movement and diffusion of polymer blocks through the membrane. It therefore functions as a kind of molecular restraint that keeps all the loose polymeric components together and in order, thereby preventing PDEA from reorienting itself towards the water layer and so stopping the vesicle from irreversibly losing its shape. The only thing that the PDEA layer can do when it attracts water is swell, which causes the entire vesicle to swell in turn. Because the water-swollen layer is less densely packed than before, water molecules pass more easily through the polymer membrane.

Polymeric vesicles that change their size in response to pH have been described before. In one case[3], a conformational change of the water-soluble block resulted in an increase in the vesicle's size. In another example[4], the structure swelled because of protonation of a PDEA block (as in Eisenberg and colleagues' polymersome[1]), but the vesicle structure was maintained by cross-linking of the polymer molecules. The unique feature of the capsule reported by Eisenberg and colleagues is that it can 'breathe' repeatedly — swell and contract in response to pH changes — without the need for chemical cross-linking of the polymer molecules to stabilize its structure.

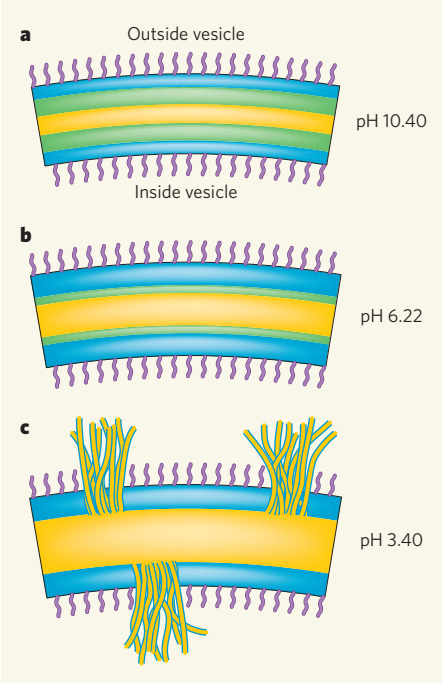

Figure 1 | A pH-responsive vesicle membrane. Eisenberg and colleagues[1] report polymeric vesicles that swell and shrink in response to changes in pH. The molecules that make up the membrane bilayer of the vesicles consist of one end block of poly(ethylene glycol) (PEG); a middle block of polystyrene; and a block of poly(2-diethylaminoethyl methacrylate) (PDEA) at the other end. **a**, At high pH, the pattern of polymer layers in the membrane is as follows: an outer layer of PEG (purple), a thin polystyrene layer (blue), a thicker polystyrene–PDEA layer (green), then a PDEA layer (yellow) at the centre of the membrane; the reverse pattern occurs moving from the PDEA layer to the inside of the vesicle. **b**, As the pH decreases, the PDEA and polystyrene layers separate, each becoming thicker, whereas the polystyrene–PDEA layers become thinner. **c**, At low pH, the polystyrene layer might rupture in places, allowing some PDEA to break through.

The authors' study[1] also demonstrates that there is still much to learn about the aggregation of macromolecules that contain hydrophilic and hydrophobic regions. Because such aggregations are often non-equilibrium processes, this makes them especially difficult to predict and control. Much more work is therefore required before these polymeric self-assembly processes can be used to make nanometre-scale devices. Moreover, polymeric aggregates are often stable structures, even when not in equilibrium, which means that many different aggregation morphologies might be obtainable from the same polymer, if the correct formation process is applied. This could lead to many exciting possibilities for drug delivery, or for making templates for objects such as nanowires.

Of course, if Eisenberg and colleagues' polymersome is to be useful in a biological setting, the vesicle's size must be controllable under physiological conditions, and its membrane must allow through not only water molecules, but also biologically active compounds. The second issue is already being tackled by an increasing number of scientists, who have succeeded in making polymersomes that irreversibly trap enzymes, but which allow the enzyme's substrates and products to pass through the vesicle membrane. The selective permeability originates from the clever design of the polymer molecules[5], or from trapping protein channels in the vesicle's membrane[6].

Eisenberg and colleagues' work[1] breathes new life into the branch of polymer science that studies the aggregation phenomena of apparently simple block copolymers. By combining lessons from polymer science with those from nature, nanometre-scale device manufacturing will be elevated to a higher level. ■

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STRUCTURAL BIOLOGY

A channel with a twist

Valeria Vásquez and Eduardo Perozo

Mechanosensitive channels release tension in cell membranes by opening 'pressure relief' pores. The structure of a partially open channel suggests a gating mechanism and delivers an unexpected architectural twist.

