad Corporation Ltd) and χ-conotoxin MrIA (Xen2174, which is being developed by Xenome Ltd). In 2000, Richard was appointed Associate Professor at the Institute for Molecular Bioscience at The University of Queensland, and Director of Pharmacology at Xenome Ltd, of which he is a founding scientist. His current research interests focus on the molecular pharmacology of conopeptides acting at voltage-sensitive sodium, calcium and potassium channels, acetylcholine- and *N*-methyl-D-aspartate (NMDA)-gated channels, the noradrenaline transporter and the α_1-adrenoceptor.

the advent of direct structure determination, we can see (still with a lot of work and a dose of luck) a global picture at higher resolution.

Dennis Dougherty. Molecules derived from natural sources will become less and less useful. My feeling is that natural products, most notably various toxins, were extremely valuable tools for isolating channels and for initial studies. But, in these days of molecular biology, heterologous expression and crystallography, that role is diminished. There are ongoing efforts to characterize toxin–channel interactions, but personally I feel that the drug discovery effort would be better served by studying actual drug–receptor interactions.

6

Biophysical and structural methods are the very opposite of high throughput. How can they be adapted to aid discovery of new lead compounds?

Kenneth Chien. New technology for patch-clamp arrays now exists, and I think these will be the prototype of the future.

Richard Lewis. High-throughput patch clamping can gain biophysical data, and crystallography continues to become more automated. However, structures of much of the 'low-hanging fruit' in the area of membrane proteins will likely be completed in the next five years.

Michael Sanguinetti. High-throughput patch-clamp instruments are about to be made commercially available and should revolutionize the process of drug discovery (Axon Instruments, Molecular Devices, Nanion Technologies). These instruments should be able to accommodate 396-well format plates and be fully automated.

Alan North. There are many potentially useful tools in development for high-throughput, cell-based ion channel screening. For example, Axon Instruments, Molecular Devices and Sophion Bioscience are all developing such tools.

Francisco Bezanilla. This will change with the aid of more sophisticated optical techniques, such as genetically encoded, cell-type-directed fast optical probes, to follow the membrane potential of cell populations subjected to experimental manipulations, and the possibility of patch clamping arrays of cells using a multiple and automated patch-clamp system.

David Clapham. Various people are attempting to make structural methods 'high throughput'. But this is relative to the current case-by-case approach. If the key components of ion channels that control gating could be isolated in their native configuration, one could envision rapid testing of occupancy of the key binding sites by small molecules and peptides. This is probably not around the corner.

Trevor Smart. It might be worth segregating the aims of these methods into screening for broad drug selectivity on the one hand, and determining the mechanism of action of the drug and the precise location of binding sites on ion channels on the other. The former would lend itself to rapid screening in combination with drug/compound libraries, if automated methods for patch-clamp and intracellular electrophysiology continue to be realized. However, for details regarding the mechanisms of action and determination of binding sites at the structural level, it is unlikely that rapid screening methods will be suitable, as there are requirements for detailed, tailor-made experimental designs and data interpretation that are rarely in accord with the concepts of mass screening.

Dennis Dougherty. Advances in higher-throughput electrophysiology are very encouraging. They will inevitably be an important part of the drug discovery process. I have two tools in mind. One is the OpusXpress from Axon Instruments. This allows electrophysiological studies of ion channels expressed in *Xenopus laevis* oocytes in a more nearly automated way. We have been using one in my lab for about six months now, and it has led to an increase in throughput by roughly a factor of ten. This is still nowhere near that seen for soluble proteins, but it is an important advance. The *Xenopus* oocyte system is not ideal, but it can be quite valuable in certain situations.

The second tool, and the one that will ultimately have a greater impact, is higher-throughput patch clamping using planar patch technology (Molecular Devices/Essen Instruments, Axon Instruments). This will work on mammalian cells, which are often of greater relevance to the drug discovery process, and could be significantly higher throughput than the OpusXpress — still not 100,000 compounds per day, but a meaningful, functional screen with much greater speed. These instruments are just now becoming commercially available. I am certain that the pharmaceutical industry will embrace these devices, and that they will greatly facilitate the drug discovery process around ion channels and other CNS targets.

Although unlikely to become ultra-high throughput, when coupled with structural insights, the newer methods promise the evaluation of focused, well-designed libraries with adequate throughput. Current work in the pharmaceutical industry suggests that such focused libraries are preferable in the drug discovery process, and so the trends are converging.

Sarah Lummis. There are increasing indications of attempts to increase throughput in both biophysics and structural biology. In biophysics, semi or fully automatic electrophysiological systems are increasingly being developed, such as the OpusXpress (Axon Instruments), which can independently impale and record from many oocytes simultaneously. In terms of structural methods, researchers in some crystallographic facilities are being encouraged to allow their crystals to be analysed by researchers based at SYNCHROTRONS, thus saving time and energy for those that produce protein crystals to

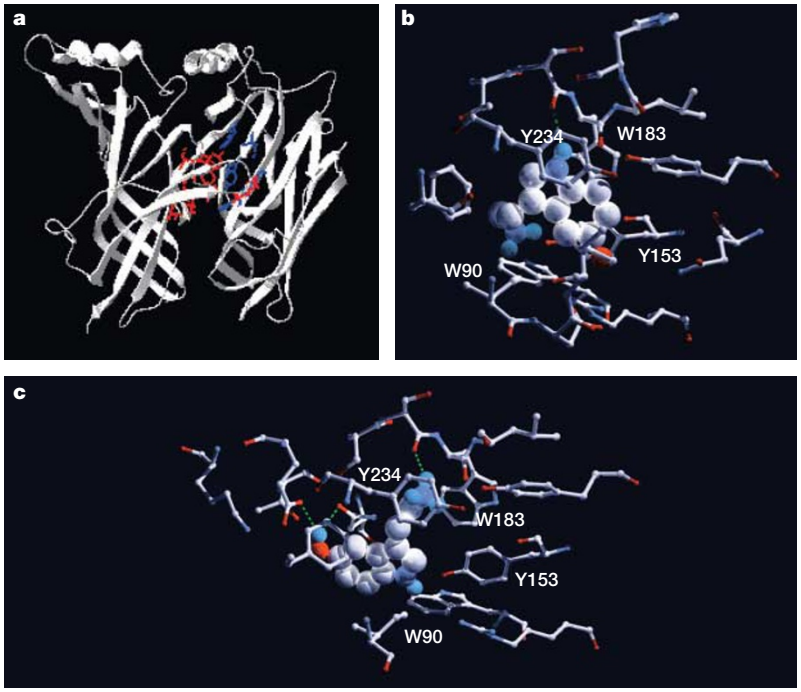

Figure 6 | **Accurate knowledge of the structure of a ligand-binding protein can yield structural information on the ligand-binding domain of related ion channel proteins.** The extracellular domain of the 5-hydroxytryptamine (5-HT_3) receptor shows significant structural and functional homology to other members of the Cys-loop ligand-gated ion channel superfamily, and to the acetylcholine-binding protein (AChBP) found in the snail *Limnaea stagnalis*, for which an X-ray crystal structure is available[9]. **a** | The putative ligand-binding site of the 5-HT_3 receptor was created by homology modelling using the known structure of AChBP. Two of the five subunits are shown here, with the binding site located at the interface. Amino-acid residues located less than 5 Å from 5-HT in one subunit are shown in red, and those in the adjacent subunit are shown in blue. **b,c** | Two possible orientations of 5-HT docked into the binding site of the 5-HT_3 receptor. 5-HT was docked into the binding site using AUTODOCK software[53]. The software found both orientations possible, but only the orientation shown in **c** is supported by experimental evidence. 5-HT is shown in a space-filling view, and the 5-HT_3-receptor binding pocket is shown in the same three-dimensional orientation in both panels. Potential hydrogen bonds are shown as dotted green lines. All other atoms are coloured according to the Corey–Pauling–Koltun (CPK) system (blue, nitrogen; red, oxygen; white, carbon). Figure prepared by Sarah Lummis.

concentrate on producing more of them. Multi-recording devices such as those described above can undoubtedly assist in drug design, but I believe it is less critical for structural methods, as accurate knowledge of a single ion channel structure can yield much information on many potentially active compounds. The structure of AChBP, for example, has now yielded model structures of the binding sites of nACh[50,51], $GABA_A$[52] and 5-HT_3 receptors[53] (FIG. 6). These have defined the locations of critical residues, and experiments are now beginning to show how different ligands associate with distinct residues.

Senyon Choe. I do not quite agree that biophysical and structural methods are the opposite of high-throughput methods. Although the determination of crystal structures might be a seemingly endless process, I believe the field has rapidly progressed thanks to the advancement of high-throughput methods. Screening a large number of related gene fragments from related organisms would be of benefit to the critical step of the structural analysis: protein production and preparation for biophysical studies. To me, the idea of scanning through a variety of genomic databases is by itself a high-throughput strategy employed for biophysical and structural studies. Furthermore, high-throughput drug discovery will take advantage of knowledge obtained from analyses of protein–protein interfaces and drug–protein complexes on the one hand, and from the genomic knowledge base on the other.

7 What, if any, will be the importance of ion channels for bionano-technology?

Sarah Lummis. The development of the patch-clamp technique has allowed us to examine the characteristics of a single protein molecule *in vivo*, which was remarkable when it was developed and still is today. Ion channels could therefore play a big role in bionanotechnology; for example, as sensors.

Senyon Choe. Developing miniaturized devices for detecting molecular identity combined with electrical or optical signals seems to be a reasonable approach towards nanotechnology. I cannot elaborate on how new technology will emerge, but a better understanding of the physical principles of ion channel conduction and regulation could probably provide a solid foundation from which to generate novel ways of engineering protein-based biotechnology products, or even artificial circuitry using ion-selective functionality.

Francisco Bezanilla. The combination of nanotechnology and channel function will be the future in understanding integrated cell physiology. It will also be important in diagnosis; for example, this could be achieved by using automated patch clamping combined with microfluidics and nanodetection devices, which can be manufactured to act as sensors for specific molecules. Such systems could be used in diagnosis by using live cells from the suspect tissue.

Alan North. Channels have the potential to revolutionize bionanotechnology. Membrane channels are a potential sequencing technology; for example, α-haemolysin channels can detect the permeation of individual bases of nucleic acids[54], and channels on chips are a way of developing the biological–silicon interface for *in vivo* multi-electrode recording.

Dale Benos. If regions of ion channels that act as mechanical or chemical 'sensors' can be identified and functionally isolated, these could theoretically be coupled to microcantilevers and/or piezoelectric devices to sense local concentrations of odorants, contaminants or changes in pressure in very restricted locations. For example, using microelectromechanical systems into which specific ion channel receptor domains have been incorporated, the binding or adsorption of a given molecule could theoretically be detected in small aqueous or gaseous compartments, such as blood vessels or other body cavities. Moreover, appropriately constructed devices could be used to detect levels of specific molecules or pressure changes in closed environmental compartments, such as those found in a space capsule.

David Clapham. Laboratories have been using ion channels in attempts at high-throughput DNA sequencing — individual nucleotides can be deduced from changes in electrical resistance as they snake through the pores of some ion channels.

Many researchers are also interested in using ion channels as biosensors for the detection of toxins (for example, neurotoxins) — ion channels are the frequent targets of lethal agents, so can be placed on artificial platforms as direct electronic detectors of toxin binding. The key problem is making a sturdy scaffold for a protein that was designed by nature to span a fragile, 30-Å-wide lipid bilayer. These problems might be solved by adapting toxin-binding sites to more conventional semiconductor elements, or by detecting binding of toxins to protein substrates by alteration of light scattering.

Ion channels might also be used for the generation of batteries, as in cells, or for biocompatible switches.

8

Is there a bifurcation taking place in ion channel research between physiology/pharmacology and structural biology/molecular biophysics? And if so, how does one re-integrate ion channel research?

