Ghost of speciation past

Thomas D. Kocher

Lurking in the rivers of Botswana are the remnants of a diverse flock of cichlid fishes, whose origins can be traced to a lake that vanished more than 2,000 years ago.

Just add water. A simple recipe, but when applied to cichlid fishes it reliably causes spectacular evolutionary radiations of hundreds of new species. Generalist riverine cichlids have given rise to flocks of highly specialized fishes in each of the major lakes in East Africa. Now, on page 90 of this issue, Joyce and colleagues[1] describe a previously unrecognized radiation of cichlids in the extinct Lake Makgadikgadi, whose descendants still haunt the rivers of southern Africa today.

Joyce and colleagues' evidence[1] consists of mitochondrial DNA sequences determined from fishes collected from rivers throughout southern Africa. Rivers in East Africa typically hold just one haplochromine cichlid species (haplochromine is the name given to one of the major lineages of cichlid fishes in Africa). But the Okavango, Cunene, Limpopo and upper sections of the Congo and Zambezi rivers in southern Africa each harbour several species of haplochromines. Evolutionary analyses show that these fishes share a common ancestor quite separate from that which gave rise to the better known radiations in lakes Malawi and Victoria (Fig. 1). The diversity of these riverine cichlids peaks in the Okavango delta, which geological evidence suggests was, until about 2,000 years ago, part of a lake larger than Switzerland.

The extent of this newly recognized adaptive radiation is truly impressive. The remnants of the Lake Makgadikgadi flock display a morphological diversity nearly as great as that of the extant cichlids in lakes Malawi and Victoria. The flock includes ambush predators with long heads and jaws, and snail crushers with deep heads and massive teeth. Only a few specialized morphologies are missing — such as those of pelagic plankton eaters and benthic algal scrapers — probably because these niches are not available in the remaining river habitat.

What is it about lakes that facilitates these rapid radiations? Certainly, large lakes provide a range of new ecological habitats not present in rivers. The still waters of lakes probably also reduce rates of gene flow between different groups of fish, enabling them to diverge genetically from each other and eventually to speciate. But other groups of fishes have not responded to these physical factors with similar bursts of speciation. It seems there is also something special about haplochromine cichlids that causes them to diversify in these large lakes. What are the key innovations that make them particularly prone to rapid adaptive radiation?

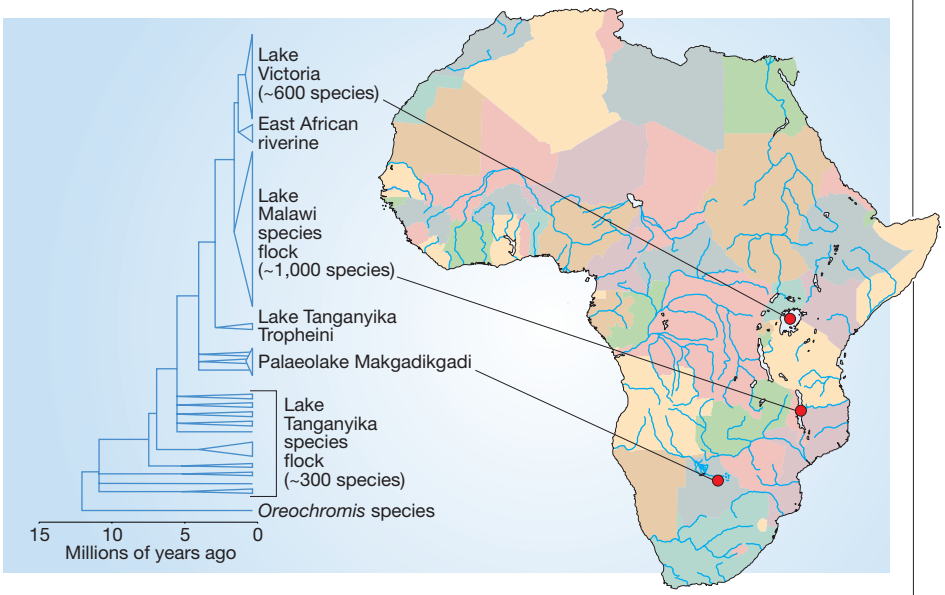

Figure 1 **Repeated diversification of cichlid fishes in southeast Africa. Mitochondrial DNA sequences reveal a radiation of cichlids in Lake Tanganyika beginning 7–9 million years ago. Over the past 3–4 million years, one of these lineages has spread through the rivers of southern and eastern Africa. These haplochromine cichlids gave rise to independent radiations in lakes Victoria and Malawi, and, as Joyce *et al.*[1] now describe, in the palaeolake Makgadikgadi. In Lake Tanganyika, these fishes also gave rise to a recent radiation of the Tropheini. The tree is rooted with tilapia (genus *Oreochromis*), a popular food fish that is closely related to the haplochromine cichlids of the region. (Tree modified from ref. 10.)**

A long-standing hypothesis is that cichlids have a malleable feeding apparatus that can be quickly altered to take advantage of new feeding opportunities[2]. Cichlids have a second set of jaws in the back of the throat — the pharyngeal jaws — that process food before it enters the gut. The oral jaws are therefore relatively free to evolve specializations for acquiring food. This decoupling of functions has clearly contributed to the rapid evolution of feeding specializations in each of the species flocks that inhabit lakes.

Another key innovation is maternal mouth-brooding. Most non-haplochromine cichlids lay large numbers of small eggs on the lake or river bottom, and both parents cooperate in the care of the eggs and fry. In contrast, female haplochromines lay small numbers of large eggs, and carry the eggs in their mouths for several weeks until the young are released to fend for themselves. Haplochromine males provide no post-spawning care of their offspring.

This unequal parental investment results in strong sexual selection. Males must compete for females, and as a consequence have evolved an astonishing variety of nuptial colorations and behaviours to attract mates. These differences in colour are often enough to prevent interbreeding (hybridization) among existing species[3]. But it is not clear whether sexual selection on male traits is sufficient to drive speciation in the first place. Other genetic conflicts, including those between females and their offspring over the sex ratio, could also be important[4].

Joyce and colleagues[1] suggest that hybridization may itself have played a part in the origin of the Lake Makgadikgadi species flock. This lake was probably colonized by distantly related cichlids from previously unconnected rivers. If these lineages hybridized, the resulting pool of genetic diversity might have facilitated the generation of new morphological or behavioural characteristics, enabling the colonization of newly available ecological niches in the lake[5]. This hypothesis is difficult to prove, but may be testable through analysis of variation

in the genes underlying important adaptive traits.