In the constant struggle with their environment, free-living cells have evolved a variety of mechanisms to deal with sudden variations in the physicochemical properties of their surroundings[1]. But few environmental challenges require more assertive responses than a hypo-osmotic insult. Faced with a sudden decrease in osmotic pressure, as might be caused by a spring downpour, free-living cells are subjected to a rapid influx of water. This sharply increases the pressure of the cell contents against the membrane, potentially compromising the integrity of the cell.

Most prokaryotes (bacteria and archaea) have therefore evolved a 'pressure-release valve' mechanism in which changes in membrane tension open up channels to form large, aqueous pores in the membrane. Once formed, the short-lived pores allow the passage of both solute and solvent at very high rates, quickly equilibrating hypo-osmotic imbalances across the cell wall. Different variants of these mechanosensitive (MS) channels exist, each of which forms pores that have distinct conductance and tension thresholds[2–5]. On page 120 of this issue, Rees and colleagues[6] provide evidence for the gating mechanism in the mechanosensitive channel of large conductance of *Staphylococcus aureus* (SaMscL) by determining the crystal structure of a truncated form of the channel.

To solve the molecular mechanism of gating in prokaryotic MS channels, two crucial questions must be addressed: how are membrane deformations sensed by the channel, and what is the mechanism by which the channel physically opens its permeation pathway? Although we have some idea of the types of force at the membrane–channel interface that might trigger MS channel opening[7], the principles underlying tension sensing through the membrane remain largely unknown. We do, however,

NATURE|Vol 461|3 September 2009

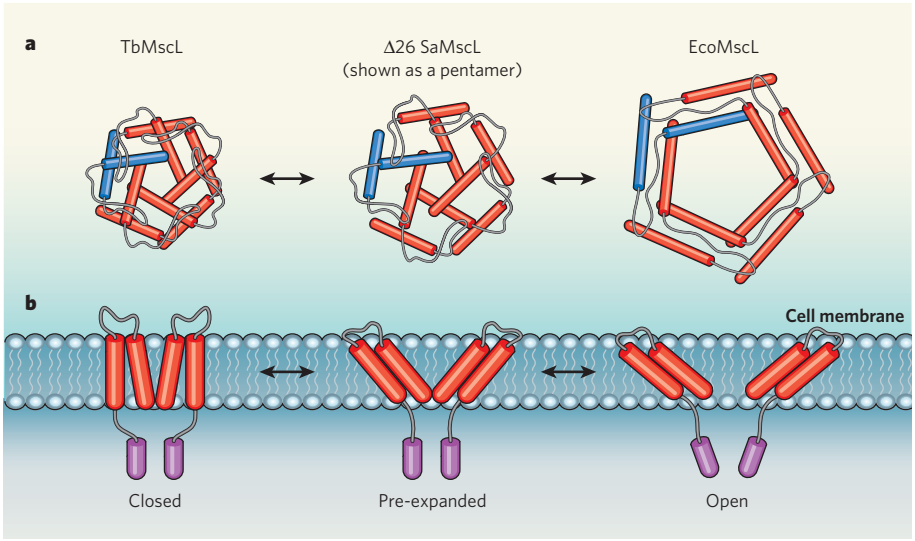

Figure 1 | Opening of the MscL channel. The MscL channel opens in cell membranes to release osmotic pressure in the cell. The crystal structure of the MscL from *Mycobacterium tuberculosis*[8] (TbMscL) revealed the closed state of the channel, whereas the open state was seen in the spectroscopically derived structure[10] of EcoMscL (the MscL from *Escherichia coli*). Rees and colleagues[6] now report the crystal structure of an intermediate, 'pre-expanded' state of a truncated MscL from *Staphylococcus aureus* (Δ26 SaMscL). **a**, In these extracellular views looking down at the MscL structure, the movements of the helices that line the pore as the channel opens (or closes) are visible. The cross-section of the channel expands from left to right, but the pore remains closed in the pre-expanded state. The Δ26 SaMscL channel is actually a tetramer, but is shown here as a pentamer for comparison with the open and closed states. An individual monomer is highlighted in blue. **b**, Side views reveal that the helices lining the inside of the pore tilt towards the plane of the membrane as the channel moves from the closed to the pre-expanded state, and that the whole channel structure becomes flatter. The helices maintain this orientation as the cross-section of the channel expands to reach the open state.

have a better handle on the conformational rearrangements in the channels that underlie their opening.