David Clapham. Not by those who read the literature. As in all areas, the problem is that the literature is vast.

Alan North. No, I do not think that there is such a bifurcation. The leading figures in structural work (Rod MacKinnon, Thomas Jentsch, Eric Gouaux and Mark Mayer) all have a solid grounding in functional principles. If re-integration is necessary, it is educational. Students should keep in mind the function in cells and tissues while probing the molecular structure.

Jamie Vandenberg. Quite the opposite — I think the great strides being made in structural biology/molecular biophysics are informing a great deal of pharmacological research (for example, the studies from Mike Sanguinetti's group and others on the structural basis of drug-induced LQTS[36]). The key to further progress is for physiologists/pharmacologists to embrace the new structural methodologies and at least understand what they are telling us, even if we don't actively pursue such experiments.

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Birgit Liss gained First Class honours degrees (M.Sc. and Ph.D.) in Biochemistry/Molecular Biology at the University of Hamburg, Germany. In 1999, she moved to the University of Oxford, UK, to work in the Institute of Physiology with Professor Frances Ashcroft, and in the Medical Research Council's Anatomical Neurophysiology Unit with Professor Jochen Roeper, where she developed ways of combining electrophysiological and molecular biology techniques to analyse ion channels in single neurons. In 2001, she was awarded a Royal Society Research Fellowship at the University of Oxford to continue studying the role of differential ion channel expression in dopaminergic function and neurodegeneration. In 2003, Liss moved back to Germany to the Institute for Physiology at the Philipps University of Marburg. She currently holds one of the first tenure-track Professorships in Germany.

Dennis Dougherty. I see no such bifurcation; just the opposite. Arguably the most exciting aspect of modern ion channel research is the effort to reconcile the extensive literature and ongoing work in physiology/pharmacology with the new insights being provided by structural work. Question 4 not withstanding, the K_VAP structure highlights that this effort can be challenging[55]. Inevitably, the structural insights will reflect back on pharmacology as the first opportunities for structure-based drug design become feasible. These results, coupled with higher-throughput functional assays (see question 6), will revolutionize the drug discovery process for ion channels.

Trevor Smart. I am not aware of an intentional bifurcation any more than exists already. However, in some areas, it could be argued that there is a convergence of technology, as exemplified in the K^+-channel field, in which structural biology (X-ray crystallography) is being used to try to solve the gating properties of some of these ion channels. Fields will naturally converge when it is appropriate, but this can be expedited by explaining methods to broad audiences. This can be difficult at a time when many scientists have become more focused, with a heightened sense of 'tunnel vision' in their own techniques. Promotion of funding for closer links at the biological/physical science interface could help.

John Peters. Not necessarily. The recent elucidation of the structure of AChBP[9] provides a good example of a structural study that has been particularly revealing from a pharmacological perspective. The newly defined structure provides a framework for interpreting decades of previous studies using chemical modification and site-directed mutagenesis of amino-acid residues within the amino-terminal ligand-binding domain of the nACh receptor. The correspondence between structure and function is gratifyingly good. The structure has also spawned a series of modelling

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Sarah Lummis completed her B.Sc. (Hons) degree in Biochemistry at the University of Bath, Bath, UK. During her degree, she spent six months working on GABA (γ-amino-butyric acid) receptors at the University of California, Riverside, California, USA, which initiated her interest in the Cys-loop family of neurotransmitter receptors. Her subsequent M.A. and Ph.D. at the University of Cambridge, Cambridge, UK, and postdoctoral studies at the University of Sydney, New South Wales, Australia, were concerned with GABA and acetylcholine receptors in insects, and, during the latter, she was also part of a team at the Commonwealth Scientific and Industrial Research Organisation (CSIRO), developing novel insecticides. She returned to Cambridge University with a Junior Research Fellowship at King's College and a Royal Society University Research Fellowship, which enabled her to continue her work in neurotransmitter receptors in both insects and vertebrates. Sarah soon turned her attention to the 5-hydroxytryptamine $(5\text{-HT})_3$ receptor, which, as she demonstrated, is not only interesting in its own right, but is also an excellent model for understanding all Cys-loop receptors. She is now one of the most active researchers in this field, and currently holds a Wellcome Trust Senior Research Fellowship in Basic Biomedical Science to continue this work. Her group in Cambridge has clarified, among other things, many aspects of the relationship between structure and function in this protein, in addition to developing new tools and techniques to study these proteins. She is also a Fellow, Lecturer in Biochemistry and Director of Studies in Natural Sciences at King's College.

studies of related receptors of the Cys-loop family that will help direct future functional investigations and the generation of PHARMACOPHORES.

Senyon Choe. I am not sure if these two areas are bifurcating at the level of scientific questions, but I think it is certainly so with respect to experimental means and scientific terms to describe them, and the ways in which hypotheses are presented. Only a relatively small number of groups can readily move over the boundary as they wish. But the scientific questions are often shared; for example, what underlies the molecular basis for the assembly of channels, and how does this affect their biological functioning. So, I think the two fields are probably inseparable and that people in the two fields will continue to look in the same direction anyway. They will become co-dependent for their progress rather than being re-integrated. To me, this is a natural evolution as the field matures and sub-fields emerge that are more specialized.

Michael Sanguinetti. Because of the different educational and research backgrounds of scientists, it is inevitable that there will remain some gap between traditional physiological/pharmacological approaches to characterizing ion channel function and the more 'modern' approaches that rely on structural biology and biophysics (this is not a problem unique to ion channel research). Better integration of the disciplines could be achieved by halting the current trend to marginalize departments of physiology and pharmacology in medical schools and quelling the urge to populate these departments with molecular biologists at the expense of cellular, organ and whole-animal physiologists.

Sarah Lummis. In recent years, the physiological/pharmacological approach to ion channel research in my field has largely taken second place to structural biology and molecular biophysics. However, as both these approaches (although particularly the former) have yielded a number of potential models of ion channels, some of which are defined as being in the open or closed states, it will be essential that functional data are applied to understand fully the functional implications of the molecular details of these structures. I therefore foresee a revival of the more classical approaches to studying ion channels, such as examinations of the effects of a wide range of different ligands in whole-cell and organ experiments.

Michel Lazdunski. The most important part of ion channel research is now at the interface of physiology/pharmacology.

Francisco Bezanilla. Such a bifurcation has existed for a while, but I would suggest that the division is between biophysics/structural biology/physiology and pharmacology. However, there are contact points at which pharmacology is used to understand structure/function relationships. The more we understand structure/function relationships, the more we will see integration with pharmacology.

Irwin Levitan. I believe that this bifurcation actually took place some time ago (see question 3). In fact, ion channel physiology and pharmacology have been playing second fiddle, in terms of funding and publication in high-profile journals, to molecular biophysics and structure for more than a decade. Re-integration is difficult, because it is rare to have these different kinds of expertise in a single laboratory. The pharmaceutical industry might be the best place to achieve re-integration, because large companies can assemble interdisciplinary teams that span the entire range of ion channel research.

Birgit Liss. I agree that there is a bifurcation developing in ion channel research. This might be a natural development in the post-cloning era. We now know almost all the channel subunit genes. The next critical step is to understand, on the one hand, their distinct roles in determining/modulating physiological and pathological cell function, and, on the other hand, how exactly these proteins function on the atomic scale. These two areas of research require quite different techniques and expertise.

Kenneth Chien. I agree that there is such a bifurcation. Physician scientists will ultimately plug this gap by working with structural people like Rod Mackinnon, David Clapham and Lily Jan, and then doing research with groups such as ours, which are more integrative and disease orientated. We are starting to see people like this already apply to our programme; that is, M.D.–Ph.D.

combined degree candidates who did their work in labs such as these, and then complete their clinical training and perform their postdoctoral research in our institute (Institute of Molecular Medicine, University of California, San Diego, USA).

Chris Miller. That bifurcation was already rolling a decade ago, and now the split is quite complete.

Frances Ashcroft. This bifurcation is inevitable because few individuals have the capacity or desire to embrace all aspects of channel function, from atom to animal, in their research. However, I believe that re-integration is happening across a wide spectrum of ion channel studies, fuelled by interdisciplinary collaborations and integrative training programmes like OXION, recently established at Oxford University, UK[56].

9 How will the sequencing of the human (and other) genomes affect ion channel research?

Michel Lazdunski. The sequencing of the human (and other) genomes will affect ion channel research through the discovery of new channels, or of new channel forms.

Francisco Bezanilla. It has been useful for recognizing families of channels and understanding the role of conserved regions in the sequence that may have relevance to function.

Birgit Liss. The human and other genome sequencing projects provide us with a list of the possible players. We now need to find out which specific subsets of ion channels are functionally expressed in distinct cell types, and how they interact and complement each other to generate cell-specific physiological function.

David Clapham. It has had a tremendous impact on ion channel research by providing building blocks for understanding ion channel complexes and their regulation, and for identifying previously unrecognized ion channels. It also allows the integration of data from more readily achievable genetic experiments in *Drosophila melanogaster*, *Caenorhabditis elegans*, zebrafish and so on.

Irwin Levitan. Quite dramatically, and in several different ways. First, genome sequencing has made evident the existence of an enormous number of K^+ channels, in organisms ranging from worms to humans. This raises the fundamental cell physiological question of why so many channels that differ only subtly in their functional properties are required. Second, evolutionary conservation of specific sequences in ion channel proteins provides insight into crucial functional domains. Finally, the sequences can help to guide drug discovery by identifying regions specific to one or a few ion channels that might serve as drug targets.

Dennis Dougherty. The most significant impact has been the discovery of so many bacterial channels, which has opened the way to structural studies. Otherwise, the Human Genome Project might not be of very high impact — just knowing the sequence of a channel is not much help. These are dynamic molecules with multiple states, and it is essential to gain an understanding of the interconversions between these states — hence the need for functional assays (question 6). Furthermore, multiple subunit combinations, which can vary dynamically, in addition to extensive post-translational modifications, limit the usefulness of simple genomic information.

Sarah Lummis. The sequencing of the human (and other) genomes will (and already has in some cases) yield important information about novel proteins and subunits in addition to providing access to new regulatory DNA sequences. In my immediate field, for example, it has led to the discovery of three novel 5-HT_3-receptor subunits by similarity searches using the human sequence databases, and subsequent cloning using RAPID AMPLIFICATION OF cDNA ENDS (RACE)[57]. I believe this will ultimately have a tremendous effect on our understanding of ion channel composition and regulation.

Alan North. It already has. It has provided us with a complete vocabulary, and has been formative in setting new family relationships among channels. It has led to an explosion in the discovery of channelopathies, and the consequential new understanding of channel functions. It will continue to be influential in terms of discovering channel complexes and all the interacting proteins, and in particular for elucidating the functions of some channels that do not result directly from the 'hole-down-the-middle' mechanism (for example, kinase activity and cytoskeletal interactions).

Alan Verkman. Rapid genome screening for channels and channel analogues bypasses rate-limiting barriers in the gene-identification step, although functional

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Chris Miller graduated from Swarthmore College, Swarthmore, Pennsylvania, USA, with a B.A. in Physics, and after a stint as a high-school mathematics teacher, obtained a Ph.D. in Molecular Biology at the University of Pennsylvania, Philadelphia, Pennsylvania. After a postdoctoral research period with Efraim Racker at Cornell University, Ithaca, New York, he joined the Biochemistry faculty at Brandeis University, Waltham, Massachusetts, in 1976, where he is now a Howard Hughes Medical Institute Investigator. His research has focused on the basic mechanisms of operation of ion channel proteins, a field in which he began by working out methods for recording the activity of single ion channels in chemically defined reconstituted membranes. During the course of 25 years, his research has evolved along with the field's capacity to pose mechanistic questions at increasingly high resolution, so that he now finds himself engaging as a novice with problems in membrane protein crystallography. His main interest is currently in the area of chloride channel structure and function, and in the relationships between channel- versus transporter-mediated ion permeation.

characterization is still needed. From a practical perspective, it is seldom possible to select (without previous knowledge) the relevant gene that is responsible for a specific function. At this time, the database of functional genomics remains too incomplete in most cases to effectively exploit sequence information in target identification; however, databases of protein–protein interactions and protein function are rapidly expanding.