The present situation in the Okavango delta may also provide some context for discussing the origin of today's Lake Victoria flock. Although some geological data suggest that the Lake Victoria basin was completely dry 15,000 years ago, molecular evolutionary studies generally suggest that the flock is much older (100,000 years)[6,7]. As with Makgadikgadi, it seems likely that several genetically and morphologically diverse cichlids survived the drying of Lake Victoria, and have been able to re-radiate into more than 500 species during the 15,000 years since the lake refilled[8].

The high rates of speciation observed in these African cichlids are almost beyond belief[9], but the evidence is clear. The discovery of yet another species flock[1] emphasizes the importance of the phenomenon, and reinforces the utility of these fishes for studying evolutionary mechanisms. African cichlid fishes represent about 5% of all vertebrate species. A synthesis of the mechanisms responsible for their spectacular radiation is essential if we are to fully appreciate the origins of vertebrate diversity. ■

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Developmental biology

Morphogens hitch a greasy ride

Richard S. Mann and Joaquim Culi

Morphogen proteins guide the development of many tissues in animals, but how are these insoluble proteins ferried around the body? A well-known group of lipid transporters might be the answer.

Every once in a while, a scientific discovery results in the marriage of two previously disparate fields. On page 58 of this issue, such a romance is suggested by Eaton and colleagues[1]. On the one hand, there are morphogens — signalling proteins that play an essential part in animal development by inducing specific cellular fates in a concentration-dependent manner[2,3]. How these proteins move from the cells that synthesize them to neighbouring cells to create a concentration gradient is an area of intense debate. On the other hand, there are lipoprotein particles, most famous for their role in lipid transport and heart disease. The marriage, witnessed by intriguing experiments described in the new paper, raises the possibility that lipoprotein particles carry morphogens as they move through tissues during development.

Although biologists have had much success in measuring the biological effects of morphogens, understanding how these proteins spread through tissues to generate a concentration gradient has proved more challenging. This is in part because many morphogens are highly insoluble, which hampers their diffusion through the hydrophilic environment of tissues. In particular, two morphogens, the Hedgehog (Hh) and Wnt/Wingless (Wg) proteins, are attached to palmitic acid, and Hh is also covalently linked to cholesterol[4]; these lipids would promote the association of the two proteins with cell membranes.

Several models have been proposed to explain how morphogens move through tissues. Some suggest that they are actively transported by cells — through long cellular extensions called cytonemes, for example, or by being passed from cell to cell through cycles of secretion and uptake[2,3]. Although these models satisfactorily deal with morphogen insolubility, it has been difficult to confirm their role in morphogen spreading and activity. In contrast, accumulating biological evidence and mathematical modelling favour the idea that morphogens diffuse passively along the surface of cells, aided by local interactions with other molecules[2,3]. For such 'restricted diffusion' to be possible, lipid-modified morphogens must somehow acquire a relatively soluble form.

One potential solution, suggested previously by the Eaton group[5], posits that morphogens move in membranous vesicles called argosomes that are derived from morphogen-producing cells. Now the Eaton and Thiele labs[1] have revised this model by suggesting that morphogens move via large particles better known for their role in transporting lipids — the lipoproteins (Fig. 1). If they are right, this connection would contribute significantly to our understanding of morphogen spreading, and suggests a new role for lipoproteins during animal development.

Lipoproteins are large, globular macromolecular complexes composed of a central core of lipids surrounded by an outer layer of polar phospholipids, cholesterol and specialized proteins called apolipoproteins[6]. Most lipoproteins are synthesized in the liver and intestine of mammals and in the fat-body of insects, from where they move to peripheral tissues through the blood and lymph (the haemolymph in insects) to regulate lipid levels throughout the body.

Using their lipid adducts as anchors, Wnt and Hh proteins could in principle easily reside in the outer phospholipid layer of lipoproteins. Indeed, using biochemical fractionation of fruitfly (*Drosophila*) tissues, Eaton and colleagues[1] show that, although most Wg and Hh proteins co-fractionate with cellular membranes, a significant percentage partitions into the lipoprotein fraction. Moreover, lipoproteins can be co-immunoprecipitated with Wg and Hh, suggesting a tight association.

So, lipoproteins and morphogens have been seen together, but is this relationship a meaningful one? Eaton and colleagues suggest that it is, because lipoproteins seem to be required for the accurate establishment of morphogen gradients and activity in *Drosophila* larvae. The authors used a process called RNA interference (RNAi) to decrease apolipoprotein synthesis in the *Drosophila* fat-body — thus decreasing lipoprotein concentrations throughout the animal. This reduced the range of action of Hh and Wg in the imaginal discs, where morphogen activity is best characterized. Moreover, Hh accumulated at higher than normal levels in cells near the Hh source, perhaps as a consequence of reduced diffusion.

Although these findings are intriguing, the systemic way in which lipoproteins are synthesized and circulated, combined with their role in lipid metabolism, makes it difficult to design a watertight experiment. The authors describe two controls that help strengthen their conclusions. First, incubating the RNAi-treated discs with purified lipoproteins at least partially restored the long-range signalling activity of Hh. Second, growing larvae on a reduced-lipid diet did not affect morphogen activity or diffusion. Both experiments support the idea that the RNAi-induced defects are due to lower numbers of lipoprotein particles and not to a secondary effect such as reduced lipid levels.

