Biophysics

Dynamic ion channel defies dogma

Ute A. Hellmich

It is well established that proteins in the TRP family of ion channels assemble from four subunits. But do they always do this? A five-subunit structure has now been observed, and might be involved in channel regulation. See p.206

Receptor proteins in cell membranes are responsible for sensing signals such as temperature, pressure and the compounds that give foods their pungency – such as the 'heat' of spices or the 'coolness' of mint. A prominent group of receptors are the ion channels, which include the transient receptor potential (TRP) channels. The first high-resolution structure of a TRP channel was reported[1] in 2013 and hundreds more have since been determined[2], establishing a common core architecture: four subunits assemble to form a central pore that dilates or constricts in response to stimuli, thus modulating ion flux across cellular membranes. However, on page 206, Lansky *et al.*[3] now provide the first evidence that TRP channels can also form from five subunits – prompting the question of whether these pentameric structures are involved in a previously unknown layer of TRP-channel regulation.

The fact that proteins can form structurally well-defined complexes composed of multiple subunits, arrangements referred to as protein quaternary structures, was first demonstrated by Theodor Svedberg in 1926 using his own invention: the ultracentrifuge[4]. Subsequent seminal work by Jacques Monod and others established that the quaternary structure not only is important for protein function and stability, but also has a key role in regulating protein activity[5,6]. Remarkably, nature seems to strongly favour complexes that have an even number of subunits, particularly dimers (two subunits) and tetramers (four subunits)[6]. By contrast, only about 0.6% of all experimentally determined protein quaternary structures are pentamers (http://pqs.ebi.ac.uk/).

But is the number of subunits of a protein always set in stone? The idea that the quaternary structures of soluble proteins are not static has long been described – for example, the subunits of enzymes can dissociate on binding to a small molecule[7]. In the current study, Lansky *et al.* looked at the dynamics of subunit assembly for TRP vanilloid 3 (TRPV3) – a TRP channel that is activated by heat, and by herbs such as thyme and oregano. The channel is involved in hair development, wound healing and in the resilience and sensory perception of skin[8].

The authors used a technique called high-speed atomic force microscopy (HS-AFM), which allows individual proteins to be monitored under near-physiological conditions and in membranes[9]. This revealed the co-existence of TRPV3 tetramers and pentamers in lipid bilayers. Strikingly, the tetrameric and pentameric channels interconvert continuously by exchanging subunits, which diffuse through the surrounding membrane.

Ion flux across cell membranes is tightly regulated. On stimulation of an ion channel, the pore typically opens briefly, and just wide enough to allow the ions of choice to pass. Dysregulation of ion channels can lead to cell damage or death, a mechanism that often underlies severe diseases[10]. For example, a process known as pore dilation occurs under certain conditions, causing the permeability of ion channels to increase greatly – thereby allowing many ions and molecules with molecular masses of up to hundreds of daltons to pass through[11].

TRPV3 also undergoes extreme pore dilation when treated with a small molecule known as diphenylboronic anhydride (DPBA), resulting in a loss of ion-flux directionality and an increase in permeability to large cations. This pore dilation is established and maintained on timescales of seconds to minutes, and it was originally thought that it must occur in tetrameric TRP channels[11,12]. Lansky *et al.* observed that the equilibrium between the tetramers and pentamers in their HS-AFM experiments is modulated by DPBA (Fig. 1),

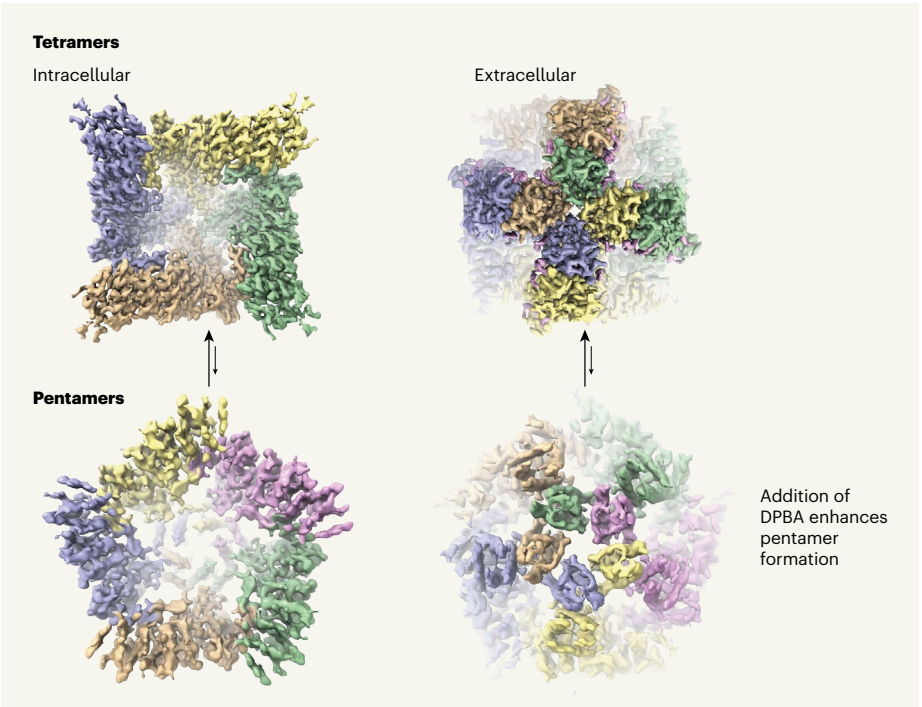

Figure 1 | TRPV3 ion channels can assemble from either four or five subunits in membranes. Lansky *et al.*[3] used high-speed atomic force microscopy (HS-AFM) to observe that the TRPV3 protein interconverts between tetrameric (four subunits) and pentameric (five subunits) assemblies in lipid membranes by exchanging subunits, which diffuse through the membrane. The authors then obtained structures of the assemblies using cryo-electron microscopy, shown here as they would be seen from inside and outside the cell (intracellular and extracellular views, respectively). The small molecule diphenylboronic anhydride (DPBA) is known to dilate the pore of TRPV3 ion channels in cells[11,12], a slow process that lasts seconds to minutes. Lansky and colleagues' HS-AFM experiments show that DPBA promotes the formation of TRPV3 pentamers through diffusive subunit exchange on the same timescale as that of DPBA-dependent pore dilation, and that pentamers have a much wider pore than the previously known tetramers – consistent with the idea that pentameric assemblies have a role in pore dilation.