Trevor Smart. Sequencing of the genome will affect ion channel research by revealing novel receptor or channel subunits, and also relevant chaperone or interacting proteins. It will take some time to work through the system. It is also possible that such screens will reveal that we have discovered all of the subunits for a particular channel/receptor family, thus stopping any further wasted effort. The proteomics approach has value in rapidly establishing a whole network of proteins (and possibly identifying signalling pathways) that might interact with individual ion channel species (as evidenced by the proteomic screen of the *N*-methyl-D-aspartate (NMDA) receptor, for example[58]. To accomplish this work for the NMDA receptor using yeast two-hybrid methods would have taken years.

Kenneth Chien. The sequencing of the human (and other) genomes is helping to identify new classes of channel regulators and signalling complexes.

Michael Sanguinetti. The most important impact will be in the search for proteins that interact with ion channels, either as accessory subunits that affect channel gating or as scaffolds that link channels with other regulatory proteins, such as kinases.

Chris Miller. Perhaps it is better not to speculate. Many past public predictions of the outcomes of genome sequencing have failed to occur, such as an immediate leap to the understanding of disease states, and some really amazing current outcomes of genomics were not predicted, such as the existence in prokaryotic genomes of homologues of neurobiologically familiar ion channels.

10

How might microarrays and proteomics contribute to research on ion channel diseases and/or to basic research on channel function at the single-cell level?

Michel Lazdunski. They will certainly be an essential part of the toolbox used in ion channel research; they will become routine techniques.

Sarah Lummis. Microarrays and proteomics are already contributing to research on ion channel diseases, and it is easy to see them being extended to the single-cell level, at which the presence of a particular gene or gene product could have significant implications.

Francisco Bezanilla. Proteomics and microarrays will play a major role in understanding the role of ion channels in cell homeostasis. There are already numerous examples of how the population of ion channels changes in health and disease, depending on many factors, such as age, sex or pregnancy.

Dennis Dougherty. Information on the spatial and temporal distribution of specific ion channels and specific subunit types would be invaluable in developing therapeutic approaches. The key to drug discovery in ion channels is subtype specificity, and proteomic strategies promise to reveal which subtypes are the correct targets.

Alan North. Channels are expressed at different levels during the life history of a cell and during disease. They have different functional roles depending on cell cycle, cell connections and cell development. Microarrays can be used to probe this. In addition, channels are critically affected by interactors, and proteomics can deliver here.

Kenneth Chien. State-dependent changes in channel function — that is, covalent modification or regulation by dynamic signalling complexes — will, I believe, be key in understanding diseases of excitability in the future. The field will move beyond cataloguing mutations in channel genes themselves, towards a more integrated view into the mainstream of cell signalling, particularly as channels relate to acquired forms of disease, such as heart failure and sudden death from lethal arrhythmias, atrial fibrillation and so on (see question 1 and REF. 12).

David Clapham. Microarrays and proteomic approaches will be more useful as errors and noise are reduced in these systems. They currently provide correlations that give ideas about regulatory pathways that affect ion channels and modulators of ion channel transcription, translation and targeting.

Richard Lewis. Diseases such as pain are complex, involving the up- and downregulation of a wide range of membrane proteins, including ion channels[59,60]. This up- and downregulation varies between the different pain states modelled *in vivo*, adding further layers of complexity to our understanding of pain. Microarrays and proteomics are able to address this complexity in a direct way, though they can present more questions than answers. Access to biopsies (which is problematic for non-cancer nerve biopsies) to look at target regulation in human diseases using gene arrays may assist in the validation of therapeutic targets before commencing clinical trials for agents with new pharmacologies.

Birgit Liss. Gene-expression profiling has particular strength in combination with the patch-clamp technique, as the combined approach allows the functional differences of individual cells to be correlated and compared with their specific ion channel gene-expression profiles. A prerequisite for this microarray approach is the unbiased amplification of single-cell mRNA or cDNA to generate enough material for array hybridization (see question 2). Using laser-microdissection techniques, single cells from

fixed (post-mortem) material are accessible, but without the information on cellular function/excitability.

Trevor Smart. The use of microarrays and proteomics will undoubtedly help our understanding of ion channels by providing further evidence of how these receptors and channels exist in cell membranes, which are unlikely to be isolated entities floating free in the membrane. One of the advances being made is in understanding how channels are tethered at precise locations (for example, synapses) in the membrane and how they physically/functionally link to signalling pathways. This can be achieved by the dynamic binding of rafts of different proteins to the intracellular surface of the channels, such as protein kinases that phosphorylate the proteins. It seems increasingly likely that channels are dynamically anchored to a protein framework inside cells that provides support and targeting, and is capable of modulating function. As such, this could represent a whole series of new therapeutic targets (the targeted delivery of drugs to the inside of cells, however, is quite another matter!).

The question arises as to what these targets are likely to be. At present, an incomplete understanding of the protein scaffolds and their roles hinders precise identification; however, it is unlikely that new drugs will target molecules that have widespread roles in many signalling pathways, such as 'generic protein-kinase blockers', for example. A greater degree of selectivity would be achieved by identifying where on the receptors/channels these proteins actually bind and then trying to influence this process with suitable ligands. For example, for receptors with four transmembrane (TM) domains, such as $GABA_A$ receptors, this would require targeting drugs to the intracellular domains between TM3 and TM4 (REF. 61). In essence, proteomic approaches will help by allowing us to know what pieces are necessary to complete the 'intracellular protein jigsaw' that couples to ion channels. We will then require an understanding of their functional roles and precisely where these proteins link to the ion channels. It is at this stage that therapeutic possibilities may become apparent.

11 What are the barriers to achieving specificity using ion channel ligands?

Dennis Dougherty. Lack of structure, lack of throughput (see questions 1 and 6) and poor understanding of ion channel function, even when we have a structure.

Michel Lazdunski. The similarity of the pore regions, at least in the K^+-channel field. One has to remember that there are channels that as yet have no pharmacology at all.

Irwin Levitan. The major barrier is the enormous number of closely related ion channels and the difficulty in identifying a specific molecular target — a drug that targets the pore of one K^+ channel, for example, is likely to hit many others. A related problem is the possibility of redundancy of function, so that even if a particular channel could be targeted specifically, another closely related one might step into the breach.

Dale Benos. I think that the biggest barriers to achieving ion channel ligand specificity are the large redundancy in structural domains among different ion channels; the lack of knowledge concerning the tertiary and quaternary structures of ion channels at the atomic level; and the gross lack of information concerning which proteins actually comprise an ion channel in different tissues. Depending on the molecular mechanism of action of the ligand, the specificity may in large part be determined by the tissue itself, not only because of ion channel heterogeneity, but also because of possible tissue uniqueness in cytoplasmic signalling complexes.

Richard Lewis. Many therapeutic classes of ion channel modulators, including local anaesthetics, target membrane-spanning hydrophobic domains of ion channels, which are usually the structurally more conserved elements found within a class of ion channel[62]. As major pharmaceutical drug libraries are composed of relatively hydrophobic molecules, most of the 'hits' identified are likely to target these hydrophobic domains. Access to the diverse and novel chemistry provided by natural-product libraries offers opportunities to broaden the classes of interactions identified. For example, the venom peptides that target ion channels typically act at the least-conserved structural elements that are found in the loops that extend extracellularly from the membrane, such as -conotoxins,

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Alan North is Professor of Molecular Physiology at the University of Sheffield, Sheffield, UK, and Director of its Institute of Molecular Physiology. He graduated with a B.Sc. (Physiology, 1969), M.B. Ch.B. (Medicine, 1969) and Ph.D. (Pharmacology, 1973) from the University of Aberdeen, Aberdeen, UK. North worked for 18 years in the United States, as Associate Professor of Pharmacology at Loyola University Stritch School of Medicine, Chicago, Illinois; as Professor of Neuropharmacology at the Massachusetts Institute of Technology, Boston, Massachusetts; and as Senior Scientist and Professor at the Vollum Institute, Oregon Health Sciences University, Portland, Oregon. For five years he was Principal Scientist at the Geneva Biomedical Research Institute, a division of GlaxoWellcome Research and Development in Switzerland. He has also held Fellowships or Visiting Professorships at the Max Planck Institute for Psychiatry in Munich, Germany; the John Curtin School for Medical Research at the Australian National University in Canberra; Flinders University in Adelaide, Australia; the Bogomoletz Institute of Physiology in Kiev, Ukraine; the Johann Wolfgang Goethe University in Frankfurt, Germany; and the University of Melbourne in Australia. At present, he is President of the UK Physiological Society and Editor-in-Chief of the *British Journal of Pharmacology*. Professor North's work has been centred on obtaining a quantitative understanding of drug and transmitter action at the level of single cells and single molecules, primarily by biophysical and molecular biological approaches. His publications deal with drug and neurotransmitter receptors, the structure and function of ion channels, the physiology of the autonomic (particularly enteric) nervous system, pain mechanisms, and the actions of opiates and opioid receptors. His main current interest is the physiological role of P2X receptors, which are ion channels gated by extracellular ATP.

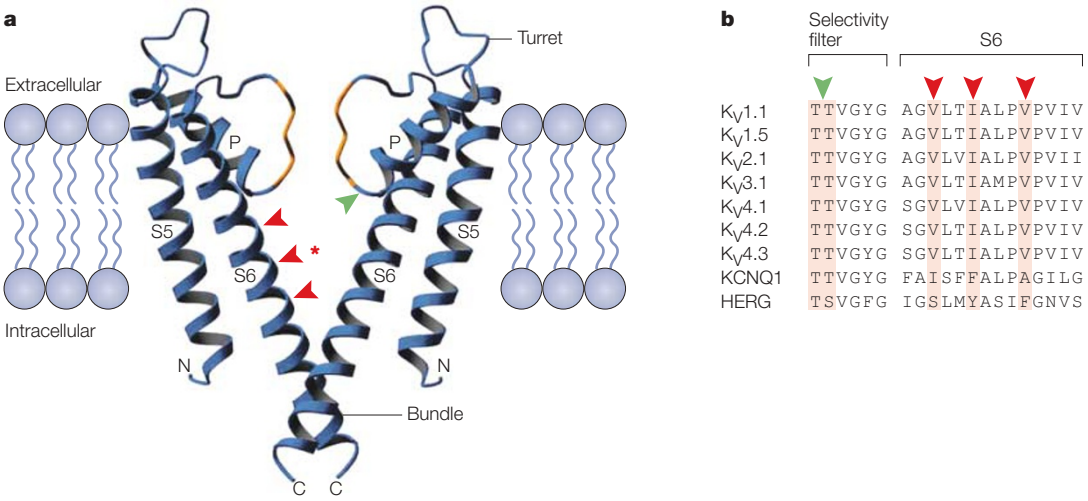

Figure 7 | **The remarkable similarity of K⁺ channel pore regions presents a challenge in the design of channel-specific blockers. a** | Typical structure of the pore region of a voltage-gated K^+ (K_V) channel elucidated by the X-ray crystal structure of KcsA[3]. For clarity, only two of the four identical subunits are shown. The pore helices are labelled P, and the central cavity is indicated by a red asterisk. The green triangle points to the position of the two threonine residues located at the base of the pore helix. The side chains of these threonine residues and a few residues (red triangles) of the S6 helices face the central cavity and are common sites of interaction with pore blockers. **b** | The sequence alignment of key regions near the selectivity filter and S6 domains of several K_V channels are shown. Residues that face the central cavity of the channel are shaded in red. Blockers of $K_V1.5$ (REF. 114), KCNQ1 (REF. 115) and human ether-a-go-go-related gene (HERG)[36] channels have been reported to interact with these residues. The key residues of K_V1–K_V4 channels are identical, illustrating why it is so difficult to discover specific drugs for any one of these channels. Drugs that alter channel gating or block the outer pore are more likely to be channel specific because the amino-acid sequences are variable for the extracellular loops that connect transmembrane domains (for example, the turret structure in part **a**). Part **a** modified, with permission, from *Nature* (REF. 3) © (2003) Macmillan Magazines Ltd. Figure prepared by Michael Sanguinetti.

which target the extracellular pore of voltage-sensitive Na^+ channels[63].