Beyond providing a new mechanism by which lipophilic morphogens might spread through tissues (Fig. 1), the marriage between lipoprotein metabolism and animal development, if correct, hints at additional relationships. For example, the diffusion of most morphogens also requires heparan sulphate proteoglycans (HSPGs) — large molecules consisting of protein and carbohydrate that are found on the cell surface[7]. Lipoproteins are already known to interact

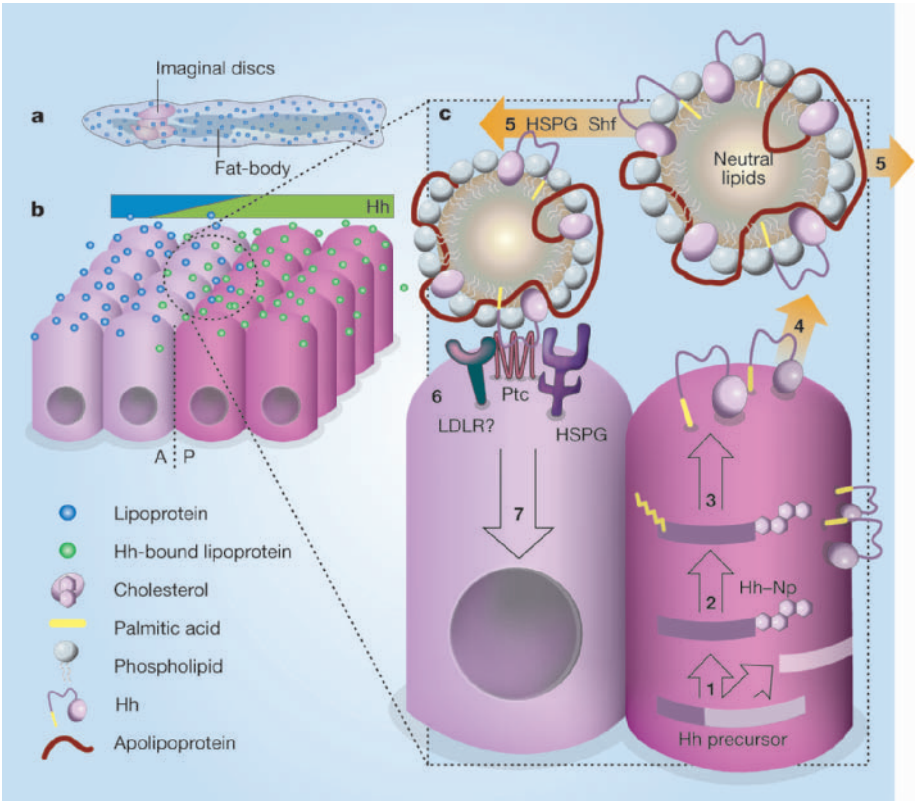

Figure 1 **Morphogen movement. How a gradient of Hedgehog (Hh) protein might form, as proposed by Eaton and colleagues[1].** a, **Lipoprotein particles are made in the fat-body and distributed throughout a fruitfly larva, coming into contact with the imaginal discs.** b, **A blow-up of an imaginal disc close to its anterior (A)–posterior (P) boundary. Posterior cells secrete Hh, producing a gradient of Hh-charged lipoprotein particles and a counter gradient of uncharged particles.** c, **Blow-up of A and P cells, showing some of the steps that produce and transduce the Hh gradient. 1: The Hh precursor cleaves itself and is added to cholesterol to form Hh–Np. 2: Palmitic acid is added. 3: The protein is transported to the plasma membrane. 4: The Dispatched protein might help to load Hh into lipoproteins. 5: Loaded particles diffuse somewhat, with the help of heparan sulphate proteoglycans (HSPGs) and the Shifted (Shf) protein[19,20]. 6: Charged lipoproteins bind to receiving cells, aided by the Hh receptor Patched (Ptc) and possibly HSPGs and low-density-lipoprotein receptors (LDLRs). 7: The signal is transmitted to the nucleus.**

with HSPGs, which are also important in lipid metabolism[8]. Thus, HSPGs might affect morphogen diffusion, at least in part, by interacting with their lipoprotein carrier. Consistent with this idea, diffusion of a form of Hh that cannot be modified by lipids is independent of HSPGs[7].

Another fascinating potential link concerns how target cells might bind and take up morphogen–lipoprotein complexes. Lipoproteins have their own receptors, the low-density-lipoprotein receptors (LDLRs), and certain members of this protein family are essential for receiving the Wg signal[9,10]. But it is not clear whether they interact directly with Wg[11]. Perhaps instead they bind the lipoprotein moiety of Wg–lipoprotein particles. Other LDLRs might similarly contribute to morphogen activity[12,13].

Yet another exciting possibility concerns the mechanism that delivers Hh and Wg from the membranes of the cells that produce them to lipoproteins. Perhaps morphogens associate reversibly with lipoproteins, thus generating a source of morphogen-charged particles close to morphogen-producing cells. Alternatively, a more active mechanism may exist. Consistent with this notion, the protein Dispatched — a member of the sterol-sensing receptor family — is essential for the release of cholesterol-modified Hh from Hh-producing cells, but is not required for the release of a non-cholesterol-modified form[14]. Thus, Dispatched might be involved in charging lipoproteins with Hh. A similar mechanism involving lipid rafts — specialized regions of the cell membrane — has also been suggested for Wg secretion[15].

Despite the many possibilities suggested by this model, more questions are raised, in part because both fields have been so productive. How efficiently are peripheral tissues, such as the lumens of *Drosophila* imaginal discs, bathed by lipoproteins? Are lipoproteins the sole carriers for Hh and Wg, or are other mechanisms, such as the formation of micelle-like Hh multimers[16], also involved? Because Wg and Hh sometimes act on the same cells, do individual lipoprotein particles carry both morphogens at the same

100 YEARS AGO

On Sunday, the President of the French Republic entertained the King at the Elysée at a dinner party, at which 120 guests were present. The guests included distinguished authors, artists, musicians, and other representatives of intellectual activity, almost exclusively members of the Institute of France. By inviting leaders of literature, art, and science to meet the King, graceful recognition was given of the high place occupied by the muses in the polity of the Republic. In the days when sheer muscular force was the mainstay of a nation, bodily strength and prowess were rightly regarded as recommendations for Court favours; but now that brain-power instead of muscle determines the rate of national progress, the State that desires to advance must foster all the intellectual forces it possesses. This principle is well understood in France, and is also clearly recognised in Germany, where every man who makes notable contributions to knowledge of any kind, assists industrial progress, or creates works of distinguished merit, whatever they may be, is sure to receive personal encouragement from the Emperor. The presence of these leaders of thought is a striking characteristic of the German Court; while, on the other hand, their absence, and the overpowering influence of military interests, are distinguishing features of Russian, and, let us add, of British Court functions.

ALSO:

Satisfactory progress and general prosperity form the key-note of the report of the Zoological Gardens at Giza for the past year. The report is illustrated by the reproduction of a most interesting photograph of an aard-vark, or ant-bear, slightly marred by the effect of a shadow by the side of the nose.

From *Nature* 4 May 1905.

