with even higher energy densities exist, this constant-force configuration allows the most energy to be stored by a rotary motor that can continuously apply a maximum torque output to match the force needed to compress the spring.

Finally, the spring is even heavier than the motor in Hawkes and colleagues' jumping robot. This contrasts with biological jumpers, whose muscles are typically much more massive than the springs they use to directly power the jump[9]. This unexpectedly high ratio between spring and motor mass is a result of the relative energy limitations of these components: the energy density of the spring limits the robot's jump height, whereas the work density of muscle limits the amount of energy that biological jumpers can store in their springs.

A surprising feature of Hawkes and colleagues' robot is its relatively large size. The best jumpers propelled by springs in biology are typically limited to having masses of several grams or less[4,5], whereas jumpers with larger mass (such as humans) benefit from direct muscle power instead of spring actuation. But this robot has a mass of 30 grams, which makes it more than ten times heavier than the largest spring-actuated jumping organisms that inspired it[5]. This is due to the 10-gram mass of the rotary motor used in this jumper, and because the highest jumps possible for Hawkes and co-workers' robot require spring mass to exceed motor mass. Although the team did not explore further limitations, such as battery energy density, the study implies that robots larger than theirs, with bigger springs, would jump even higher.

Further research is still needed for these jumping systems to achieve their full potential. Hawkes and colleagues' robot is limited to a single jump height — just like the majority of previously reported jumping robots[6,10,11]. Resource constraints mean that these systems are not designed to control the amount of energy stored and released during each jump. But to operate in a 'real world' environment, these systems will need to be able to control their jumps. One way of doing that could involve a redesign of the latch that releases the energy during each jump, as has been proposed previously[12]. Another approach to precision jumps could come from controlling the launch angle[13]. It is also crucial that the jumps these robots perform are repeatable. Although Hawkes and co-workers' robot can reset itself and jump repeatedly, it takes two minutes between jumps to reload the spring. By contrast, other jumping robots that use different strategies to maximize power can jump again immediately on landing[14].

Hawkes *et al*. have succeeded in using biology as inspiration for their design, while circumventing the limitations of living systems through clever engineering. Instead of having a jump height limited by the amount of work a muscle can do in a single contraction, for example, the authors' robot is limited only by its spring and its battery. The robot also makes use of the fact that constant-force springs made from carbon fibre and rubber have much higher energy densities than do those made from the limited set of materials available to biological jumpers. The work therefore serves as a reminder that biologically inspired engineered systems need not incorporate biological limitations.

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Structural biology

Stalk twists into a hook in adhesion receptors

Antony A. Boucard

The inner workings of a family of proteins, known as adhesion G-protein-coupled receptors, have finally been visualized at high resolution — revealing the structural basis of their self-activation mechanism. **See p.757, p.763, p.771 & p.779**

Cells need a means of communicating with their surroundings to survive. Membrane proteins in the family of G-protein-coupled receptors (GPCRs) help cells to sense their micro-environment by responding to various stimuli, ranging from light to hormones. A subfamily known as the adhesion GPCRs (aGPCRs) has a role in sensing the interactions of cells with other cells and with the extracellular matrix (the material that surrounds cells). These receptors are unusual because many of them activate themselves. The structures of many types of GPCR in their active states have been reported, but not those of self-activated aGPCRs. A quartet of papers by Barros-Álvarez *et al.*[1] (page 757), Ping *et al.*[2] (page 763), Qu *et al.*[3] (page 779) and Xiao *et al.*[4] (page 771) now addresses this issue, and sheds light on the mechanism involved in initiating the sensing function of aGPCRs.

As biological systems evolved to become multicellular, the number of things they interacted with increased, thereby expanding the variety of external factors that could trigger a response. Cells needed to explore their micro-environments to seek nutrients, trigger defence mechanisms or form tissue, for example. This, in turn, required them to develop strategies to navigate constantly changing environmental conditions.

The advent of cells with molecular appendages that served as sensors, interfacing with the surroundings, aided cellular movement by allowing changes in cell behaviour to be stimulated in response to externally acquired information. Adhesion molecules extending from the cell membrane acquired this sensing ability, because their subcellular localization put them in the front line to probe myriad cell–cell and cell–matrix interactions. The evolution of a diverse array of these molecular sensors — the GPCRs — was key to ensuring that cells can respond to as many insults or encounters as possible.