Jamie Vandenberg. The problem with many ion channel ligands developed so far is that they bind to motifs that are common to many ion channels, most notably the pore region. At present, we do not know enough about the structural differences between ion channel subtypes to be able to pursue so-called 'rational' drug design strategies. However, even if we did, I am not convinced that targeting individual ion channels is necessarily the right way to go. In the cardiac field at least, the most successful drugs so far have not been specific ion channel blockers, but drugs that modulate the signalling pathways that regulate the integrated ion channel response, such as beta-blockers. Even amiodarone (Cordarone; Wyeth Pharmaceuticals), reputedly a non-specific ion channel blocker, might be exerting some of its effect by modulation of thyroid hormone signalling[64].

Michael Sanguinetti. Perhaps the biggest hurdle will be the discovery of agents that specifically affect closely related channels. For example, the binding site for pore-blocking drugs is usually located on the S6 transmembrane domain of ion channels[36,65]. The sequence of this domain is almost identical for members of the K_V1–K_V4 channels, making selectivity difficult (FIG. 7).

Alan North. A barrier to achieving specificity is the fact that certain subunits are used very widely in channel formation, and in tissues of very different function. A recent example might be the unexpected finding of α7 nACh receptors in macrophages[66]; who would have thought it? The most critical step is the molecular identification of the channel in the cell of interest, under the conditions of interest (for example, ageing, pain or inflammation). This identification, with high-throughput screening, can lead to specificity.

Sarah Lummis. Ligand-gated ion channels have relatively small binding pockets, and their specificity for many ligands, especially small ones, is defined by the number of amino acids present in this pocket. However, this limited number of amino acids is also one of the main barriers to achieving specificity, particularly between related receptors, for which critical amino acids may be conserved. For example, tryptophan (Trp)149 in the nACh receptor and the equivalent Trp183 residue in the 5-HT_3 receptor are both important ligand-binding residues and have been shown to have a similar interaction with their neurotransmitters (in this case forming a cation–π interaction with the charged group[67,68]). In addition, it is becoming clear that in this class of receptors, all the binding sites are constructed primarily of an aromatic box[69], thereby allowing the neurotransmitter to bind reversibly. The limited repertoire of aromatic amino acids that is available to create this box results in lack of specificity in a number of binding ligands, particularly small ones.

David Clapham. The main barrier is the lack of high-throughput methods that provide the same quality of information as the patch clamp. This may be solved within a few years given current commercial attempts

by several companies, including Aviva Bioscience, Axon Instruments, Molecular Devices, Nanion Technologies and Sophion Bioscience. A second barrier is the need for more high-resolution structural information in multiple channel-gating states. A third barrier is the limitation of compound libraries, which will hopefully be solved in part by combinatorial chemistry. A fourth barrier is the lack of delivery mechanisms for peptides and proteins, and our poor understanding of how to make scaffolds for small molecules.

Trevor Smart. Specificity can be interpreted at two levels: specificity between quite different channel populations, such as K^+ and Na^+ channels, and specificity for individual channel isoforms within a population (for example, the voltage-sensitive K^+ channels $K_V1.1$ and $K_V2.1$). One major barrier to achieving specificity is our limited understanding of the basis of receptor/channel heterogeneity within single-channel populations. For example, where are these channels located and what are their distinctive physiological functions, if any? It could be that heterogeneity is physiologically important or, alternatively, that we are just seeing 'Darwinian evolutionary noise' in the production of ion channels, and that these variations in structure are of no significance. Evidence suggests the former view is more likely to be correct (for example, REF. 70), thus adding impetus to the design of subtype-selective ion channel ligands.

In conjunction with specificity, we also need to be clear about when drugs elicit 'side effects' as opposed to 'unwanted effects'. Side effects are deleterious but should not be confused with unwanted effects; for example, the many actions of the benzodiazepines are clinically useful, but not necessarily when they are all evoked by one drug at a set dose used for a single, specific condition.

When we appreciate where different receptors and channels are located in cells and understand their different functions, then the design of subtype-selective drugs will have a rational basis. Using transgenic technology, and introducing subtle mutations into receptors (knock-in techniques) that leave the animal unaffected by the mutation apart from its response to particular drugs, is a valuable approach to investigate the natural roles of different isoforms of receptors and channels. For example, the benzodiazepines modulate the function of many different $GABA_A$-receptor isoforms and produce a number of clinical effects, ranging from sedation, amnesia, anxiolysis and myorelaxation to anticonvulsant effects. But what has become clearer is that the anxiolytic properties of benzodiazepines are mediated largely via α_2-subunit-containing $GABA_A$ receptors, whereas α_1-subunit receptors mediate the sedative effects[71]. This separation of benzodiazepine effects to different receptor isoforms would support different roles for these receptors in the brain and also further encourage the prospect of synthesizing drugs that specifically modulate only selected receptor isoforms.

Another major area that is becoming more important is the connection between the genetic composition of individual patients and their response to particular drugs. The field of pharmacogenetics will have several major implications for the future reduction of side effects and targeting of drug therapies[72].

12

Could there be an approach to ion channel pharmacology based around monoclonal antibodies or other biologicals, rather than small molecules?

Richard Lewis. Yes, but getting *in vivo* function without side effects can be a challenge.

David Clapham. Yes, and some are currently attempting this approach with antibodies or Fab fragments. The problems here are potential immunogenicity and proteolysis.

Kenneth Chien. These could work if there was a clear, specific link between a receptor and the activation of a given channel that is implicated in disease; for example, the muscarinic acetylcholine receptor, adenosine receptor and so on. I am bullish on exploring the role of the many novel, orphan G-protein-coupled receptors in the heart and how they relate to the control of channel function; RNAi and mouse knockouts will be informative here.

Michel Lazdunski. Yes. The feasibility of using antibodies was shown a long time ago in my lab with the discovery of monoclonal antibodies that can work just like scorpion toxins; that is, by changing the gating kinetics of Na_V channels[73].

Sarah Lummis. Monoclonal antibodies and other biologicals such as toxins have long been recognized to be highly selective for certain ion channels. Their relatively large size ensures that they have the potential to bind to many different regions of the ion channel and thus be highly specific. However, this in itself creates problems, as such molecules may also apparently bind specifically to completely different regions of the protein. Therefore, although using these molecules can be advantageous, their use should be combined with other more traditional approaches.

Irwin Levitan. In principle, this is an ideal way to achieve specificity. I think an under-exploited target is the interaction domain between ion channel pore-forming subunits and their auxiliary subunits or other associated proteins, and either antibodies or peptide sequences that interfere with association domains will be powerful pharmacological tools. The problem, of course, is the delivery of such large molecular reagents to interaction sites inside the cell.

Alan North. The advantage of monoclonal antibodies is that ion channels are expressed on cell surfaces and are therefore accessible to antibodies directed against the ectodomain. However, there are still the usual problems of delivery. But in principle it could work, as it has for tumour-necrosis factor (TNF)-α receptors. For example, infliximab (Remicade; Centocor) is a chimeric anti-TNF-α monoclonal

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John Peters gained an Upper Second Class B.Sc. (Hons) degree in Neurobiology from the University of Sussex, Brighton, UK, and subsequently a Ph.D. in Zoology from the University of Nottingham. He joined the Department of Pharmacology and Clinical Pharmacology at the University of Dundee in 1984 and has remained there ever since. Peters' primary interest is in the biophysics, physiology and pharmacology of the Cys-loop family of transmitter-gated ion channels. Most of his work has focused on the 5-hydroxytryptamine $(5\text{-HT})_3$ and $GABA_A$ (γ-aminobutyric acid) receptors, but has also included studies on nicotinic acetylcholine, strychnine-sensitive glycine and ionotropic glutamate receptors. For his work on the electophysiology of the 5-HT_3 receptor, begun as a Ph.D. student and still continuing, Peters was awarded the Sandoz (now Novartis) Prize of the British Pharmacological Society in 1992. He also has an interest in receptor and ion channel nomenclature and, as a co-editor, has produced a number of guides on the subject, including the *Guide to Receptors and Channels* (eds Alexander, S. P. H., Mathie, A. & Peters, J. A.), which is published by the *British Journal of Pharmacology*. Peters currently holds a Readership in Pharmacology in the Neurosciences Institute, Division of Pathology and Neuroscience, at the University of Dundee.

antibody with potent anti-inflammatory effects, which are probably based on apoptosis of inflammatory cells. Numerous controlled trials have demonstrated its efficacy in both rheumatoid arthritis and Crohn's disease[74].

Walter Stühmer. Definitely, given the high specificity of monoclonal antibodies and the great variety of ion channels within each family (particularly the K^+ channels). A potential drawback of this approach might be the generation of an immune response against the extraneous antibodies. However, the use of humanized or human antibodies seems to reduce this undesirable side effect to a minimum, as shown for new therapies, such as trastuzumab (Herceptin; Genentech), a humanized monoclonal antibody directed against HER2, which is an epidermal growth factor (EGF) receptor that is overexpressed in some types of tumour[75,76]. Many human or humanized antibodies are undergoing clinical trials at present, and channelopathies are ideal candidates for this type of approach, given the extracellular epitopes of ion channels.

John Peters. I take this question to mean ion channel therapeutics, rather than pharmacology, as monoclonal antibodies are currently major tools in the localization of specific ion channel subtypes. Although it is conceivable that monoclonal antibodies could be used as therapeutic agents directed towards ion channels, a logical choice for a peripheral ion channel target does not immediately spring to mind. Monoclonal antibodies are capable of great selectivity and are enjoying increasing use as therapeutic agents in the treatment of cancer, inflammatory, cardiovascular, respiratory and other disorders, although largely in hospital settings. A recent report describing crystalline monoclonal antibodies for subcutaneous delivery raises the possibility of more convenient use of these agents[77]. Perhaps more promising are peptide molecules based on the structure of naturally occurring toxins (such as α-conotoxins; mentioned in question 5), but the usual pharmacokinetic difficulties associated with peptides will need to be addressed.

Jamie Vandenberg. Given that developing specific ion channel blockers might not be the optimal strategy, at least with respect to targeting cardiac arrhythmias, the use of biologicals that inhibit ion channels with greater specificity is unlikely to be worthwhile. However, I think it is worth pursuing the idea that ligands that can specifically target (with high affinity) a particular ion channel that is preferentially expressed in a given tissue could be used to carry drugs to that particular tissue. In this scenario, the antibody/biological (or polymers using motifs based on biologicals with high specificity for the ion channel of interest) would not block the channel, but would use the channel as a homing device to enable tissue-specific delivery of its cargo of drugs. In this case, the drugs being carried to the target tissue do not necessarily have to be aimed at modulating electrical activity, but could, for example, be chemotherapeutic agents.