50 YEARS AGO

"Mathematical Association Annual Meeting." The first item in the afternoon session was a discussion of "The Disadvantages of a Mathematical Education", led by Mr. W. O. Storer (Department of Education, University of Birmingham); Mr Storer thought that the logical training supposedly given by mathematics might be arid, and that mathematical insistence on accuracy might lead to intellectual arrogance. Some members were reluctant to accept these inferences.

From *Nature* 7 May 1955.

time? Also, because Eaton and colleagues studied only *Drosophila* larvae, and examined only a few target genes, it is not known whether this mechanism also operates in *Drosophila* and vertebrate embryos. Suggestively, however, insect egg yolk contains abundant lipoprotein, and lipoprotein synthesis occurs in the yolk-sac visceral endoderm of early mouse embryos[17,18].

We also wonder which of the several varieties of mammalian lipoproteins could be involved in morphogen diffusion, and if they provide additional specificity. And, finally, could the diffusion of morphogens not associated with lipids, such as members of the transforming growth factor-β/activin/Dpp family, also be mediated by lipoproteins? Thus, from this marriage, we expect many interesting progeny. ■

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Planetary science

Saturn's retrograde renegade

J. Brad Dalton

Data from the Cassini–Huygens mission provide convincing evidence that the saturnian moon Phoebe formed elsewhere in the Solar System, and was only later captured by Saturn's gravitational pull.

In the next few years, the Cassini–Huygens spacecraft will return a wealth of data from its orbital tour of Saturn's rings and moons. Although many images are released to the public almost immediately, it takes time to conduct detailed scientific analyses of the observations. Stunning results from early encounters with the moons are now beginning to trickle in.

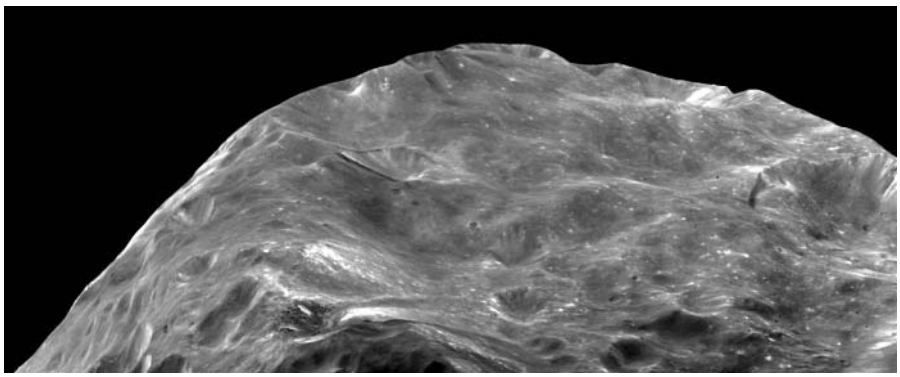
On 11 June 2004, shortly before it permanently entered Saturn's orbit, Cassini–Huygens made its closest approach to the planet's outermost moon, Phoebe (Fig. 1; see also Box 1, overleaf). Phoebe is a small (around 220-km-diameter), irregular satellite in an eccentric, inclined, retrograde orbit — moving counter to the direction of the planet's rotation, in contrast to the more common, prograde, movement. Scientists have long suspected[1,2] that Phoebe did not form alongside Saturn and its regular satellites, which occupy roughly circular, low-inclination, prograde orbits. Two papers in this issue[3,4] provide additional evidence for this hypothesis.

Johnson and Lunine[3] (page 69) use density and volume calculations, determined from navigation and imaging systems on board Cassini, to suggest that Phoebe is more similar to objects found in the Kuiper belt, a collection of small, icy bodies beyond Neptune, than it is to its siblings in orbit about Saturn. Clark *et al.*[4] (page 66) interpret spectral observations from Cassini's Visual and Infrared Mapping Spectrometer to reveal a complex array of surface materials ranging from minerals to volatile organic compounds.

A number of these compounds have not yet been observed on the regular saturnian satellites, but are typically found in primitive bodies of the outer Solar System, such as comets, certain types of asteroid and, again, objects in the Kuiper belt. As the Kuiper belt is thought to contain material left over from the formation of Saturn, Uranus and Neptune, such an origin for Phoebe still keeps it in the family, albeit as a more distant, 'prodigal' child of Saturn. But converging lines of evidence now suggest that Phoebe formed even farther from the Sun and only later ventured inwards. Its progress was then arrested by gravitational interactions with the Saturn system.

The compositional diversity of Phoebe, in fact, seems unlike that of any single object studied to date within the Solar System. Clark *et al.*[4] have identified the presence of iron-bearing minerals, and possible phyllosilicates (the family of sheet silicates that includes clays and micas). The latter, if present, would indicate some degree of aqueous processing, possibly in a parent body or even before the formation of the protoplanetary nebula — the cloud of gas and dust from which planets are formed around a newborn star, such as the Sun. Spectral evidence for two crystalline silicate families, the olivines and pyroxenes, is also consistent with the presence of primitive materials left over from the formation of the Solar System.

The spectral observations also point to a wealth of volatile compounds — among them water-ice, carbon dioxide and several organic compounds, including alkanes, aromatic compounds, nitriles and other cyanide compounds — indicating an origin somewhere in the frozen outer reaches of the solar nebula, rather than in the hotter, drier inner Solar System where the terrestrial planets and the asteroid belt formed. An origin beyond Saturn's orbit also makes more sense when considering the orbital dynamics required for outward migration from the asteroid belt, against the gravitational pull of the Sun.



NASA/JPL/SPACE SCIENCE INST.

Figure 1 **One battered rock. This stunning image of Phoebe's pitted surface, as seen by the Cassini Imaging Science Subsystem, reveals its long and complex history. The region shown is near the satellite's south pole and is around 120 km across; the largest craters are up to 4 km deep. Bright patches of water and other ices, as well as dark regions hosting complex organic compounds and minerals, indicate an origin far from its current orbit around Saturn. Results from Cassini[3,4] suggest that Phoebe may have formed as far out as the Kuiper belt, beyond Neptune's orbit, and may even contain cometary material pre-dating the formation of the Solar System.**

Box 1 The Cassini–Huygens mission to Saturn

The Cassini–Huygens spacecraft is a joint endeavour of NASA, the European Space Agency and the Italian Space Agency, ASI. With a dry weight of 5.6 tonnes, it is the largest interplanetary spacecraft ever built, and consists of two parts — the orbiter Cassini and the Titan probe Huygens. The components are named after Jean-Dominique Cassini (1625–1711), discoverer of the saturnian moons Dione, Iapetus, Rhea and Tethys, and Christiaan Huygens (1629–95), who first observed Saturn's rings and Titan, its largest moon.

