Ion channels

How to be desensitized

Christian Rosenmund and Michael Mansour

The various functional states of glutamate receptors control much of the brain's neuronal activity. Our understanding of how one of those states — desensitization — occurs has taken a leap forward.

Neurons communicate with each other through synapses, which operate in the following way. On one side of the synapse, the incoming neuron converts electrical activity, encoded in action potentials, into a chemical signal by releasing a neurotransmitter. The membrane of the receiving, postsynaptic, neuron is packed with ion channels, which transduce neurotransmitter binding into complex conformational changes that induce channel opening and closing, a process known as gating. Channel gating translates the concentration profile of neurotransmitter in the synapse into a defined time course of ion flow across the postsynaptic neuronal membrane, changing the membrane potential accordingly and, usually, resulting in onward transmission of the presynaptic action potential. The most important class of neurotransmitter-gated ion channels conveying excitatory signals in the brain are the AMPA-type glutamate receptors. On page 245 of this issue, Sun *et al.*[1] provide us with exciting snapshots of the gating process involved.

Like many other ion channels, AMPA receptors are doughnut-shaped structures, formed by the assembly of different members of the family of AMPA-receptor subunits[2], that span the insulating neuronal membrane, with an ion-conducting water-filled pore down the centre. The pore is controlled by a gate, which 'opens' to permit ion flow only when the neurotransmitter — glutamate — binds to the receptor. However, AMPA receptors share with other ion channels a property known as 'desensitization', which limits the effective ion flow to the few milliseconds following binding of glutamate. This is because the channel tends to close even though neurotransmitter is still bound to it. This process has a considerable influence on how AMPA receptors encode information, as the degree of desensitization is a major factor in shaping the time course of postsynaptic responses[3]. Moreover, the degree of desensitization of the AMPA receptor is under exquisite control in various ways, such as regulated gene expression, RNA editing, alternative splicing of receptor-encoding RNAs, and the assembly of receptors from different subunits[2]. Despite the great importance of desensitization in brain function, however, little is known about how AMPA-receptor subunits coordinate gate opening and desensitization.

The extracellular glutamate-binding portion of each subunit comprises two protein domains. Earlier studies[4] provided the crystal structure of these domains, which can best be described as resembling a clam shell. On binding glutamate, the cleft between the two shells (the two domains) narrows by the movement of the second domain towards the first[5]. This may directly trigger the opening of the gate, as amino-acid residues that link domain 2 to the plasma membrane are pulled away from the pore-forming region of the receptor. Because AMPA receptors are tetramers, and because all subunits are able to bind glutamate, they may unite in a stepwise fashion in the attempt to open the gate[6]. An unusual structural feature is that individual glutamate-binding pockets of AMPA receptors pair up to form dimers[5]. The dimer interface, which is formed by a twofold symmetric assembly through an interaction at the domain 1 interfaces, is packed with residues that change receptor desensitization dramatically if structurally modified[3], indicating that the dimer interface may be involved in desensitization.