Trevor Smart. In theory, this could be a highly selective approach to targeting specific ion channels, but the choice of the epitope will be crucial, and even then not a guarantor of the functional outcome following antibody binding. Antibodies have been shown to have effects on ion channels, notably ionotropic glutamate receptors (for which some autoantibodies to GluR3 receptors are associated with Rasmussen's encephalitis/syndrome[78,79]) and muscle nACh receptors, which can cause numerous defects in transmission[80,81]. However, there are numerous examples in which antibodies have no effect on channel function. In principle, the use of antibodies will bring considerable selectivity in targeting receptors, but they will also be problematic in that their sheer size may cause receptors to internalize inside cells. They may also impede or interfere with membrane trafficking and may not be ideal, readily reversible ligands. Moreover, their structures present considerable difficulties when considering the issue of drug delivery to specific organs/systems of the body, the CNS being a prime example. For example, the oral route is rendered unsuitable unless special delivery vehicles are considered. It would seem more likely that selective peptides to particular defined binding sites will be more useful as therapeutic aids than antibodies; however, antibodies have found clinical use in treating rheumatoid arthritis and Crohn's disease (for example, etanercept (Enbrel; Amgen/Wyeth) and infliximab), and also for treating some cancers, such as breast and prostrate tumours (for example, pertuzumab (Omnitarg; Genentech) and trastuzumab), but not by targeting ion channels. The field is clearly developing.

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What are the main hurdles that will need to be overcome to creating further successful therapeutic approaches based around the modulation of electrical activity?

Trevor Smart. The biggest single hurdle is the design of subtype-selective agents. The ability to modulate part of a population of ion channels may avoid many side/unwanted effects. We also need to better understand the physiological consequences for channel proteins after drug binding. Do they simply remain *in situ*, and are other proteins acutely upregulated? We have to remember that channels and receptors are increasingly being shown to be quite dynamic systems.

Michel Lazdunski. Problems caused by too much sedation or by too much activity, resulting in TORSADES DE POINTES in the heart or in epilepsy in the CNS, respectively.

Alan North. With regard to modulation of electrical activity, effects on cardiac function (for example, LQTS screening) and subtle effects on brain function will be barriers to overcome. But channels function by their chemical signal as well as their electrical signal, and this might be less of a problem with respect to the heart and brain.

Sarah Lummis. One of the major problems is the lack of ligand specificity (see question 11). There is also the problem of potency; that is, a ligand may completely block or hyperactivate a channel, when a more beneficial approach would perhaps be subtle modulation. Thus, compounds that modulate channel activity, such as the benzodiazepines at $GABA_A$ receptors, could potentially be more therapeutically useful.

Birgit Liss. I guess one big challenge will be to find more specific drugs that will selectively modulate channel activity in a defined target-cell population.

David Clapham. The main hurdle is the need for a more detailed understanding of the underlying physiology, particularly in the nervous system. For cardiac antiarrhythmic drugs, the main problem is potential toxicity, such as inadvertent production of LQTS in subpopulations of susceptible patients or LQTS produced by combinations of drugs. A big problem is the downregulation or adaptation of ion channels or receptors after continued drug application.

Jamie Vandenberg. We need a better understanding of the integrated response of a cell's electrical activity to environmental stresses, including how ion channels, metabolic pathways and signalling pathways interact with each other on different timescales; that is, acutely (seconds to minutes) and chronically (hours to days). The latter timescale obviously becomes much more complicated, as transcription patterns will almost certainly be affected. We also need a better understanding of the long-term effects of ion channel modulation compared with the acute effects. This is a particularly difficult problem, as it is time consuming and costly — hence the need for good model organisms.

Kenneth Chien. I think we should start to think about targeting channel modulators and the signalling pathways that control channels, rather than trying to target channels themselves. In the heart, drugs that have been designed to directly occupy channels have been associated with increased mortality. It seems that the level of channel activity is carefully controlled in the heart and that major effects in either direction (activation or inhibition) can produce a phenotype similar to arrhythmogenesis. Thus, a modulatory approach — that is, slightly inhibiting the activity of a channel by designing an inhibitor of a naturally occurring positive co-regulator of the channel, or initiating changes in upstream signalling pathways that regulate the co-regulator — might be better, because there would be no danger of turning the channel of interest on or off completely. One could envision effects on gating, activation, trafficking or inactivation, depending on the role of the modulator, rather than being restricted to designing a small molecule that binds directly to the channel protein itself and has a selective effect on just one aspect of channel function.

Walter Stühmer. I see a problem, in that for the most part only short-term effects and interactions are known for ion channels. This means that in most cases the disease-relevant, long-term effects are unknown, even if phenomenologically proven to be important. This reduces the confidence of drug companies in ion channels as potential targets, as they would like to see a mechanism linking the channel with the respective disease. An example is the link between cancer and ion channels. Cl^- and K^+ channels have for a long time been linked to cancer, and the mechanism of action has mainly been attributed to regulation of the cell cycle[82]. Ion channels have therefore been rather indirectly linked to tumour progression. Recently, however, there is growing evidence for a causative factor linking cell proliferation and certain ion channel types, such as $K_V1.3$, HERG and ether-a-go-go (EAG)[83]. EAG can be considered an oncogene, as its ectopic expression promotes uncontrolled cell proliferation[84]. However, the mechanism or the signalling cascade involved is totally unknown at present, and although ion channels are potentially relevant and valid targets, few companies, if any, have ventured into this field.

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Michael Sanguinetti received an M.S. degree in Biology from San José State University, San José, California, USA, and a Ph.D. degree in Pharmacology from the University of California, Davis, in 1982. Following a postdoctoral fellowship at the University of Rochester, Rochester, New York, he was a member of the Department of Pharmacology at Searle Research & Development in Skokie, Illinois, for three years, followed by five years at Merck Research Laboratories in West Point, Pennsylvania. In 1992, Sanguinetti joined the University of Utah in Salt Lake City, Utah, where he is now Professor of Physiology and a member of the Nora Eccles Harrison Cardiovascular Research and Training Institute. His research interests include the molecular basis of inherited arrhythmias, particularly long QT syndrome, and the pharmacology and mechanisms of gating of voltage-gated potassium channels. Most recently, Professor Sanguinetti's laboratory has concentrated on elucidating the structural basis of human ether-a-go-go-related gene (HERG) potassium-channel sensitivity to block by common medications.

14

In your opinion, what diseases are most strongly linked with aberrant ion channel function?

Kenneth Chien. Diseases of excitable cells, particularly those which are innervated, are the most obvious. However, I think that channel function will be pivotal to many human diseases outside of classic diseases of excitability. Functions such as cell homing, migration and the contractile function of both muscle and non-muscle cells are likely to emerge. The role of channels in inflammatory pathways could also become of interest.

Sarah Lummis. A variety of diseases are currently strongly linked with aberrant ion channel function. Examples in the ligand-gated ion channel field include ADNFLE (described in question 3) and inherited myoclonus of Poll Hereford cattle[85,86].

Alan North. First, channelopathies are directly linked to aberrant ion channel function, and there are several hundred of these already known[27]. We can expect this number to grow as the functional roles of other large channel families become better understood (for example, transient receptor potential (TRP) channels, ClC and P2X). Second, there are many diseases that are already successfully treated by drugs acting on ion channels; examples include antiarrhythmics, anaesthesia, epilepsy and essential hypertension. Third, there are many further diseases for which ion channels are currently under investigation as potential targets; again, the list is long, but includes inflammation (involving the TRPM2 channel and the ATP receptor $P2X_7$), neuropathic pain (acid-sensitive ion channels, TRPs and $P2X_3$), osteoporosis, brain tumours and others.

Michel Lazdunski. Diseases that are strongly linked with aberrant ion channel function will include cardiac arrhythmias; epilepsy; Parkinson's disease; hypertension, including pulmonary hypertension; hypotension; type 2 diabetes; incontinence; claudication; retinal diseases; muscle diseases; respiratory diseases; pain sensation; psychiatric diseases, including depression; and some forms of cancer.

Dale Benos. There are a number of diseases that are directly linked to aberrant ion channel function. Some are genetically based, such as Liddle's syndrome, malignant hyperthermia, polycystic kidney disease, many myotonias, including Thomsen's disease, and, of course, the prototypical ion channel genetic disease cystic fibrosis. Diseases directly related to ion channels can also result, not because of mutations in the genes coding the channel proteins themselves, but because of dysfunction in the regulatory pathways controlling ion channel activity. Hypertension resulting from adrenal medullary tumours that overproduce aldosterone is one such example. Another example is provided by certain autoimmune diseases, such as myasthenia gravis. It is clear that because ion channels are ubiquitous and subserve so many different functions, deviations from 'normal' behaviour can have dramatic physiological consequences.

Frances Ashcroft. Many diseases are linked with aberrant ion channel function, either due to mutations in ion channel genes themselves, or to mutations in proteins involved in ion channel expression (such as transcription factors), membrane targeting or regulation of channel activity. For example, there is evidence that the defective insulin secretion found in monogenic forms of diabetes arises from impaired K_{ATP} channel activity in pancreatic β-cells, due to mutations in metabolic genes and transcription factors. It also seems likely that many behavioural diseases are due to aberrant brain electrical activity, and thus to impaired ion channel activity.

John Peters. There are a host of channelopathies for which aberrant ion channel function seems to be the cause of morbidity. Anion-selective ion channels offer some excellent examples. There can be little doubt regarding mutations of the *CFTR* gene and cystic fibrosis, even though some details remain to be addressed. Multiple point mutations of the strychnine-sensitive glycine receptor result in hyperekplexia (startle disease and neonatal hypertonia). Mutations in the ClC1 Cl^- channel result in myotonia congenita, which can be either autosomal dominant (Thomsen's disease) or recessive (Becker's myotonia). Bartter's syndrome (classic (type III) and Gitelman's variant) is associated with mutations of the ClC-Kb Cl^- channel, and mutations in barttin (a ClC channel 'accessory protein') cause Bartter's syndrome type IV. Mutations within the *ClC7* gene are associated with some forms of osteopetrosis in man.

David Clapham. Most of the following are equally well-linked (the channels linked to each disease are indicated in brackets): arrhythmias due to a prolonged plateau of cardiac action potential, such as LQTS (Na_V and K_V channels); hypertension (particularly renal hypertension, Liddle's syndrome and Bartter's syndrome); polycystic kidney disease (PKD1 and PKD2); male sterility (PKDL, CATSPER2); cystic fibrosis (CFTR); muscle weakness; periodic paralysis and myotonia (Na_V and Ca_V channels); ataxia; autoimmune channelopathies, such as Lambert–Eaton syndrome (Ca_V channels); myasthenia gravis (nACh receptor); persistent hyperinsulinaemic hypoglycaemia of infancy (SUR, KIR6); malignant hyperthermia (ryanodine receptor); Dent's disease; Charcot–Marie–Tooth neuropathy; Thomsen's disease (ClC1 chloride channel); diabetes insipidus (AQ1); and glaucoma (aquaporin). Less understood but probable: intracellular lysosomal storage diseases (mucolipidosis; MCOLN); various causes of deafness and blindness; and diabetic neuropathies. The evidence indicates that all of these are more closely linked with ion channels than, say, Alzheimer's disease or cancer. The next wave of ion channels linked to diseases will be TRP channels; they have only recently been discovered and there are many of them.

Trevor Smart. There are many diseases exhibiting tentative links that still need to be firmly established;

however, those diseases with relatively good, quite clear links to ion channel dysfunction would include myasthenia gravis with nACh receptors; the Lambert–Eaton syndrome with presynaptic calcium channels; some forms of hyperekplexia with glycine receptors (the α1-subunit, for example); Bartter's syndrome with inwardly rectifying potassium channels ($K_{ir}1.1$), chloride channels (ClC-Kb) and a $Na^+/K^+/Cl^-$ co-transporter; and the LQTS with sodium and potassium (KCNQ1) channels. The involvement of ion channels in disease will reflect not only mutations of channel proteins, or their targeting by autoantibodies, but may also involve aberrant trafficking of these proteins into and out of the surface membrane.

Glossary

ARRHYTHMIA
A generalized term used to describe disturbances in heart rhythm.

ATOMIC FORCE MICROSCOPY
A microscope that non-destructively measures the forces (at the atomic level) between a sharp probing tip (which is attached to a cantilever spring) and a sample surface. The microscope images structures at the resolution of individual atoms.