- 15 October 1997: Launch from Cape Canaveral. Helped on its way by 'stealing' rotational energy in three fly-pasts of Venus (April 1998, June 1999) and Earth (August 1999).
- 30 December 2000: Sends back high-resolution images as it passes Jupiter at a distance of 9.7 million kilometres.
- 11 June 2004: First detailed images of Phoebe, on the edge of the Saturn system, from a range of 2,000 kilometres.
- 1 July 2004: Permanently enters orbit around Saturn, sending back spectacular images of the planet's rings.
- 26 October 2004: First close pass of Titan, which, with a diameter of 2,700 km, is the largest of Saturn's 34 known moons.
- 25 December 2004: The Cassini orbiter releases the Huygens probe to coast down to Titan's surface.
- 14 January 2005: Huygens enters Titan's cloudy atmosphere, touching down two-and-a-half hours later. The probe continues sending data for well over an hour from the surface, allowing a detailed picture of its composition to be made. (More will appear on this topic in a later issue.)

In the next three years, Cassini will make numerous further passes of Titan, as well as other moons — including Iapetus, Enceladus, Tethys and Rhea — in the course of 75 orbits of Saturn before its fuel supply is exhausted. **J.B.D.**

The findings of Clark *et al.*[4] dovetail nicely with Johnson and Lunine's analysis[3], which finds Phoebe's mass, volume and density to be more consistent with those of Pluto and Neptune's giant satellite Triton than those of the regular saturnian satellites (with the exception of Hyperion and Titan, which were excluded from the analysis). Because the composition of Phoebe should reflect that of the region in which it formed, this new knowledge could place constraints on the composition and evolution of the early solar nebula, as well as the various worlds spawned within it.

There is much more for Cassini to do. The nature of the proto-saturnian nebula is still not well known; complementary analyses of encounter data for the regular satellites are likely to shed further light on this problem. The formation of Saturn itself, for example, could have altered the abundances of important species — such as oxygen or water — relative to the protoplanetary nebula[5]. The ring system, as noted by Clark *et al.*, seems to contain similar iron-bearing materials to those seen on Phoebe. It has long been thought that dust from Phoebe could be migrating inwards within the Saturn system, perhaps even providing the bulk of the dark material seen on Saturn's moon Iapetus[6,7]. The similarities between these two satellites, at least as seen from ground-based telescopes and from the Voyager spacecraft, might also be explained by assuming that meteoritic or cometary infall has coated both with similar material.

The many planned encounters with Iapetus and Saturn's other regular satellites are expected to provide definitive answers to these remaining questions. Given the far-reaching implications of this first encounter with tiny Phoebe, we can expect Cassini to provide many more powerful insights during its four-year mission. ■

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Reproductive biology

Fatty link to fertility

S. K. Dey

A short delay in the attachment of embryos to the wall of the womb during early pregnancy adversely affects later developmental processes. New evidence reinforces the need for lipids to regulate this event.

In mammals, the creation of new life depends on the union of a sperm and an egg. The fertilized egg then undergoes several cell divisions to form a differentiated tissue — the blastocyst. Next, an intricate molecular dialogue between blastocyst and uterus initiates the process of implantation, by which the blastocyst attaches to the lining of the uterus[1,2]. Low implantation rates are common in women undergoing assisted reproduction, posing a challenge to both patients and doctors, so researchers are striving towards a deeper understanding of this process. On page 104 of this issue, Ye *et al.*[3] reveal a previously unknown and essential role for a small lipid signalling molecule, lysophosphatidic acid (LPA), in normal implantation.

Normal implantation is initiated only when embryonic development is synchronized with the appropriate preparation of the uterus. More specifically, the embryo must reach the blastocyst stage and gain 'implantation competency'; meanwhile, the uterus — through the coordinated actions of oestrogen and progesterone — must reach what is known as the receptive phase.

This state of uterine receptivity, also termed the window of implantation, lasts for a limited period. It is only during this time that the womb is able to support normal embryonic growth and implantation[1]. Previous studies have shown that when blastocysts implant beyond this window, the delay creates an adverse ripple effect: embryos crowd near the cervix; placentas fail to form correctly; fetuses are resorbed; and the development of remaining fetuses is retarded[4,5]. In other words, the quality of implantation determines the quality of pregnancy and fetal well-being; failure to achieve 'on-time' implantation risks an adverse pregnancy outcome. In humans, implantation beyond the normal window leads to spontaneous pregnancy losses[6].

Tracing the hierarchical landscape of the molecular signalling pathways that govern embryo–uterus interactions during human pregnancy is not easy, because of experimental difficulties and ethical considerations. Experiments in mice, however, have directly shown that lipid molecules known as prostaglandins, generated by the enzyme cyclooxygenase-2 (COX-2), are essential for implantation[5,7]. Their role in reproduction is further illustrated by the poor fertility — caused by deferred implantation — seen in female mice lacking cytoplasmic phospholipase $A_{2\alpha}$ ($cPLA_{2\alpha}$; ref. 4). This enzyme uses the phospholipids that make up cell membranes to generate arachidonic acid, which is in turn used for prostaglandin synthesis by COX-2. These studies establish the importance of lipid signalling through the $cPLA_{2\alpha}$–COX-2 axis in implantation.