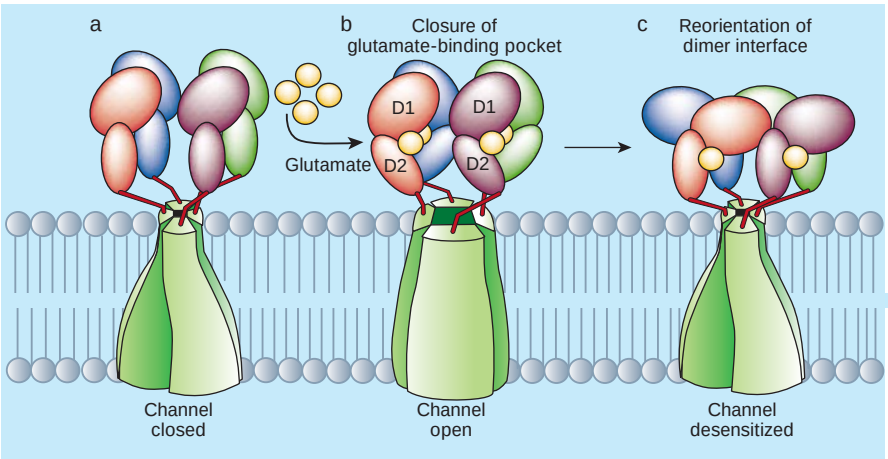

Figure 1 **Activation and desensitization of AMPA-type glutamate receptors.** a, **The glutamate-binding domains are organized as a 'dimer of dimers', with the red and blue glutamate-binding cores forming one dimer and the purple and green subunits forming the other. The two dimers sit side-by-side so that the inter-dimer local twofold axis is in the centre of the four subunits and coincides with the fourfold axis of the ion channel.** b, **In the scheme of events proposed by Sun *et al.*[1], binding of glutamate (yellow particles) induces domain closure at the glutamate-binding core: movement of domain 2 (D2) towards domain 1 (D1) causes conformational changes at the channel gate and opens the channel.** c, **Reorientation of the dimer interface decouples glutamate-binding-induced domain closure from the channel gate, and leads to receptor desensitization. (Modified from a graphic provided by Y. Sun.)**

Next, with the aim of revealing the structural basis of the dimerization process, Sun *et al.* compared the crystal structures of mutant receptors that had dramatically different desensitization properties. The increased dimer affinity of the non-desensitizing mutant can be nicely explained by the formation of a strong hydrophobic pocket in the dimer interface. An equivalent effect can be seen in crystals of normal — wild-type — desensitizing receptors in the presence of cyclothiazide.

So what does the desensitization process look like? The authors' view of events is shown in Fig. 5 of the paper (page 251) and in Fig. 1 here. Activation of the receptor by glutamate, and subsequent movement of domain 2, produces tension on the

connecting portion between the extracellular binding pocket and the receptor's transmembrane domains. This leads to conformational changes within the membrane, forcing the gate to open. But in consequence there is an equivalent strain on the glutamate-binding pocket, facilitating an ensuing conformational rearrangement to escape tension. This causes rearrangement at the dimer interface and movement of the receptor into the desensitized state.

In receptors that do not desensitize, the strengthened dimer interface prevents rearrangement within the ligand-binding pocket. The linker between binding pocket and gate remains under tension and leaves the pore open as long as glutamate is bound. It is therefore no surprise that the glutamate-binding domain of the wild-type receptor and that of the non-desensitizing mutant both crystallize into the same 'open-channel' state, because the link, and therefore the tension force, from the membrane is missing.

Finally, Sun *et al.* surprise us with crystal structures of a mutant receptor that was designed to disrupt the dimer interface. The accelerated desensitization properties of the intact mutant receptor nicely confirm the importance of dimer affinity in desensitization. As expected, the structure does not dimerize at the usual interface. But it does reveal another, previously unknown, subunit interaction — hinting at a possible way in which pairs of dimers can assemble into a tetramer. In this interaction, domain 1 connects with a different region (lateral to that involved in dimerization) of domain 2 of a neighbouring receptor. If this interaction indeed takes place in the assembled receptor complex, the tetrameric structure represents a pair of dimers that are shifted parallel to one another.

This would be an unprecedented form of channel subunit assembly — one in which the dimers facing each other follow a different symmetry from the assumed (though not proven) fourfold symmetry of the receptor in the transmembrane channel pore. This arrangement may, however, provide a positional clue to how different isoforms of AMPA-receptor subunits assemble into a complex *in vivo*[8], as chemically equivalent subunits may prefer to assume positions of equivalent symmetry, namely diagonally across from each other. It is yet not clear how these arrangements would affect receptor gating. But it is clear that glutamate receptors are likely to remain a rich source of surprises. ■

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Developmental biology

Signalling polarity

Randall T. Moon and Kavita Shah

Cell signalling pathways triggered by Wnt proteins control gene expression and cell behaviour, especially during development. Work on frogs reveals another specific role for these proteins, and the signalling involved.

