Transmembrane Signalling: Membrane Messengers

Membrane messengers

Life has evolved elaborate means of communicating essential chemical information across cell membranes. Inspired by biology, two new artificial mechanisms that relay chemical signals across lipid membranes have now been demonstrated using minimal synthetic messenger molecules.

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Cell membranes protect the microreactors of life from harmful substances while enabling the transport of biomolecular building blocks and the establishment of electrochemical gradients for energy transduction. Such transmembrane flux is facilitated in living organisms by specialised protein channels and carriers. For example, nerve cells communicate with each other via small-molecule neurotransmitters that bind to, and open protein channels thereby modulating the transmembrane voltage (Fig. 1a). However, other membrane-spanning proteins such as kinases (Fig. 1b) and G-protein-coupled receptors (GPCRs, Fig. 1c) facilitate signal transduction without the transmission of any ions or molecules across the membrane. Inspired by such biological transmembrane processes, chemists have already established synthetic systems that mimic channels, carriers, and kinase signalling (Figs. 1a-b).[1-3] Now, two independent teams of researchers have shown that chemical signals can be communicated across lipid membranes by two new synthetic mechanisms, as reported in the current issue of *Nature Chemistry*.[4-5]

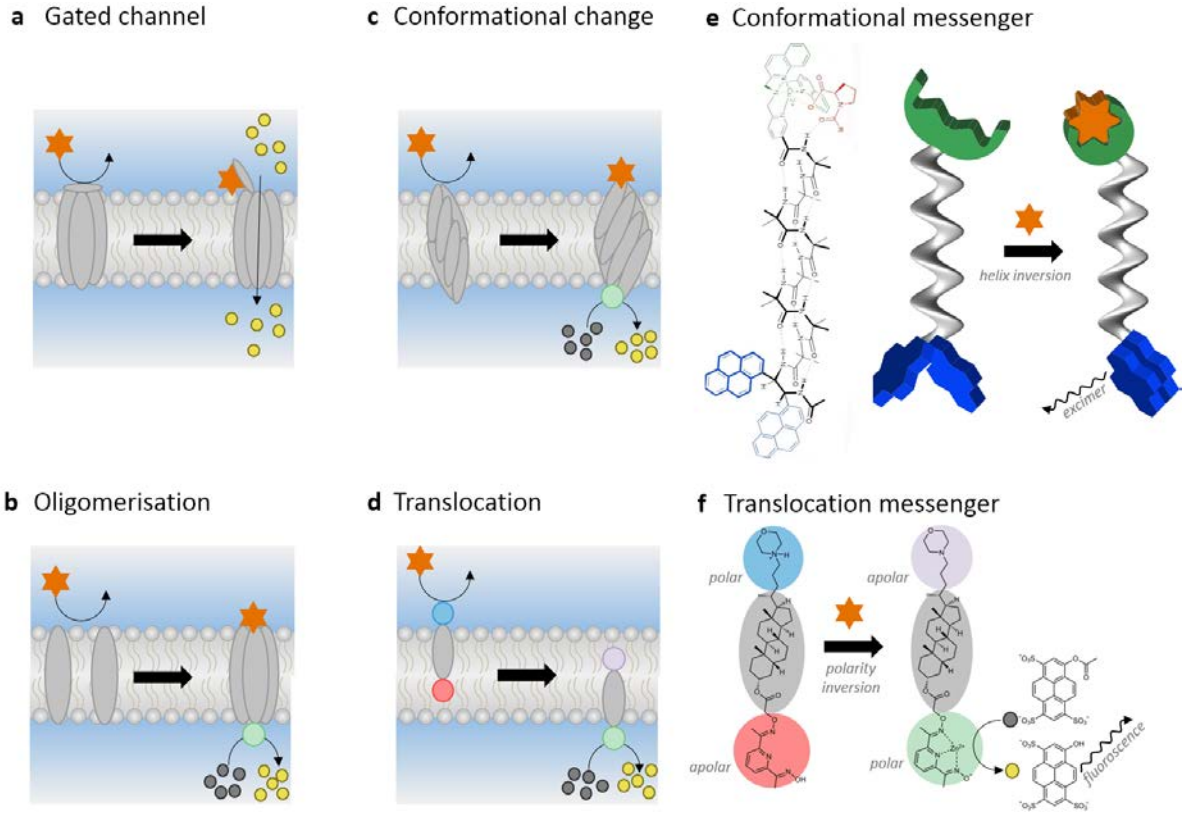

Figure 1 | Transmembrane signalling mechanisms. Mechanisms (**a**) to (**c**) have been established in biological and synthetic mimics, while mechanism (**d**) has only recently been demonstrated in an artificial system (**f**).[5] A chemical stimulus (star) may trigger signal transduction by the opening of a channel (**a**) (e.g. acetyl choline receptors in nerve cells), or the creation of a catalytic binding site (green) via aggregation (**b**) (e.g. kinase signalling) or a conformational change (**c**) (e.g. GPCR signalling). Signal amplification occurs by changes in the transmembrane potential (**a**) or the catalytic turn-over of a substrate (grey circles to yellow circles (**b** to **d**). (**e**) A synthetic conformational messenger that operates via ligand-triggered helix inversion to give a fluorescent excimer signal.[4] (**d** and **f**) A synthetic messenger that responds to a pH change (light blue to purple) facilitating translocation from the outer to the inner leaf of the membrane and generating a catalytic site (red to green) for fluorescent signal amplification (grey circles to yellow circles) on the opposite side of the membrane..[5]

