Ion channels and stem cells

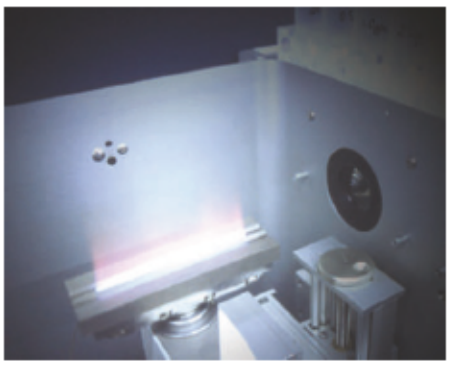
Ion channels, stem cells and cell signalling are the focus of intense interest in both cell biology and drug discovery. Pete Moore takes a look at what's on offer for the researcher.

Ion channels act as electrical gatekeepers in cell membranes, and are responsible for the generation and propagation of nerve impulses, muscle contraction, and many other biological processes. With more than 400 ion-channel genes identified in the human genome, interest in detecting and measuring their activity is burgeoning.

A high-throughput method of assessing the function of outward-rectifying potassium channels is to monitor the flow of tracer ions through them. In the case of potassium channels, rubidium ions (Rb^+) are used as a tracer because Rb^+ has similar characteristics to K^+ but is not present in biological systems and so there is no background noise. Trace amounts of Rb^+ (as low as 0.05 mg l^{-1}) can be detected using flame atomic absorption spectroscopy with the Ion Channel Reader (ICR) from Aurora Biomed of Vancouver, British Columbia. The ICR can be used to study voltage- and ligand-gated potassium channels as well as sodium channels and chloride channels.

Another way of studying ion-channel activity is to monitor changes in membrane potential. Invitrogen of Carlsbad, California, and PerkinElmer of Boston, Massachusetts, have

AURORA BIOMED



Aurora's Ion Channel Reader measures Rb^+ flow.

recently joined forces to offer a combination of Invitrogen's Voltage Sensor Probes ion-channel reagents and PerkinElmer's CellLux Fluorescence Cellular Screening Platform. This assay is based on fluorescence resonance excitation transfer (FRET); it uses a coumarin-phospholipid FRET donor that binds to the exterior of the cell membrane and a negatively charged FRET acceptor. In resting cells the two probes associate with the membrane exterior, resulting in efficient FRET and a red fluorescence signal. When a cell becomes depolarized as ions flow through channels, the FRET acceptor rapidly translocates to the other membrane face. Exciting the donor probe now generates a blue fluorescence signal.

Tracking channels

Ion-channel localization can affect cell function dramatically, and ChanTest of Cleveland, Ohio, offers antibody-based tests for detecting intracellular ion-channel trafficking. "In cystic fibrosis, 50% of families have a defect that prevents the CFTR channel protein being transported to the cell surface, and for the hereditary form of the hERG disease, about half of the mutations in the hERG channel protein affect trafficking," says ChanTest's chief executive officer Arthur 'Buzz' Brown. Blocking the function of the hERG potassium ion channel in cardiac muscle may be a major adverse drug effect as it can cause arrhythmia and sudden cardiac death, and all new drugs must be tested for whether they block this channel. In ChanTest's HERG-Lite assay, human embryonic kidney (HEK) cells express a version of the hERG channel carrying a hemagglutinin epitope. Protein turnover replenishes hERG channels about every 12 hours, so the cells are incubated overnight with

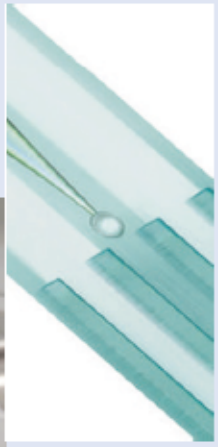
MAXIMIZING RETURN

CELLECTRICON

Although most patch-clamp technologies seek to maximize the number of cells rushed through the system, Owe Orwar and his colleagues at Cellectricon, a start-up company based in Gothenburg, Sweden, have developed a platform that maximizes the information gained from each cell. The result is a powerful tool for secondary screening in drug discovery. Their Dynaflow technology uses conventional glass pipette patch clamping, in combination with a novel microfluidic device for controlled delivery of drug solutions.

Solutions of drugs or drug combinations are placed in up to 48 wells, each of which is connected to a measurement chamber by micrometre-diameter channels. These solutions can be directed through the chamber with high precision. "Dynaflow uses the unique properties of fluids when they are running at very low Reynolds numbers. When the fluids come out from a tiny channel in the open volume they behave as if they are still in channels — they do not mix," says Orwar.

Cellectricon's Dynaflow patch-clamp system combines microfluidics and patch-clamping technology.



With no turbulence, diffusion would be the only chance of mixing between solution batches, but the timescales used are too short for that to occur. Consequently, Dynaflow can provide step changes in drugs or drug concentrations, with a change every 30 milliseconds if desired. "It is the most precise technology in the world to titrate receptors," claims Orwar. "You can see it as a microfluidic device that generates a barcode of chemicals, and the cell effectively reading the barcode," he adds.

The ability to squeeze so much data out of a single cell enables some users to claim a ten-fold increase in productivity. By using carefully considered combinations of drugs in each well, cells can be taken through physiologically relevant conditions that relate to many different disease states. "In effect, it gives you the option of passing a chemical waveform over the cell while constantly recording from it," says Orwar. P.M.