DELAYED-RECTIFIER K^+ CHANNEL
Slowly activating and very slowly inactivating channels that preferentially pass K^+ out of the cell.

DEPOLARIZATION
A decrease in the electrical potential across a membrane, such as when the inside of a cell becomes less negative.

DESENSITIZATION
The mechanism by which a ligand becomes less effective on a receptor during a prolonged application.

ELECTROCARDIOGRAM
A graphic record of the electrical currents of the heart recorded at the body surface.

ELECTROGENIC PROCESSES
Processes involving the production of electrical impulses in living tissues or organs.

ELECTRON PARAMAGNETIC RESONANCE
(EPR). When an atom with an unpaired electron is placed in a magnetic field, the spin of the unpaired electron can align, either in the same direction as the field or in the opposite direction. EPR spectroscopy is used to measure the absorption of microwave radiation that accompanies the transition between these two states.

EXOCYTOSIS
The discharge by a cell of intracellular materials into the extracellular space through the fusion of vesicles (containing these materials) with the plasma membrane.

FLUORESCENCE RESONANCE ENERGY TRANSFER
(FRET). A spectroscopic technique that is based on the transfer of energy from the excited state of a donor moiety to an acceptor. The transfer efficiency depends on the distance between the donor and the acceptor. FRET is often used to estimate distances between macromolecular sites in the 20–100-Å range, or to study interactions between macromolecules *in vivo*.

GREEN FLUORESCENT PROTEIN
An auto-fluorescent protein, originally isolated from the jellyfish *Aequorea victoria*, that can be genetically conjugated with proteins to make them fluorescent

INWARDLY RECTIFYING K^+ CHANNEL
K^+ channels that allow long depolarizing responses, as they close during depolarizing pulses and open with steep voltage dependence on hyperpolarization. They are called inward rectifiers because current flows through them more easily into than out of the cell.

MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT
(MALDI-TOF). A mass spectrometric technique that is used for the analysis of large biomolecules, in which analyte molecules are laser-desorbed from a solid or liquid matrix containing a substance that shows high resonant absorption at the laser wavelength used. A laser beam passes through the substances to be analysed, and causes these elements to vaporize and their molecules to fly upwards into a tube. Time of flight through the tube correlates directly to mass, with lighter molecules having a shorter time of flight than heavier ones.

METASTABLE CONFORMATION
An unstable and transient but relatively long-lived state of a chemical or physical system.

PATCH CLAMP
A technique whereby a very small electrode tip is sealed onto a patch of cell membrane, thereby making it possible to record the flow of current through individual ion channels or pores within the patch.

PDZ DOMAIN
Protein–protein interaction domain that often occurs in scaffolding proteins and is named after the founding members of this protein family (PSD-95, DLG and ZO-1).

PHARMACOPHORE
The ensemble of steric and electronic features that is necessary to ensure optimal interactions with a specific biological target structure and to trigger (or to block) its biological response.

PROGENITOR CELL
In development, a 'parent cell' that gives rise to a distinct cell lineage by a series of divisions.

QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP
The quantitative relationship between the structures of a set of compounds and their biological activity.

QUANTITATIVE TRAIT LOCI
A genetic locus or chromosomal region that contributes to variability in a complex quantitative trait (such as body weight), as identified by statistical analysis.

QT INTERVAL
On an electrocardiogram, the QT interval represents the time between the electrical activation and repolarization of the ventricles, the lower chambers of the heart.

RAPID AMPLIFICATION OF cDNA ENDS
(RACE). A polymerase chain reaction (PCR)-based technique that was developed to facilitate the cloning of full-length cDNA 5′- and 3′-ends after a partial cDNA sequence has been obtained by other methods.

REPOLARIZATION
Recovery of the electrical potential across a membrane back to the resting level following a depolarization stimulus.

RNA INTERFERENCE
(RNAi). A method by which double-stranded RNA that is encoded by an exogenous vector can be used to interfere with normal RNA processing, causing rapid degradation of the endogenous RNA and thereby precluding translation. This provides a simple way of studying the effects of the absence of a gene product in simple organisms and in cells.

SINGLE NUCLEOTIDE POLYMORPHISM
A specific location in a DNA sequence at which different people can have a different DNA base. Differences in a single base could change the protein sequence, leading to disease (for example, sickle-cell disease), alter the expression of a gene, or have no known consequences.

SMALL INTERFERING RNA
(siRNA). Small antisense RNA molecules (20–25 nucleotides), generated from specific double-stranded RNAs, which trigger targeted mRNA degradation.

SYNCHROTRON
A synchrotron accelerates charged particles in a circular orbit. This produces very intense X-rays, which allows the use of smaller and more easily obtained crystals than can be used with conventional X-ray crystallography, and also boosts relevant signals while minimizing noise. The wavelength of synchrotron X-radiation can be varied to perform multiple anomalous dispersion (MAD) experiments.

TORSADES DE POINTES
A form of polymorphic ventricular tachycardia characterized by QRS complexes that gradually rotate around the isoelectric baseline. Torsades de Pointes is usually associated with a long QT interval on the ECG and an absolute or relative bradycardia.

TRANSIENT OUTWARD K^+ CURRENT
A rapidly activating and inactivating K^+ current.

X-RAY CRYSTALLOGRAPHY
A technique that uses the diffraction of X-rays passed through a crystallized protein to determine the atomic structure of the protein.

15

What are the pitfalls and advantages of animal models that seek to recapitulate the human channelopathies?

Sarah Lummis. Animal models will never completely recapitulate the problems of human channelopathies, but if this is appreciated, they can be tremendously useful in testing a variety of hypotheses and/or drugs.

Michel Lazdunski. There are only advantages if you are reasonable enough not to expect everything from these models. Plus, in the near future, we will probably see types of knockout other than mice that might be more informative (I would expect to see rat knockouts first).

Alan North. Most human channelopathies are not great therapeutic targets. Take the lesson from cystic fibrosis, for which the animal model(s) (*Cftr*-null/mutant mice) are not much help in understanding the pulmonary manifestations of the disease[87]. Models may be more useful for educating us about the real physiological roles of channels.

Jamie Vandenberg. There will always be differences between model organisms and humans, and so any data obtained in model organisms have to be, where possible, verified in a human setting. The major potential advantage of using model organisms is the ability to study modifier influences in detail, particularly at a genetic level.

Irwin Levitan. The advantages are, of course, enormous. The use of genetically tractable organisms, such as worms, flies and mice, allows the rapid testing of potential therapies for both efficacy and toxicity. A major disadvantage is that results from such model organisms do not always extrapolate to humans (see question 18), for reasons that are not yet clear.

Birgit Liss. With general knockout/transgenic mice, there is always the possibility of functional compensation — for example, by related ion channels — or of unspecific effects due to the genetic modification. However, animal models of channelopathies provide an accessible tool to study the molecular mechanisms of the related disease. I believe that general physiological concepts will be similar between mice and men.

Frances Ashcroft. Animal models can shed light on the physiological function of a given ion channel in the whole organism or organ (if tissue-specific expression is used). They have often identified new and important information about ion channel function and its role in human disease. However, a human is not a mouse (or indeed a worm), and an animal model does not necessarily recapitulate the human disease phenotype. Ultimately, there is only one gold standard — human beings.

David Clapham. The advantages are clear — it is much more acceptable to experiment on animals than humans. There is currently no practical substitute for testing in animals. The pitfalls are, of course, that, depending on species, significant differences exist between humans and other animals. Compensatory mechanisms are often different between animals. A major pitfall is that assays, particularly of higher-order functions such as memory, are limited in animals and are predicated on assumptions that may be incorrect.

Kenneth Chien. The electrophysiology of the mouse, the preferred system for genetically based models of human disease, is quite different to humans, so there is inherent disparity there. More needs to be done with other systems; I think we will see alternative models emerge, such as the use of PROGENITOR CELLS from the heart and brain, which may open the door to direct studies in human cells themselves — this would be ideal. Understanding why some patients that harbour LQT mutations do not have the phenotype of sudden death could be quite informative, particularly if this reflects how the mutation influences the action potential in other subtle ways.

Richard Lewis. Animal models are a direct and relatively inexpensive means of determining whether compounds with interesting *in vitro* activity have *in vivo* efficacy and/or unwanted side effects. For pharmacological classes proven to have therapeutic relevance in humans, models are an excellent means of establishing likely efficacy in humans. For compounds within known chemical classes, side-effect issues can be easier to predict. However, with the development of new pharmacologies and chemistries, the risks are higher. Although the pharmacology of human versus animal-model targets is easily assessed through cloning and expression, animal models are unlikely to be predictive

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Trevor Smart graduated with a First Class B.Pharm. honours degree from London University, London, UK, and joined the National Health Service (NHS), before completing a Ph.D. in Pharmacology at The School of Pharmacy, University of London. He eventually became the Wellcome Professor of Pharmacology at The School of Pharmacy. Smart moved to University College London in 2002, and is now Head of the Department of Pharmacology and occupies the Schild Chair in Pharmacology. His work has centred on the molecular pharmacology and physiology of the $GABA_A$ (γ-aminobutyric acid) receptor, the major inhibitory neurotransmitter receptor in the brain, which is pivotally involved in controlling nerve cell excitability. In particular, Professor Smart has used a multidisciplinary approach to understanding how endogenous regulators in the nervous system modulate the function of GABA receptors and inhibitory synapses. Detailed structure/function studies have revealed where ligands can bind to these receptors and how they can affect receptor function in a subtype-selective manner. Moreover, monitoring the intracellular regulation of GABA receptors by anchoring and trafficking proteins, and after modification of receptor structure by phosphorylation, has enabled a detailed, dynamic profile of this receptor to be assembled. Professor Smart has been awarded the Sandoz prize in Pharmacology, the Lilly Award for Pharmaceutical Sciences and the Royal Pharmaceutical Society of Great Britain Conference Science Medal. He became a Fellow of the Royal Pharmaceutical Society of Great Britain in 2000.

if the physiological/pathophysiological role of the specific target in diseased tissue deviates significantly from normal tissue, such as potentially exists for the neurokinin-1 (NK1) receptor, for which antagonists are useful in rodents, but not in the human pain states investigated clinically (see REF. 88 and subsequent discussions).

Alan Verkman. A potential pitfall of studies on transgenic mice is that they may give false phenotypic information as a consequence of compensatory changes in other transporters (such as the replacement of a deleted/mutant protein) or developmental changes. However, agreement in phenotype comparisons between a transgenic mouse model and drug-treated normal mice is reassuring. Another pitfall in animal models is the differences between animal and human biology. For example, cystic fibrosis (*Cftr*-null/mutant) mice develop little lung disease because of differences in airway submucosal-gland anatomy and the expression of alternative Cl^- channels in the airways/lung[87]. Large-animal models with human-like physiology, such as pigs, sheep or primates, are desperately needed, and work is in progress to create such models using genetic and pharmacological approaches. A different approach that our lab has found to be successful in studying lung and airway phenotypes in human cystic fibrosis is to treat freshly obtained human tissues (autopsy or surgical specimens) with a potent CFTR inhibitor to pharmacologically create the cystic fibrosis phenotype[89].

Michael Sanguinetti. The biggest problem is whether the mouse is an appropriate model for humans. In some cases, such as cardiac arrhythmias, it certainly is not. The molecular and cellular basis of cardiac excitability in rodents is very different from larger mammals. Rodents have heart rates that are almost ten times higher than in humans, and this necessitates significant differences in how action potentials are repolarized and how the cycling of intracellular Ca^{2+} is handled to permit the most efficient means of excitation–contraction coupling. It is therefore not surprising that the types of channel and their density in the membrane differ between mice and humans. For example, delayed-rectifier K^+ channels are far more important for cardiac repolarization in humans than in mice, whereas the opposite is true for transient outward K^+ currents[90]. However, the role of the fast Na^+ channel is relatively similar in the two species. Thus, transgenic mouse models for studying the physiological consequences of mutations in the Na^+ channel can be much more informative than models of delayed-rectifier K^+-channel mutations.