MscL is nonselective for the ions and small molecules it transports and is activated at membrane tensions close to the breaking point of the membrane bilayer. It is thus usually thought of as a bacterium's last line of defence against hypo-osmotic shock. Over the past decade, genetic, structural and biophysical data have provided a fairly detailed picture of the channel's basic architecture[8] and functional behaviour[9], and have defined the types of structural rearrangement that could support the formation of very large pores characteristic of MscL[10,11]. Rees's group previously reported the MscL crystal structure from *Mycobacterium tuberculosis*[8] (TbMscL), which is generally agreed to represent the closed conformation of the channel. The new structure[6] looks like an intermediate conformation, somewhere between the open and closed states.

It has been proposed[12,13] that a domain of MscL known as the cytoplasmic bundle acts as a sieve that limits the passage of large molecules through the pore. By removing the last 26 amino-acid residues of this cytoplasmic domain from SaMscL, Rees and colleagues[6] were able to drive the (typically stable) closed conformation of the channel to an expanded, partially open state. This state is characterized by a significant tilt of the TM1 helices — transmembrane helices that line the pore of the channel — towards the plane of the membrane. The conformational change shortens the length of the water-permeation pathway and makes the whole channel flatter than it is in the closed state (Fig. 1). The authors also found that the activity of, and the current through, their truncated channels in functional measurements is greater than that of wild-type channels, supporting the idea that the structural changes observed in the crystal structure also occur in functional channels.

Rees and colleagues' crystal structure[6] provides an explicit conformational pathway from the closed to the open state of SaMscL: the first physical transition generates a 'pre-expanded' state in which the cross-sectional area (A) of the channel is slightly larger than in the closed state (Fig. 1). This is likely to be the most tension-dependent state of the mechanism, as the probability that the channel will open should be proportional to $-\gamma\Delta A$, where γ is the lateral tension in the membrane[9]. The pre-expanded state maintains a narrow pore, still flanked by the inner TM1 helices, and is therefore predicted to be non-conductive. The present structure[6] is thus in excellent agreement with earlier models of the pre-expanded state[9,11,14].

Although Rees and colleagues' structure does not have a pore wide enough to conduct, the fact that the transmembrane helices are tilted away from the normal of the membrane, together with the expansion of the

50 & 100 YEARS AGO

50 YEARS AGO

The Human Response to an Expanding Universe. By Harlow Shapley — [The author of this book] is a world-renowned figure in the fields of astronomy and cosmography... Dr. Shapley begins by attempting an obituary of the anthropocentric view that man is the centre of the cosmos, and continues by interpreting the consequences to man (or rather to certain aspects of rational thinking) of the latest scientific discoveries in the cosmos...

Dr. Shapley's displacement of human life from its once supreme position does not make him a pessimist, for he argues cogently that there must be at least a hundred million planets capable of supporting some form of life. Dr. Shapley concludes his book with what he calls "a Martian look" into the future. He dismisses the prospect of the Earth's collision with a star, or of wandering from its orbit and getting too near or too far away from the Sun. Nor does he envisage a biological calamity wiping out the whole human race. The real danger is man himself, who is busy perfecting the tools for performing an operation which is unlikely to be performed by natural forces.

From *Nature* 5 September 1959

100 YEARS AGO

In February last Dr. N. Annandale obtained on the Orissa coast of India a number of small more or less nearly globular organisms in the tide-wash. When placed in water their shape changed from globular to conical, and indicated that they were evidently pelagic sea-anemones, although devoid of tentacles. The mouth is conspicuous, forming a relatively long, narrow slit expanded at one end, and the whole organism presents a milky appearance... As these actinians, which are apparently adult, although no gonads are visible, evidently indicate a new generic and specific type, Dr. Annandale has described them under the name *Anactinia pelagica*.

From *Nature* 2 September 1909

cross-sectional area of the pore, clearly suggests that the observed conformation is an intermediate stage on its way to the open state. Indeed, the observed tilts of the helices match remarkably well with those predicted by spectroscopic studies of channels trapped in the open state[10].

Perhaps the most surprising result of this work[6] is that, defying all expectations, the channel is arranged as a tetramer, not a pentamer as observed for the crystal structure of TbMscL in a closed state[8]. This is in contrast to the vast majority of oligomeric membrane proteins, each of which maintains the same oligomeric architecture across species and physiological states, at least for those studied so far. Still, controversy regarding the oligomeric state of MscL is not new — it was originally thought to be a hexamer, on the basis of biochemical experiments[15] and electron-crystallography data[16]. It is, however, unlikely that the present result[6] will lend further support to the idea that MscL gating is the consequence of monomer aggregation in the plane of the bilayer (which could support variable oligomeric states).