When a cell is activated in response to a stimulus, membrane phospholipids can be used to generate numerous lipid signalling molecules, such as eicosanoids and lysophospholipids. Prostaglandins belong to the eicosanoid class. The lipid featuring in Ye *et al.*'s study, LPA, belongs to the lysophospholipid group, and is characterized by a 3-carbon backbone, to which is attached an

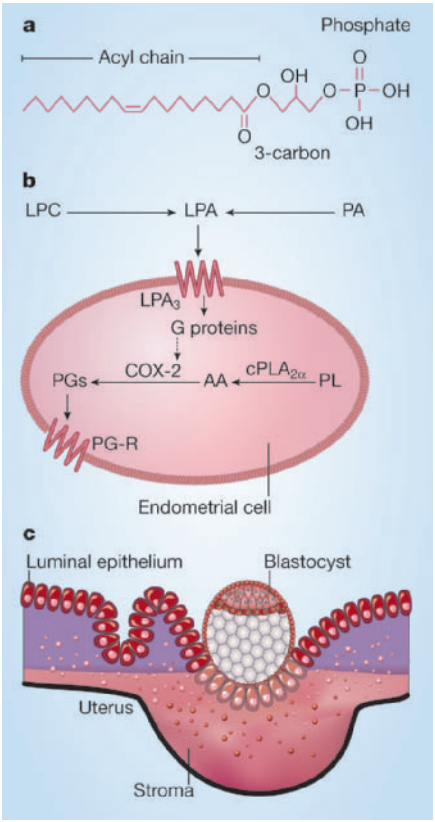

Figure 1 **Lipid signals in implantation. Ye *et al.*[3] find that lysophosphatidic acid (LPA), through its receptor LPA_3, is crucial for on-time implantation.** a, **LPA consists of a 3-carbon backbone, attached to an acyl chain and a phosphate head-group.** b, **How LPA and other lipid signals might converge in the uterus. LPA is formed from lysophosphatidylcholine (LPC) or phosphatidic acid (PA). Binding of LPA to LPA_3 in the endometrium (the uterine wall) activates specific G proteins. Ye *et al.* suggest that these in turn activate the enzyme COX-2, leading to greater production of prostaglandins (PGs), which interact with their receptors (PG-Rs). This leads to increased vascular permeability and increased adhesiveness of the uterine lining[1,2]. Cytosolic $PLA_{2\alpha}$ ($cPLA_{2\alpha}$) is also needed for on-time implantation[4]; it generates arachidonic acid (AA) from membrane phospholipids (PL).** c, **Sites of LPA_3, COX-2 and $cPLA_{2\alpha}$ expression. LPA_3 is detected in the endometrial luminal epithelium, with peak expression occurring before implantation. $cPLA_{2\alpha}$ and COX-2 are expressed in the endometrial stroma, but also overlap with LPA_3 at the site of blastocyst implantation.**

acyl chain and a phosphate head-group (Fig. 1a). Lysophospholipids influence a range of processes — including blood-vessel formation, vascular maturation and neural development — by activating cell-surface receptors that are coupled to common molecular switches termed G proteins[8]. LPA uses four different receptors, LPA_1 to LPA_4, to produce different effects[8,9].

Mice missing LPA_1 and LPA_2 reproduce normally. But Ye *et al.*[3] now find that LPA_3-deficient mice show strikingly similar problems to $cPLA_{2\alpha}$-deficient ones[4]: deferred implantation, retarded fetal development, embryo crowding, and the sharing of one placenta by several embryos. The result is a much-reduced litter size. Treating these mice with prostaglandins resumes on-time implantation, although embryo crowding persists.

Given the similarities between LPA_3- and $cPLA_{2\alpha}$-deficient mice, the reduced levels of uterine COX-2 in mice missing LPA_3, and the presumed reduction in arachidonic-acid levels (providing less substrate for COX-2) in the $cPLA_{2\alpha}$-deficient animals, Ye *et al.* conclude that a molecular component that is affected in both cases is COX-2. Thus, both $cPLA_{2\alpha}$ and LPA_3 seem to feed into COX-2 (although how LPA_3 does so is not yet known), thereby influencing the production of prostaglandins and so the timing of implantation (Fig. 1b, c). Ye and colleagues' results also lend further credence to the ripple-effect concept — the idea that embryo implantation occurring past the normal window leads to a poor pregnancy outcome.

It remains to be seen, however, how uterine prostaglandins coordinate with embryonic signals to ensure on-time implantation. Another unresolved issue is the embryo crowding seen in the LPA_3-deficient mice. This raises questions such as: what signal attracts the embryo to its specific site of attachment in the uterus? Does this finding have any relevance to placenta praevia in humans — an undesirable condition in which the placenta is attached close to or covers the cervix — or to the 'multiple pregnancies' that often arise from assisted reproductive technologies? Although the LPA_3/$cPLA_{2\alpha}$–COX-2 signalling axis is clearly important for proper embryo spacing, we so far have no clue as to how to rescue the crowding defect. The molecules downstream of prostaglandin signalling that participate in the ripple effect also remain unknown.

Defects in blastocyst competency, uterine receptivity and embryo–uterine dialogue all compromise implantation and hence fertility. Mapping the molecular landscape seen during the period of implantation — a period so vital for later development — necessitates well-thought-out experiments to elucidate embryonic and uterine contributions. Past studies have shown a requirement for lipid signalling; Ye and colleagues' work[3] now adds LPA signalling to the mix, as an essential player on the maternal side. Ye *et al.* also speculate that still other lipid-based mechanisms may influence fertility, given the reduced fertility, of unknown cause, that is observed when other lysophospholipid receptors are defective[10]. Although it is not known whether all these pathways function independently or converge with others to ensure on-time implantation, one thing is clear: fat matters to fertility. ■

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Materials science

A hard look at glass

Paul Madden

The topology of amorphous glasses has generally been considered only at the level of atoms and their nearest neighbours. A larger-scale view gives a fresh perspective on the structure and formation of these glasses.

Glass — window glass being the most familiar example — constitutes an 'ill-condensed' state of matter. Writing on page 75 of this issue, Salmon and colleagues[1] present unprecedentedly detailed studies of the structure of two glassy materials. Their findings may help to explain why the molecules in the materials that form glasses fail to find the lowest-energy arrangement when cooled to low temperatures, and this should aid our understanding of these scientifically and technologically important materials.

Most liquids, as they are cooled, arrange themselves so as to minimize the interaction energy between their constituent molecules — they crystallize, forming a solid in which the molecules are held in a periodic lattice with long-range order. Distorting this lattice involves sliding whole planes of molecules past each other — giving rise to the familiar rigidity of a perfect crystalline solid.

A glassy material is rigid too, but the arrangement of its molecules is aperiodic ('amorphous'), and does not minimize the energy of the structure[2]. But we can still understand rigidity in amorphous arrangements in the absence of molecular motion (that is, at a temperature of absolute zero) by appealing to mechanical analogues. A random packing of particles with a density above a certain value — a pile of sand, for example — can be rigid. A less-dense collection of particles, in which each element is held to its neighbours by springs, also cannot change its shape if the number of constraints exceeds a critical value. But even with such pictures in mind, fundamental questions remain. Why have amorphous arrangements been adopted at low temperatures in glassy materials? Why not take on the configuration with the lowest energy?