Living organisms tend to be highly economical, using the same proteins or cell-to-cell signalling processes in different contexts and for different purposes. Take, for example, the family of proteins known as NFATs. These are transcription factors — they regulate gene expression — and they have traditionally been implicated in controlling genes involved in immunity. The events leading up to such regulation include an increase in the levels of calcium ions in a cell; the NFAT transcription factors then move into the nucleus and form a complex with relevant regions of DNA (and, in some cases, with other transcription factors[1]). Several different signalling pathways are known to activate NFAT by changing calcium levels, and to this list can now be added one of the pathways that is triggered by Wnt proteins, as Saneyoshi and colleagues[2] describe on page 295 of this issue. Rather than being involved in immunity, however, these events are key to early vertebrate development.

Members of the Wnt protein family are secreted from cells and picked up by receptor proteins on the surface of cells, triggering intracellular signalling pathways. These pathways regulate cell proliferation, death, fate and behaviour in contexts ranging from early embryonic development to colorectal cancer. The best-understood Wnt pathway is the 'canonical' one: here, Wnt-induced signalling suppresses degradation of the β-catenin protein, enabling it to accumulate in the nucleus[3]. Nuclear β-catenin then binds to particular transcription factors (not the NFATs) and thereby modulates gene expression.

Over the past few years it has become apparent that certain Wnt proteins and their receptors (proteins of the Frizzled family) can also activate 'non-canonical' pathways. Although the details of the canonical Wnt/Frizzled pathway are firmly supported by genetic, biochemical and cell-biological experiments in many species, the non-canonical pathways are less well understood. Nonetheless, it is known that a non-canonical 'planar cell polarity' pathway is used in fruitflies to orientate cells[4].

There also seems to be more than one

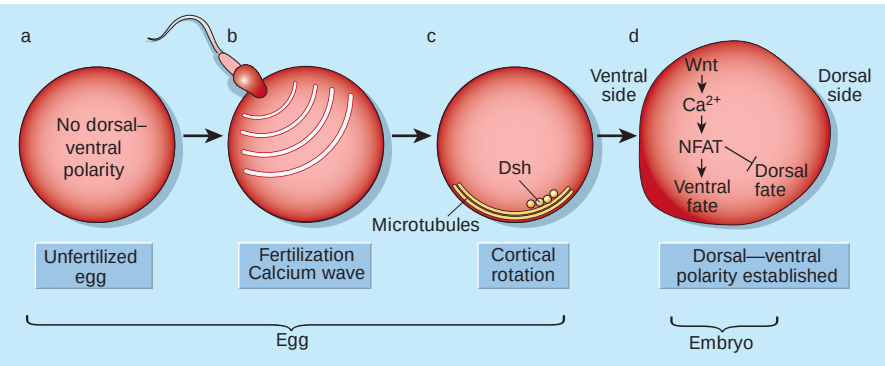

Figure 1 **Opposing signalling pathways, triggered by different members of the Wnt protein family, may help determine the polarity of *Xenopus* embryos.** a, **Before fertilization, there is no evident distinction between the dorsal and ventral sides of the egg.** b, **Fertilization by a sperm anywhere in the upper half of the egg initiates a wave of Ca^{2+} ions, which originate in what will become the animal's ventral side.** c, **During the first hour after fertilization, the cortex (the plasma membrane and associated structures and molecules) rotates by about 30° relative to the inner cytoplasm. The Dishevelled (Dsh) protein, a downstream target of 'canonical' Wnt pathways, moves along filamentous tracks (microtubules) to the future dorsal side, where it will ultimately result in stabilization of the β-catenin protein in cell nuclei, leading to transcription of relevant genes.** d, **Saneyoshi *et al.*[2] predict that sometime between fertilization and the establishment of dorsal–ventral polarity, NFAT is activated in response to Wnt-triggered rises in Ca^{2+} levels in cells on the future ventral side. This promotes ventral cell fates, and antagonizes the signal that promotes dorsal cell fates.**