The observed tetrameric structure of truncated SaMscL raises several questions. Does the oligomeric state of MscL vary in different species? Does the channel reside in the membrane as different multimers? If so, does each multimer have different functional properties? And are particular multimers favoured by particular physiological conditions? Regardless of the answers, the plasticity of this family of ion channels seems to be exceptional. More time and experimental insight will surely be needed to realize the full implications of this structure[6]. ■

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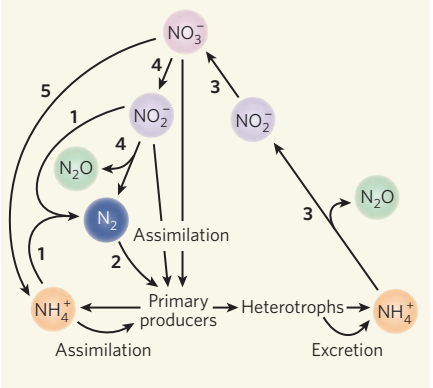

Figure 1 | Principal features of the marine nitrogen cycle. 1, Anammox, involving the generation of N_2 from inorganic constituents by autotrophic microbes. **2,** Nitrogen fixation. **3,** Nitrification. **4,** Heterotrophic denitrification, in which N_2 is produced from NO_3^- used in microbial respiration of organic substrates; this process also results in the production of NH_4^+ and CO_2 (not shown). **5,** Dissimilatory nitrate reduction to ammonium (DNRA). NO_3^-, nitrate; NO_2^-, nitrite; N_2O, nitrous oxide; NH_4^+, ammonium. The N_2-output pathways, **1** and **4,** were the subject of Ward and colleagues' study[3].

NITROGEN CYCLE

Oceans apart

Maren Voss and Joseph P. Montoya

Reactive nitrogen is lost from the oceans as dinitrogen — N_2 — produced by microbial metabolism. The latest twist in an ongoing story is that different pathways dominate in two of the oceanic regions concerned.

The availability of nitrogen limits biological production in much of the world ocean[1]; this in turn affects the strength of the 'biological pump' that converts carbon dioxide into organic matter that can sink and be sequestered in the deep sea[2]. The main input to the marine nitrogen cycle comes from the fixation of nitrogen gas (N_2) into biologically available forms. The main output is through biological processes that generate a return flux of N_2. Both input and output remain poorly constrained[1], and there is a pressing need to define them better.

On page 78 of this issue, Ward *et al.*[3] describe how they have investigated the output side of the budget. They report on a comprehensive field study aimed at identifying the processes and organisms responsible for N_2 production in two of the major sites of nitrogen loss in the world ocean. The sites concerned are the oxygen minimum zones (OMZs) of the Arabian Sea and the Eastern Tropical South Pacific (ETSP). At these sites, microbial degradation of sinking organic matter obtained by primary production — mainly photosynthesis — in the surface ocean completely removes oxygen from large parts of the water column. The resulting conditions favour metabolic pathways that convert nitrogen from its biologically reactive forms (for example, nitrate and ammonium) to N_2.

In one of the N_2-conversion pathways, termed heterotrophic denitrification, nitrate acts as a terminal electron acceptor in microbial respiration; this was long viewed as the main metabolic pathway for N_2 production in midwater OMZs. But the picture was complicated by the discovery that another route — anammox, a pathway that produces N_2 by coupling the reduction of nitrite to the oxidation of ammonium — has an important role in the oceanic nitrogen cycle[4,5]. The autotrophic microbes in this pathway produce biomass from inorganic molecules by harvesting energy from the anammox reaction. Both pathways are shown in Figure 1, which summarizes the overall marine nitrogen cycle.

Studies in the Black Sea[5], the Benguela upwelling system[6] and the ETSP[7] suggested that anammox may in fact be the dominant pathway removing reactive nitrogen from the ocean. If so, however, what supplies the necessary substrates? Heterotrophic denitrification can potentially do so — it can supply both nitrite, an intermediate in the denitrification pathway, and ammonium, a product of heterotrophy, to support anammox[4]. Earlier this year, however, Lam *et al.*[7] suggested a major revision of the nitrogen cycle in which a process called dissimilatory nitrate reduction to ammonium (DNRA) provides the necessary ammonium for anammox. In this revised cycle, heterotrophic denitrification has a minor role and is no longer a significant source of N_2.

Part of the uncertainty surrounding heterotrophic denitrification is due to the difficulty of measuring the rates of the processes involved. Even low levels of oxygen contamination suppress the activity of denitrifying microbes, and the high concentration of N_2 in the water greatly reduces the sensitivity of experimental methods that are based on following the movement of the ^{15}N isotope tracer into the N_2 pool.