Furthermore, overexpression or knockout of a channel subunit could lead to abnormal partnering of accessory subunits in some tissues, which could change both the biophysical and pharmacological properties of the heteromultimeric or homomultimeric (for example, if the β-subunit was knocked out) channel.

Trevor Smart. Animal models can be helpful in enabling the link between channel function and altered behaviour to be addressed. However, not all animal models adequately reproduce human disease phenotypes, and we must also be aware that the genetic constitution of the animal model may have a crucial effect on the disease pattern, and also on the responsiveness to drug treatments. For example, this could be manifest by promising drug treatments in mice proving to have only poor efficacy (effectiveness) in humans.

16

What methods are optimal for probing the correlation between genotype and phenotype in ion channel-linked diseases?

Michel Lazdunski. There is no optimal method that would work for all channels.

Chris Miller. The tedious and unglamorous work of old-fashioned cell/organ physiology, which is vanishing from our medical schools in a tidal wave of massive parallel-processing techniques.

Sarah Lummis. A range of methods are available at present, and useful — and different — information is gained from each of them. However, perhaps the optimal method is the use of transgenic animals.

David Clapham. Transgenic animals, with the animal being the closest possible in its genotype to humans.

Alan North. Transgenic and knock-in mice; mutations and heterologous expression; human and animal genetics; and genetics in simple organisms, particularly vertebrates such as zebrafish.

Trevor Smart. Optimal methods would include the ability to generate conditional knockout animals to mimic the disease state, and also to create knock-ins that may correct the deficiencies. Analyses of the connection between the genotype and phenotype would involve behavioural, molecular and electrophysiological methods, as indicated in my previous answers.

Alan Verkman. Transgenic knockout and mutant mice remain an important model for genotype–phenotype investigations of ion channels because of the ability to selectively alter the function of a target channel in a tissue- and time-specific manner. If compounds with appropriate selectivity are available, larger animal models are preferred that better recapitulate human physiology, comparing phenotype before and after drug administration.

Kenneth Chien. We need to understand the relationship between specific changes in channels and the *in vivo* phenotype. This will require more than just a knowledge of the structure/function relationship within a given channel, such as an understanding of how these changes relate to the cellular and, more importantly, organ system into which they are integrated.

Birgit Liss. Methods that allow the direct analysis of function and gene expression for the same cells are well suited to correlating genotype and phenotype at the cellular level. To correlate genotypes and phenotypes of whole animals, the combination of detailed standardized behavioural tests and high-throughput SINGLE NUCLEOTIDE POLYMORPHISM (SNP) analysis (for example, MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT (MALDI-TOF)-based or real-time PCR-based) might be one approach.

Jamie Vandenberg. All of the commonly used methods (heterologous expression, integration into accurate kinetic models and gene knockouts in model organisms) contribute to our understanding of genotype–phenotype relationships. At present, the main area in which we lack information is in our understanding of the chronic changes induced by mutated or inhibited ion channels. This information can be obtained only from model organisms. Anything in mammals will always be expensive (and time consuming). Furthermore, the mouse might not be the optimal organism to use for cardiac arrhythmia studies. We therefore need to look into the possibility of developing other model systems; see, for example, the recent studies using zebrafish from Calum MacRae's group[91].

17 How will the almost ubiquitous expression of some ion channels affect their potential as therapeutic targets?

Kenneth Chien. This is a major potential problem.

David Clapham. If the ion channel alone is targeted, it can be quite limiting. However, many ion channels exist in unique protein complexes, or are heteromeric in a tissue-specific way, thus mitigating nonspecificity.

Michel Lazdunski. In most tissues, channels are not identical (different genes, different splice variants, different partners). For example, L-type Ca^{2+} channels are everywhere, so it would be thought that blocking them, even partially, would be dangerous. Nevertheless, Ca^{2+}-channel blockers are very useful drugs for hypertension.

Richard Lewis. This has enormous implications for drug development that are typically addressed only by use-dependent drugs (ligands that prefer to bind to the channel state found in diseased rather than normal tissue; for example, local anaesthetics). Another approach is through the development of drugs with limited distribution, such as ion channel modulators that avoid central effects through selective peripheral distribution. Peptides are one such class of molecules for which limited distribution could be used advantageously.

Michael Sanguinetti. It will have the same impact that has always existed for ion channel modulators. For example, local anaesthetic agents block Na^+ channels in the nervous system and the heart, and L-type Ca^{2+}-channel blockade affects almost all excitable, and many types of non-excitable, tissue. Despite this problem, some agents, such as verapamil (Calan, Isoptin), are effective simply because of the presence of the blood–brain barrier, or because accessory subunits alter the response to drugs in a cell-dependent manner.

Sarah Lummis. With appropriate modulation and/or different agonists or antagonists, which might work quite differently with the same ion channels expressed in different cellular or organ locations (due, for example, to different extracellular and/or intracellular stimuli resulting in different post-translational modifications), I believe that there is good potential for ion channels, even ubiquitous ion channels, as therapeutic targets.

Frances Ashcroft. It rather depends on the functional role of the channel in other tissues, on the ability of the drug to access the different tissue types, and on the rate at which it is metabolized. For example, sulphonylureas stimulate insulin secretion in individuals with type 2 diabetes by blocking K_{ATP} channels in pancreatic β-cells[92]. Although K_{ATP} channels are found in many other cell types, side effects of these drugs are reportedly few. This may, in part, be because K_{ATP} channels in cardiac cells are largely closed (except in ischaemia), and the drug is not thought to cross the blood–brain barrier.

Irwin Levitan. A protein that is expressed ubiquitously is unlikely to be a useful therapeutic target. However, there are examples of ion channels and channel-associated proteins with tissue and cellular distributions that are much more restricted — for example, the β_4-subunit of large-conductance Ca^{2+}-dependent K^+ channels, which is found only in the brain — and it is these that the pharmaceutical industry appropriately focuses on. A more serious problem than ubiquitous expression is

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Walter Stühmer obtained his diploma in applied nuclear physics from the Technical University in Munich, Germany. He completed the experimental work for his Ph.D. in Camogli, Italy, under the supervision of Franco Conti. He then did postdoctoral training in the laboratory of Wolf Almers at the University of Washington in Seattle, Washington, USA, where he developed the loose patch-clamp technique for studying the lateral mobility of voltage-gated ion channels. In 1983, he joined the laboratory of Erwin Neher at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, where he focused on the study of structure/function relationships in sodium channels. At that time, Shosaku Numa in Kyoto, Japan, had cloned the first voltage-gated channel, a sodium channel, and the main functional regions involved in voltage sensing, inactivation, block by tetrodotoxin, the selectivity filter and inactivation were identified at the molecular level through collaboration between the two groups. In 1993, Stühmer was appointed Director of the Max Planck Institute for Experimental Medicine in Göttingen, where his present work focuses on the physiological and pathophysiological role of a voltage-gated potassium channel, the ether-a-go-go (EAG) channel. Together with Luis Pardo in 2001, he founded iOnGen, a start-up company dedicated to the treatment of cancer using EAG, which has the properties of an oncogene, as a specific target.

that even those channels with limited expression might be difficult to target specifically (see question 11).

Trevor Smart. The ubiquitous expression of ion channels need not necessarily affect their potential as therapeutic targets if ligands can be devised that selectively block or modulate particular states of the ion channel (FIG. 8). Thus, ligands that target open or inactivated states of a channel will tend to limit channel function only in areas where there is intense operation of the channel, or where the channel adopts a particular state for prolonged periods of time (similar thinking has been used to indicate why phenytoin (Dilantin; Pfizer) is effective in epilepsy by blocking the ubiquitous Na^+ channel[93]). This would argue for the development of ion channel-state-dependent drugs. However, such an approach is not always a guarantee of success, as the abortive clinical development of MK801 as a protection against neurodegeneration proved with the NMDA receptor (see, for example, REF. 94). If channels are overactive, such as in epilepsy, it might be worth attempting to target inactivated states (for voltage-gated channels) or desensitized states (for ligand-gated ion channels), rather than simply open channel states.

John Peters. The prospect might not be as bleak as it seems. Selectivity, in a functional rather than strict pharmacological sense, can be influenced by factors other than the molecular structure of the target *per se*, and these can provide a sufficient therapeutic window in some instances. An example is class I antidysrrhythmic drugs, which achieve at least modest selectivity of action through physiological, rather than pharmacological, considerations (for example, REF. 95). These agents block

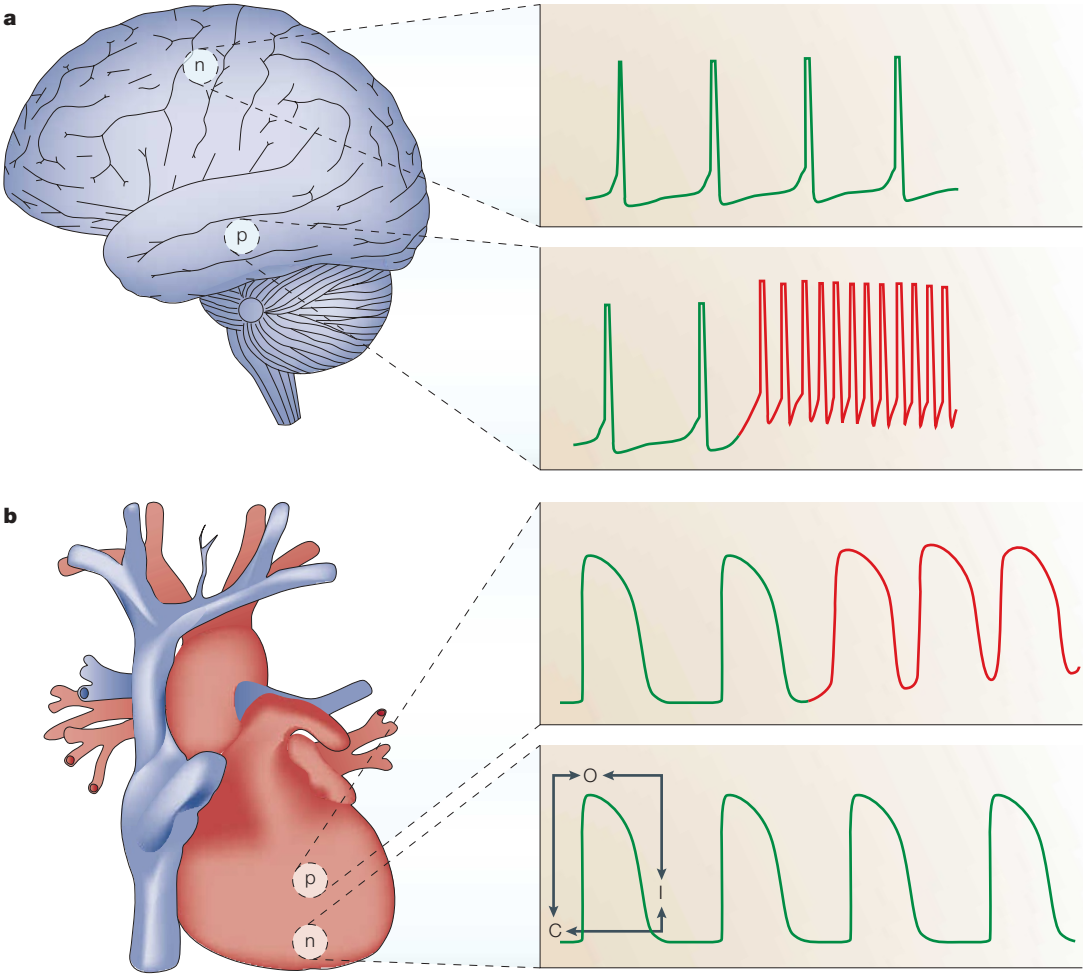

Figure 8 | **Selectivity of drug action can be achieved by blocking, or modulating, particular states of an ion channel.** This figure depicts, in a schematic manner, normal (n, green) and pathological (p, red) electrical activity in the brain (**a**) and the heart (**b**). In the brain, the pathological activity could represent an epileptic focus, whereas in the heart the aberration could underpin a ventricular tachycardia or arrhythmia. In either instance, voltage-dependent sodium channels, and in the heart also calcium channels, mediate the action potential cycling between closed (C), open (O) and inactivated (I) states, as indicated in the lower panel of part **b**. In regions of pathological activity displaying increased impulse frequency, sodium channels will enter open and inactivated states with increased probability. As such, drugs that preferentially block the open state, or modulate the inactivated state, of the sodium channel, such as the anti-epileptic phenytoin, or the antidysrrhythmic lignocaine, selectively target regions of intense activity and suppress high-frequency discharges. Figure prepared by John Peters.

voltage-activated Na^+ channels in a use-dependent manner (the more frequently a Na^+-channel population is activated, the more effectively it is blocked). Basically, this is due to the drug having higher affinity for the open and inactivated conformations of the Na^+ channel than for the resting state. As a consequence, high-frequency excitation of cardiac muscle, as occurs in tachyarrhythmias, can be blocked without seriously compromising normal cardiac rhythm. A similar use-dependent blockade of voltage-activated Na^+ channels underpins the effectiveness of carbamazepine in certain forms of epilepsy and trigeminal neuralgia[96]. Selective distribution of a drug (for example, the active secretion of certain diuretics into the nephron tubule) can also help achieve selectivity of action, even though the ion channel target might have a wider tissue expression.