The structures of real (as opposed to computer-modelled) glasses are not known beyond the shortest length scales. Yet it is precisely at longer distances that the consequences of 'ill-condensation' during the cooling process are reflected — and where the answers to these questions, and an understanding of the relationships between different materials, lie.

Salmon *et al.*[1] compare detailed measurements of the atomic arrangements of two simple binary glass-formers — zinc chloride ($ZnCl_2$) and germanium selenide ($GeSe_2$) — out to large inter-atomic separations, and demonstrate strong similarities between them. This is remarkable for two reasons. First, the nature of the interatomic interactions in the two materials is expected to differ, because $GeSe_2$ is more strongly covalent than $ZnCl_2$. Second, the two materials seem, from the temperature dependence of their viscosities (see ref. 3 and references therein), to approach the transition to the rigid glassy state in characteristically different ways. $ZnCl_2$ is the less 'fragile' liquid — its approach to rigidity is more continuous than that of $GeSe_2$, and similar to that of the silica of window glass. One might expect this difference to be correlated with a structural difference in the glasses at longer length scales.

Compared with a crystalline structure, the molecules in a normal liquid will be found at any given instant in a disordered, high-energy configuration — although at the level of the nearest neighbours the structure is almost always similar to that adopted in the solid. At high temperatures, the crystalline state, which minimizes energy, is not favoured because the number of disordered configurations, which give the liquid a higher entropy, is far greater. If a liquid is cooled slowly, the transition between the two states occurs at a sharply defined temperature (the freezing point), where the tendencies to maximize entropy and minimize energy are equal. At this point, groups of molecules adopt the same low-energy structure as the crystal; once these 'nuclei' exceed a critical size, they can grow spontaneously. The time taken to form such nuclei depends on the rate at which the molecules can reorganize themselves. This rate itself decreases as the temperature of the liquid is lowered — if this is done sufficiently rapidly, the system may reach such low temperatures that reorganization stops before the critical nuclei have a chance to form. The system is left in a rigid, amorphous state — it forms a glass.

This scenario could theoretically occur in any liquid. For a glass to form at practical rates of cooling (those that can be achieved in a laboratory), it is necessary for the formation of the critical nucleus to be 'frustrated', so that the process takes longer than usual. This can be brought about because the preferred local arrangement of the molecules does not readily propagate periodically out to the length scale required to form a crystal nucleus of the critical size. Therefore, experimental attention focuses on the intermediate-range structure of the glass-forming materials, where the frustration of nucleus formation should be apparent.

Salmon and colleagues' experiments are based on neutron diffraction, in which oscillations in the number of neutrons scattering at different angles reflect the relative positions of pairs of atoms. In binary systems such as $ZnCl_2$ and $GeSe_2$, the intensity pattern includes information on the distribution of all atom pairs (in the case of zinc chloride, for example, Zn–Zn, Zn–Cl and Cl–Cl). But Salmon *et al.* exploit the fact that neutrons scatter from different isotopes of the same element with different amplitudes: by performing experiments on three samples with the same chemical but different isotopic compositions, they were able to separate the diffraction pattern connected to each atomic pair and therefore examine the structure of the glass in exquisite detail.

Two characteristic length scales can be distinguished in the observed structures: first, a strong preference for the Zn or Ge atoms to have four Cl or Se nearest neighbours arranged at the local level as a tetrahedron, generating a chemical ordering that extends to large distances; second, an intermediate-range order, associated with the way these tetrahedra are linked together. Despite the differences in the chemistry of $ZnCl_2$ and $GeSe_2$, their structures are remarkably similar on both the short and intermediate length scales. Thus, different types of chemical interaction seem to have given rise to similar structures that, from the observation that both materials are good glass-formers, allow for the frustration of crystallization. On the other hand, even at this resolution there is no readily discernible structural feature that can account for the difference in the 'strength' of the two liquids, as seen in the temperature dependence of their viscosity. ■

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Cell biology

Sterol sensor comes up for air

Renee M. Garza and Randolph Y. Hampton

In one example of a feedback mechanism in mammals, cells switch cholesterol synthesis on or off depending on the availability of sterol. A rewired version of this pathway in yeast acts instead as an oxygen sensor.

Writing in *Cell*, Espenshade and colleagues[1] describe a previously unknown strategy by which cells sense oxygen levels. The mechanism uses an evolutionarily conserved and medically relevant pathway for sterol regulation in an unexpected way.

Over the past few years, a molecular drama has been unfolding in our understanding of how cholesterol synthesis is regulated in mammals. The overarching idea is simple: when cells need more cholesterol, they increase the levels of enzymes that make it, by increasing the expression of the enzyme-encoding genes. But the underlying mechanism for this is quite unexpected. Thanks to heroic work led by Brown and Goldstein[2], we now have a clear idea of how cholesterol-regulated gene transcription occurs. Perhaps the only unsurprising feature of this transcriptional regulation is that it centres on a transcription-factor protein: SREBP (for 'sterol-regulatory-element-binding protein').

The SREBP molecule contains a portion that carries out transcription ('TF' in Fig. 1a, overleaf), connected to a transmembrane domain. Freshly made, full-length SREPB is anchored in the membrane of a cellular compartment called the endoplasmic reticulum (ER) — tethered like a pit bull on a chain, and unable to reach its targets in the nucleus. Active SREPB is liberated from its membrane anchor by cleavage, and it is this cleavage that is controlled by sterol levels. Although SREBP resides in the