Today, GPCRs are the largest family of membrane proteins encoded by the human genome[5]. Groundbreaking advances have revealed how GPCR structures change on contact with an external stimulus, and how they communicate these changes within cells to alter cell behaviour through the induction of signalling cascades. But, until recently[6], the membrane-spanning structures of one subfamily of GPCRs remained conspicuously unexplored: the aGPCRs. This knowledge gap is now decisively being filled with the publication of the current breakthrough studies.

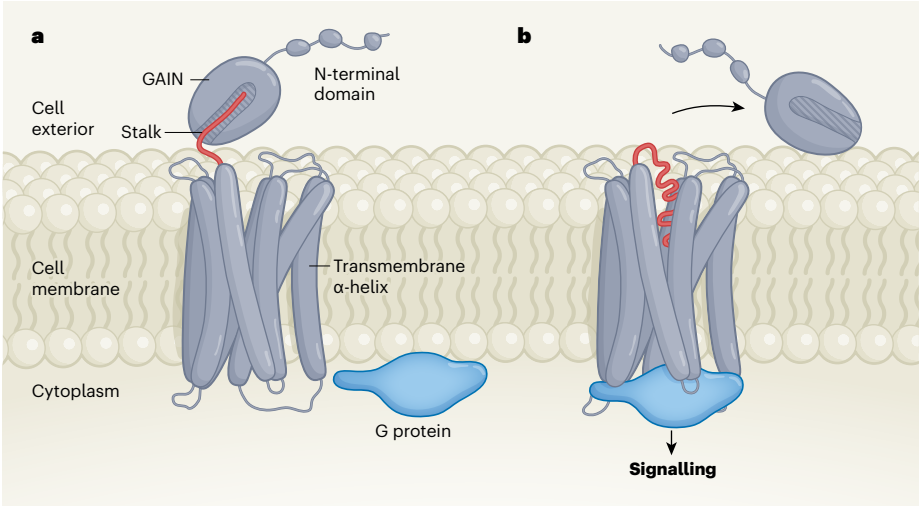

Figure 1 | Stalk-mediated sensing in adhesion G-protein-coupled receptors (aGPCRs). a, Proteins in the aGPCR family contribute to cell adhesion, and also activate cellular G proteins in response to stimuli at the cell membrane, thereby initiating intracellular signalling cascades. Signalling is carried out by the seven-transmembrane (7-TM) domain, a system of seven α-helices that span the cell membrane. The amino-terminal domain is found outside the cell membrane and is responsible for adhesion. In the inactivated receptor, a region known as the stalk is embedded in the GPCR auto-proteolysis-inducing (GAIN) domain. The embedded stalk adopts a β-strand conformation. Here, the GAIN domain has cleaved the receptor at the N-terminal end of the stalk. **b**, On activation, the stalk moves into the 7-TM domain, and the GAIN and N-terminal domains can dissociate from the rest of the receptor. Four papers[1–4] now report that the stalk adopts a twisted, hook-shaped conformation in the 7-TM domain. The hook forms contacts within the receptor that leads to coupling of a G protein, which activates signalling. The exact position and structure of the GAIN domain are unclear from the reported structures.

To understand the findings, consider the nature of aGPCRs. As their name implies, these receptors have two functions: they contribute to cell adhesion by participating in cell–cell or cell–matrix interactions; and they respond to the stimulus of cell adhesion by activating cellular G proteins, thereby initiating intracellular signalling cascades. The adhesion function is restricted to an extracellular region of the receptor (the amino-terminal domain; Fig. 1a), which is often larger than whole receptors from other GPCR subfamilies. The signalling function is carried out by the seven-transmembrane (7-TM) domain: a system of seven α-helices that spans the cell membrane.