which causes the fraction of pentameric ion channels to increase on the same slow timescale as is observed when pore dilation occurs.

The authors also used cryo-electron microscopy (cryo-EM) to obtain a structure of pentameric TRPV3 with a resolution of 4.4 ångströms. Not all of the details can be resolved, but the structure clearly shows that the pore of the ion channel at its narrowest constriction point is more than 8 Å across — much larger than the pore in the open tetramer, which is about 3.5 Å in diameter at its narrowest point. Taken together, the authors' findings show that TRPV3 pentamers meet both the temporal and architectural criteria associated with pore dilation of these channels.

So why have pentameric TRP channels not been observed previously? The answer could be that expectations often drive observations: that is, the overwhelming success story of the structural biology of TRP channels might have shaped scientists' expectations of what these channels look like.

Furthermore, the protocols used to prepare proteins for structural-biology experiments typically include a final step in which the proteins are separated by their size and shape. The expectation that proteins will form a complex from a particular number of subunits could therefore have resulted in protein species with higher molecular weights being discarded as possible contaminants or non-functional aggregates; removing such material is generally regarded as good biochemical practice. In contrast, Lansky and co-workers were specifically looking for DPBA-enriched pentamers as a result of their HS-AFM experiments, highlighting the need for structural biologists to use complementary methods that account for the highly dynamic nature of TRP channels[2,3,9].

It remains to be seen whether the ability of TRPV3 to exchange subunits is universal among TRP channels and whether researchers can now retroactively identify pentamers in their cryo-EM data sets — possibly even for non-TRP channel types that undergo pore dilation[11]. Perhaps subunit exchange will add another layer of complexity to the already complicated array of processes known to regulate ion-channel activity.

Further studies are now needed to verify the existence of pentameric TRP channels in cells, and to determine the role of naturally occurring molecules and lipids in promoting pentamer formation. The energetics and structural changes associated with subunit release from, and re-incorporation into, protein assemblies also warrant investigation. In the meantime, Lansky and colleagues' study shows that there is still much more to learn about the proteins that spice up our lives.

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Metabolism

A road less travelled for lipid synthesis

Jean E. Schaffer

The pathway used by mammalian cells to make triglyceride lipids when supplies of fat molecules are high has long been known. A route that works when fat supplies are low has now been discovered. **See p.171**

Triglycerides are key lipids used in the transport of fatty acids through the bloodstream in animals, and serve as the most efficient medium for energy storage in both animal and plant cells. In humans, accumulation of triglycerides in lipid droplets in non-fat tissues is commonly observed in lipid-overloaded states, such as obesity-related metabolic syndrome and type 2 diabetes. It can also be associated with cardiovascular disease[1] and non-alcoholic steatohepatitis[2] (a disease of the liver). Thus, the characterization of molecular pathways for triglyceride synthesis and their regulation is crucial for understanding human physiology and disease. On page 171, McLelland *et al*.[3] uncover a previously unknown pathway for triglyceride synthesis in mammalian cells.

Triglycerides are complex lipids composed of a glycerol molecule linked to three fatty acids. Given the many possible combinations of fatty acids of differing length and saturation and the three positions to which they can bind on glycerol, a vast number of triglyceride molecules can potentially be made.

Since the first description[4] of the enzymatic synthesis of triglycerides in liver extracts in 1960, two enzymes that catalyse the final step of the synthesis have been extensively characterized in mammalian cells, mice and humans[5] (Fig. 1a). Known as DGAT1 and DGAT2, these 'acyltransferase' enzymes add a fatty-acid derivative (a fatty acyl-CoA molecule) to a diacylglycerol — a glycerol molecule that already has two fatty-acid-derived groups (fatty acyl chains) attached to it. DGAT1 is embedded in the membrane of the endoplasmic reticulum, the cellular organelle that acts as a main site of lipid and protein synthesis, and also catalyses the reaction of acyl-CoA molecules with long-chain alcohols, vitamin A and monoacylglycerols (glycerol molecules with just one fatty acyl chain attached). DGAT2 localizes to both the endoplasmic reticulum and to growing lipid droplets and is involved mainly in triglyceride synthesis.

Mice that have been genetically engineered to have non-functional DGAT enzymes have striking traits (phenotypes), providing strong evidence that triglyceride-synthesis pathways involving DGATs are crucial for regulating lipid levels in mammalian tissues[6,7]. Other pathways for triglyceride synthesis that are independent of fatty acyl-CoAs, and which instead use phospholipid molecules as fatty acyl donors, have been described in yeast and plants[8]. But whether alternative routes for triglyceride synthesis exist in mammals has been largely unexplored — until now.

McLelland and colleagues used a method known as sequential CRISPR loss-of-function screening to identify one such alternative pathway in a human cell line (HAP1 cells). To search for pathways that are independent of known DGATs, they engineered HAP1 cells to be deficient in both DGAT1 and DGAT2. The