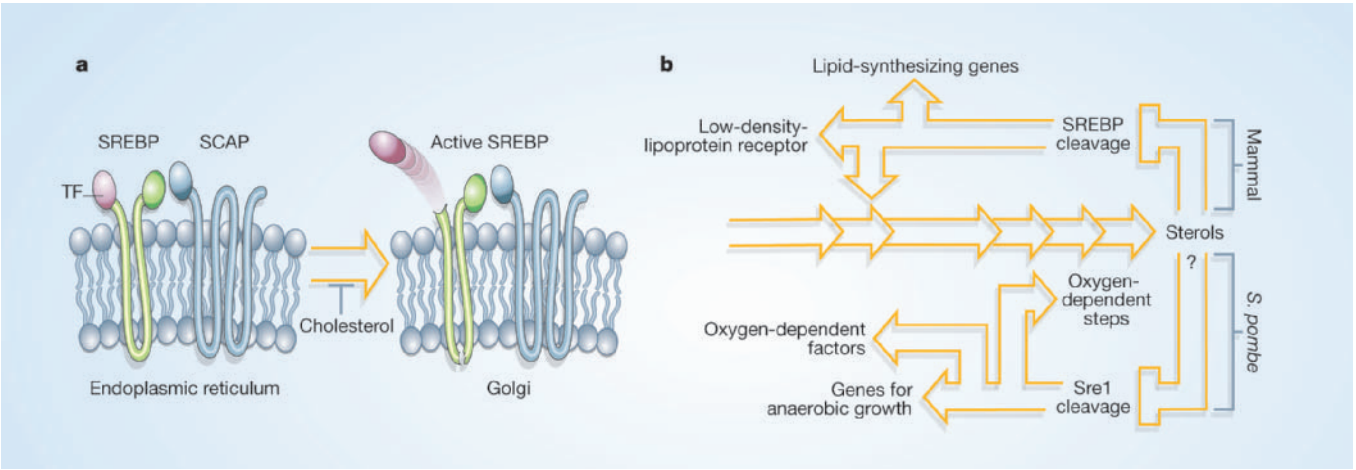

Figure 1 **New tricks for a familiar pathway.** a, **The SREBP-processing pathway. The gene-transcription factor SREBP, which regulates sterol biosynthesis, resides in the endoplasmic reticulum in mammalian cells. It is transported to the Golgi by SCAP, where it is cleaved twice to release an active transcription factor (TF). Binding of cholesterol to SCAP inhibits such transport[2].** b, **Top, mammals use SCAP-dependent cleavage of SREBP to provide feedback control of cholesterol synthesis, and to regulate other genes involved in lipid metabolism. Thus, if sterol levels are high, SREBP cleavage is inhibited, and these genes are no longer activated. Bottom, the newly discovered 'rewired' pathway from fission yeast[1]. The membrane-bound SREBP counterpart Sre1 is regulated by sterols (or possibly other signals) through Scp1, to control genes involved in oxygen-dependent functions and those required for anaerobic growth. This pathway seems to function to sense and respond to oxygen.**

ER membrane, the two protein-cleaving enzymes that liberate the active fragment occur in another compartment, the Golgi. When sterol levels are sufficiently low, SREPB is carried from the ER to the Golgi, where cleavage — and thus activation — occurs. When sterol levels are high, the movement of SREBP to the Golgi (and hence cleavage) is blocked.

This 'traffic control' is effected by the protein SCAP (for 'SREBP-cleavage-activating protein'). SCAP binds to SREBP and carries it out of the ER by entering the vesicular pathway that shuttles between the ER and the Golgi. SCAP also has a membrane-embedded motif that directly binds cholesterol. Cholesterol-bound SCAP no longer exits the ER, and so cholesterol inhibits production of active SREBP (Fig. 1a), thereby governing its own synthesis.

Genome sequencing studies show that *Schizosaccharomyces pombe* (fission yeast) also has versions of SREBP and SCAP, called Sre1 and Scp1, respectively. Given that this fungal species synthesizes cholesterol's cousin ergosterol, this is perhaps to be expected. But the surprises came when Espenshade and colleagues[1] explored the molecular details of the *S. pombe* feedback pathway.

The authors found that Sre1 is cleaved to a soluble form and that this cleavage is regulated by sterol in an Scp1-dependent manner, just as in mammals. However, the transcriptional targets of Sre1 are quite distinct. Testing of candidate genes, combined with microarray techniques to analyse gene expression more broadly, showed that the early, rate-limiting reactions of sterol synthesis — those regulated by mammalian SREBP — were completely unaffected by changing levels of active Sre1. Consistent with this, loss of either Scp1 or Sre1 through mutation had no effect on the growth of *S. pombe*, whereas in mammals loss of the SREPB pathway renders cells totally dependent on added sterols.

Instead, Espenshade and colleagues' analysis indicated that the relevant regulatory function pertains to oxygen. Genes regulated by Sre1 include those encoding: enzymes in the late, oxygen-dependent parts of the sterol-synthesis pathway; enzymes required for the biosynthesis of haem (an oxygen-binding molecule); and several other oxygen-related factors, including some that are needed for yeast cells to survive low oxygen levels. Thus, it seems that sterol-regulated cleavage of Sre1 is used to signal oxygen availability (Fig. 1b).

The authors go on to show that several predictions of their oxygen-sensing model are borne out. First, *S. pombe* cells that lack Sre1 or Scp1 cannot survive in anaerobic conditions. These mutant cells do survive, however, if allowed to express the cleaved form of Sre1 (the 'TF') by molecular-biological trickery. Furthermore, the presence of Sre1 and Scp1 enables the cells to adapt to low-oxygen conditions in ways that would be predicted if these proteins were involved in oxygen sensing — for instance by increasing the cells' capacity for the oxygen-dependent reactions of ergosterol synthesis. Finally, the cleavage of Sre1 is massively stimulated by lowering the oxygen concentration, indicating that the relevant perturbation indeed causes the proposed physiological response.

Although we don't know what signal regulates Scp1-mediated cleavage of Sre1, it is reasonable to suppose that it is a sterol — perhaps ergosterol (the end result of the sterol pathway in *S. pombe*), but possibly some other sterol generated in the pathway, or a derivative of one of the pathway molecules. And perhaps a second oxygen-dependent signal works together with sterols to regulate Sre1 cleavage. There seems to be great flexibility in the kinds of molecules that can be sensed by the sterol-sensing motif found in SCAP and related proteins[3–5], so it may be best to await the next chapter in this new use of the SREPB pathway.

Whatever the specific signals, this 'rewiring' of the SREBP pathway makes a lovely kind of sense. Synthesis of sterols is absolutely dependent on oxygen. Thus, the choice of sterol synthesis as a fiduciary indicator of oxygen levels is a good one, and one that probably exists in more than one yeast species.

This ground-breaking study raises some fascinating questions. Is this a broadly used mode of oxygen sensing in nature? Does it occur in many fungi, and thus provide an Achilles' heel that can be exploited to develop new antifungal drugs? What is the range of molecules sensed by the many SCAP counterparts found in nature? The emerging picture is that these membrane proteins may be widely used sensors of intramembrane signals that affect any aspect of biology in which lipid molecules are involved. ■

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