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What would you describe as the major concerns surrounding the development of ion channel-targeting therapies?

Richard Lewis. Safety and side effects.

David Clapham. The risk of nonspecific interactions with channels in the heart or brain that can potentially cause LQT-type arrhythmias, which might not be detected in animals.

Jamie Vandenberg. With respect to the heart, we need more information about the long-term side effects of ion channel-blocking drugs. The complement of ion channels expressed in different regions of the heart varies with disease states. Furthermore, ischaemia and/or development of cardiac hypertrophy/failure is likely to modify the response to drugs. Thus, a drug that is useful in a given patient at a given time might not be useful (or indeed, could be harmful) a short period of time later.

Michel Lazdunski. There are no more concerns to have with ion channels than with the seven-transmembrane-domain G-protein-coupled receptors, which are favourite targets for drug development in drug companies.

Alan Verkman. Specificity, because ion channels generally have a wide tissue distribution and substantial structural similarity to related family members.

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Jamie Vandenberg gained his B.Sc. in Medical Science and First Class Bachelor of Medicine and Bachelor of Surgery (MBBS) degrees from the University of Sydney, New South Wales, Australia. He received his Ph.D. in Biochemistry from the University of Cambridge, UK, where he worked on the regulation of intracellular pH in the ischaemic myocardium, using nuclear magnetic resonance spectroscopy. His interest in cardiac ion channels developed during a postdoctoral placement with Professor Powell at the Laboratory of Physiology at the University of Oxford, where the main focus of his work was on cardiac chloride channels and the effects of cell swelling on cardiac ion channels. In 1996, he joined the department of Biochemistry at the University of Cambridge, where he worked on the regulation of cardiac ion channel function and expression during cell growth and hypertrophy. His work on cardiac ion channels has been recognized by awards from the British Heart Foundation and the National Health and Medical Research Council of Australia. In 2002, he was appointed Laboratory Head of the Electrophysiology and Biophysics Program at the Victor Chang Cardiac Research Institute in Sydney, Australia, where his work focuses on the structure/function relationships and pharmacology of human ether-a-go-go-related gene (HERG) potassium channels.

Trevor Smart. The development of subtype-selective agents and the effective delivery of these agents to targeted tissues. The latter is often overlooked, although it really is important for all drug-based therapeutics.

Walter Stühmer. The presence of many subtypes with varying functions in many different tissues, and the consequent need to develop very subtype-specific interaction partners. However, the great advantage is that cell-membrane channels are accessible from outside the cell, which is of great importance for any drug.

Dale Benos. Clearly, the ubiquitous expression of certain ion channels will limit the development of therapeutic strategies that target these channels in a specific tissue or organ. However, if targeting molecules can be coupled to another agent that does show tissue specificity, such as an antibody directed against an ion channel-associated protein that in turn is tissue specific, then perhaps overall specificity could be achieved.

Irwin Levitan. Other than the issues of specificity and redundancy of function referred to above (see question 11), a serious issue is the failure rate of ion channel-targeting therapies in clinical trials. A recent K^+-channel opener tested for the treatment of stroke by Bristol-Myers Squibb[97] was based on a rational choice of target and elegant drug design, and was very effective in a rodent model of acute ischaemia, but failed in clinical trials because of lack of efficacy in humans. The reasons for this and other failures remain to be understood.

Birgit Liss. We still do not know enough about the complex functional roles of distinct ion channel subunits. For example, the A-type K_V4 K^+ channel β-subunit KChIP3 has additional functions as a transcription repressor and a presenilin-interacting enzyme (calsenilin). As a K^+-channel β-subunit, KChIP3 increases the surface expression of K_V4 channels and modulates their biophysical properties[98]. But KChIP3 is also identical to DREAM, a transcription repressor that regulates the expression of dynorphin and is involved in modulating pain sensation[99]. Finally, the sequence of KChIP3 is also identical to calsenilin, which regulates the levels of a proteolytic product of presenilin-2. Mutations of presenilin cause a form of early-onset familial Alzheimer's disease[100]. Thus, if KChIP3 is used as a target to modulate the electrical activity of neurons, one might unintentionally affect, for example, pain reception or even neurodegeneration.

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What outstanding questions in ion channel research are likely to be sorted out in the next few years?

Jamie Vandenberg. It is always dangerous to predict the future.

Michel Lazdunski. Many questions have now been solved. The big question now stands at a more complex level, and concerns the coordinated function of ensembles of ion channels in a single excitable cell or in a single dendrite.

Chris Miller. The mechanism of voltage dependence in S4-type channels.

Birgit Liss. The structure/function relationships of ion channels.

Frances Ashcroft. It seems likely that we will see the structure of bacterial equivalents of most channel types, in both the open and closed states.

Sarah Lummis. More detailed structural information, which will allow a better understanding of the molecular details of channel function and modulation.

Michael Sanguinetti. The structural basis of ion selectivity, permeation and gating in response to changes in transmembrane voltage or ligand binding.

David Clapham. The mechanisms of Na^+, Ca^{2+}, K^+ and Cl^- ion selectivity; the gating mechanism of voltage-gated ion channels (S4 movement); the regulation of ion channels by associating proteins; and the mechanisms for targeting ion channels to specific regions of a cell, or to specific tissues.

Irwin Levitan. More ion channel structures are likely to become available as this field matures and more laboratories contribute to it. Time-resolved structural analysis is likely to provide an understanding of the protein conformational changes responsible for channel gating. In my own area of channel modulation, I believe that structural approaches will begin to provide insight into detailed molecular mechanisms of modulation.

Trevor Smart. Likely gains will be better resolution of the structure/function relationship, identification of the intracellular protein framework that links to ion channels and the realization that this offers another (but more difficult) set of therapeutic targets.

Alan North. Cell-based high-throughput screening; structures of a wider range of channel families, including atomic understanding of permeation and gating mechanisms; cell biology of channels, including trafficking and assembly of channel complexes; local chemical (as distinct from electrical) signalling by channels; and changes in channel expression and function during development and disease states.

Alan Verkman. Regulation of ion channel function and targeting by protein–protein interactions is a developing paradigm. For example, significant new understanding about regulation is likely to come through analysing PDZ-BINDING DOMAINS and interactions with the actin cytoskeleton. In addition, rapid advances in ion channel high-resolution structure/function are anticipated, with increasing emphasis on the time domain to obtain useful molecular dynamics models of channel gating.

Richard Lewis. Crystal structures of ion channels of clinical relevance will start to be produced. These will provide much-needed templates for accurate pharmacophore mapping and *in silico* screening. Such models will provide insights into how to modify lead structures rationally to introduce novel elements that can improve potency and/or selectivity. All the key auxiliary proteins associated with the main pore-forming α-subunits of clinically relevant ion channels will be identified. The role of these modulator proteins in disease and their influence on pharmacology will start to be fully appreciated.

Kenneth Chien. We need to understand more about how human physiological diversity, which is extensive, interplays with the activity of specific channels to create susceptibility to specific human diseases, such as arrhythmogenesis. I believe channel biology holds great promise for understanding this diversity, as it represents the most exquisite way to quantitatively and qualitatively monitor function. The heart could be a great system for this as new human-based systems are developed, particularly if it becomes possible to drive human-based cardiac progenitors into forming differentiated, beating cardiac muscle cells over the next several years.

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Alan Verkman received undergraduate degrees in Biology and Physics from the Massachusetts Institute of Technology in Boston, Massachusetts, USA, a Ph.D. in Physics from Harvard University in Boston, and an M.D. from Harvard Medical School. His clinical training in Internal Medicine was done at the Brigham and Women's Hospital in Boston and in Nephrology at the University of California, San Francisco (UCSF). Verkman has remained at UCSF, and is now Professor of Medicine and Physiology and Senior Scientist in the Cardiovascular Research Institute, as well as Director of the Cystic Fibrosis Research Development Program. He directs a large research group funded by five National Institutes of Health (NIH) grants, including a MERIT award, and two programme grants from the Cystic Fibrosis Foundation, including a drug discovery grant. He has authored more than 300 journal articles and 60 reviews, and is a recognized authority in membrane transporter physiology and molecular genetics. Professor Verkman's research is focused on the biology of aquaporin water channels and the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel in cystic fibrosis, and the development of novel fluorescence methodology to study diffusion and protein–protein interactions in living cells. He has established a unique academic drug discovery programme with in-house resources to carry out high-throughput screening, combinatorial chemistry and small-animal pharmacology and efficacy testing. In drug discovery, his recent focus is on the development of CFTR inhibitors as antidiarrheals, and on activators of mutant CFTRs for treating cystic fibrosis.

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What areas are unlikely to be clarified by research over the next few years?

David Clapham. A detailed understanding of the role of ion channels in the CNS.

Michael Sanguinetti. The molecular and cellular basis of brain function is too complex to be solved in the near future.

Trevor Smart. The link between ion channels and disease phenotypes. There is still much more fundamental research required before the link between 'cause and effect' can be clearly established (see question 3).

Birgit Liss. The plasticity of ion channel expression, function and interaction.

Irwin Levitan. Questions relating to cellular physiology, which have been given short shrift in recent years, are likely to take a long time to address. In particular, the roles of specific subsets of ion channels in regulating membrane excitability will have to be worked out in order to predict the effects of pharmacological agents that target ion channels.

Frances Ashcroft. Precisely how mutations in some specific ion channels cause human disease may continue to elude us, as may the correlation between genotype and phenotype. Polygenic diseases that result from impaired channel regulation will also be difficult to study, because large cohorts will be needed for both genetic and functional studies in order to achieve significance.

Kenneth Chien. I believe that the development of new drugs that directly target the channels themselves will be an increasingly difficult proposition because of neuronal and cardiac side effects. As I mentioned earlier (see question 1), the hope is that integrating a more signalling-based approach to understanding channel function and disease will open the door to influencing channel function indirectly by targeting points in the regulatory pathways that control channel function.

Richard Lewis. A clear and detailed understanding of the role played by ion channels in animal models and humans. The crystal structure of each class of ion channels will be determined, preferably with bound ligands that define the pharmacophore for drug interaction.

Michel Lazdunski. Probably none in the next five to ten years; I am optimistic that many areas will be clarified.

Alan North. Don't be a pessimist!

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Competing interest statement

Some of the authors declare competing financial interests: see Web version for details.

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