Webb, Clayden and co-workers set out to mimic the mechanism of biological GPCR signal transduction using a minimal synthetic system (Fig. 1c).[4] The GPCR superfamily of proteins communicate across cell membranes via conformational changes induced by the binding of a diverse range of ligands; from flavour and scent molecules to hormones and neurotransmitters. These conformational changes in the receptor modulate the binding to proteins on the other side of the membrane, thereby amplifying a single binding event by activating various enzyme-catalysed cascades within the cell. Indeed, such revelations led to the award of the Nobel prize for Chemistry to Lefkowitz and Kobilka in 2012. Webb and Clayden's team hypothesised that a peptide-like oligomer that can fold into a helical conformation could be used to communicate conformational change across a membrane (Fig. 1e). The team synthesised such oligomeric 'foldamers' with the appropriate dimensions to span the ~3 nm width of the bilayer. A Cu (II) complex was appended on one end of the oligomer, while a pair of pyrene fluorophores was attached to the other. The molecule was designed such that the formation of a left-handed helix resulted in stacking of the terminal pyrene groups to form a spectroscopically distinct excimer complex. Extensive studies of the foldamers in organic solution showed that specific chiral carboxylate input ligands bound to the Cu (II) receptors with sub-micromolar affinity inducing either left- or right-handed helices that propagated along the full length of the foldamer (Fig. 1e). When the foldamers were administered to vesicles, changes in the fluorescence of the pyrene moieties indicated that the oligomers had inserted into the lipid membrane. Most strikingly, the team found that adding water-soluble carboxylate ligands known to bind the Cu (II) receptor resulted in changes in the excimer-response. Binding of the D-proline and L-proline-derivatives to the Cu (II) receptor gave opposing changes in the excimer response indicating that the different enantiomers induced different chiral helicities in the membrane-soluble oligomer region. Comparison of the excimer intensity of the pyrene fluorophores with those observed in organic solvents suggested that the pyrene moieties lie within the membrane but close to the interface with water. Since the charged Cu (II) receptor site is exposed to the aqueous phase then the implication is that binding of specific signalling molecules induces a helical conformational change, which communicates the binding event to the pyrene fluorophores positioned some ~3 nm away, likely on, or close to the opposite side of the membrane. Further investigations are underway to examine whether this mechanism of communication via intramolecular conformational change can be used to trigger responses within vesicles and living cells.

Not content with mimicking biology, Williams, Hunter and co-workers set out to develop an entirely new, artificial mechanism of transmembrane signal transduction (Fig. 1f).[5] In their system, the synthetic messenger molecule was based around a membrane-soluble steroid core (grey in Fig. 1f) to which was appended a basic morpholine headgroup on one end (blue/purple in Fig. 1f), while on the other was attached a neutral tetradentate pyridyl-oxime ligand (red in Fig. 1f). In contrast to Webb and Clayden's system, the messenger molecule shown in Fig. 1f is not long enough to fully span the membrane. Instead, the signalling mechanism depends on the ability of the messenger to be controllably switched between being localised in either the inner or the outer leaves of the membrane (Fig. 1d). When the solution outside a membrane-bound vesicle had a pH less than 8, the morpholine headgroup is charged due to protonation (blue) and is therefore prevented from partitioning through the apolar interior of the membrane (Fig. 1d, left). Upon the addition of a basic input signal that raises the pH, the morpholine headgroup is deprotonated (blue to purple) allowing the messenger molecule to partition between the outer and inner leaves of the lipid bilayer. Zn^{2+} ions were present in the aqueous solution inside the vesicles, which bind strongly to the pyridyl-oxime head group (red to green), which prevents the now polar headgroup from shuttling back to the outer leaf of the membrane. In addition to playing a role in polarity inversion of the headgroups, the Zn (II)-complex formed catalyses the hydrolysis of the ester groups to afford a fluorescent pyrene dye within the vesicle (grey to yellow circles in Fig. 1f). Thus, the team demonstrated that a chemical signal occurring on one side of the membrane (change in pH) could be transduced into a catalytically amplified response on the opposite side of the membrane.

Together, these independent investigations published in the current edition of *Nature Chemistry* demonstrate how biological processes can inspire the construction of novel functional chemical systems. Biology both created and solved the problem to transmembrane signalling billions

of years before chemists. However, the contrast between biology and technology is starkly underscored by comparison of the evolutionary solution to locomotion versus the minimal human invention of the wheel. The development of minimal biomimetic systems is both important and useful because synthetic chemistry allows for transferrable, functional components that can be readily repurposed and redesigned to suit man-made technological specifications. Indeed, this aspect is exemplified in the context of the present investigations by the recent synthesis of a light-switchable (rather than ligand-switchable) variant of Webb and Clayden's membrane-bound helical switch (Fig. 1e).[6] Moreover, there is also substantial potential for these synthetic membrane messengers to be employed within biological systems to facilitate communication with living cells, or the development of semi-synthetic living systems that blur lines between the synthetic and the biological. Indeed, such lines are already starting to blur; both the helical oligomer and the steroid moieties that lie at the core of the aforementioned synthetic messengers are themselves repurposed from biology. However, much remains to be done to develop transmembrane supramolecular molecular systems that attain the types of far-from-equilibrium functionalities that are so characteristic of life.[7]

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