Separating these two regions is a cleavage site engulfed by a structural domain known as the GPCR auto-proteolysis-inducing (GAIN) domain[7]. Most aGPCRs cleave themselves at this site, generating two fragments that are then held together through non-covalent interactions mediated by the GAIN domain. Next to the cleavage site, nested in the GAIN domain, is a 'stalk' region (also known as a Stachel sequence) that is evolutionarily highly conserved in aGPCRs and has been unequivocally linked to the receptors' activities[8,9].

A question about the stalk region has long persisted: how can this activation-inducing region mediate contact with the 7-TM signalling domain if it is deeply buried in the GAIN domain? The 'cryptic ligand' hypothesis[8] suggests that, aided by self-cleavage of the receptor, the stalk region dissociates from the GAIN domain and then moves to another enclave in the 7-TM domain. This hypothesis has been unilaterally supported by biophysical evidence[10]. The four new studies provide the first visualizations of the position of the stalk region in self-activated aGPCRs.

In each of these studies, the authors used cryogenic electron microscopy to obtain high-resolution structures of two different aGPCRs coupled to their respective G proteins. The high flexibility of the very large N-terminal domain could potentially produce non-uniform structures that would make characterization difficult, and so the research teams mainly characterized mutant versions of the receptors that correspond to cleavage-generated products, comprising just the stalk region and the 7-TM region. The results are surprisingly concordant: the studied receptors all share an overall similar structure, with the stalk region embedded between transmembrane domains (Fig. 1b), thus confirming the cryptic-ligand hypothesis.

Although the stalk region is known to maintain a β-strand conformation when embedded in the GAIN domain[7], the structures show that it adopts a twisted, hook-shaped configuration when bound in the 7-TM enclave – which contains a clearly defined binding pocket enclosed by transmembrane helices and extracellular loops of the receptor. Using a combination of techniques, the four teams identified amino-acid residues in the binding pocket that make contact with the stalk and contribute to the sensing activity of aGPCRs.

Importantly, contrary to what was previously thought, Qu and colleagues' analysis of aGPCR mutants that do not undergo self-cleavage reveals that such cleavage is not a prerequisite for the stalk to insert into the 7-TM binding pocket. This is of particular interest for two main reasons: it provides clues as to why receptors reported not to self-cleave still possess signalling activity; and it unveils a mechanism through which stalk insertion could occur while maintaining the anchoring of the N-terminal domain to the 7-TM region, even in cleavable activated receptors, to preserve cell–cell or cell–matrix adhesive junctions. The alternative mechanism for stalk translocation, in which the stalk is extracted from the GAIN domain, remains unknown.

Three of the teams[1,3,4] attempted to determine the structures of the N-terminal regions of aGPCRs in constructs that also contained the 7-TM region. However, all were unsuccessful. This suggests either that the N-terminal domains were actually absent from the constructs, or that these domains have highly dynamic and flexible structures. Such dynamic flexibility would be handy in the context of these domains being used by cells to explore the surrounding environment, because it would increase their chances of coming into contact with something.

The sequence of events involved in the mechanism of action of aGPCRs is still incomplete. For this, a fully resolved structure of a whole receptor is needed, so that the N-terminal domain is pictured alongside the other domains. Furthermore, aGPCRs bind to a diverse range of ligand molecules, each of which might modulate signalling in different ways. Structures of aGPCRs in complex with both their ligands and their G proteins are therefore also needed – although obtaining these structures will be a colossal task.

Because aGPCRs are involved in many human diseases, there is a need to develop compounds that help to regulate their activity. The structures reported in the four current studies will aid the rational design of molecules that target these receptors, specifically at the stalk's binding pocket or, alternatively, at allosteric sites – regions distant from the binding pocket, but at which the binding of a small molecule can still modulate receptor activity. A major challenge in any drug-discovery programme is to make compounds that bind highly selectively to the target protein, thus preventing side effects caused by binding to other proteins. This will be particularly difficult in the case of aGPCRs, because the amino-acid sequences of stalk regions are highly evolutionarily conserved in the different aGPCR families[9]. Nevertheless, the current structures are sure to attract the attention of scientists in the pharmaceutical industry.

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Palaeontology

A colourful view of the origin of dinosaur feathers

Michael J. Benton

Birds and their dinosaur ancestors had feathers, and now it seems that a distantly related group called pterosaurs had them, too. The finding extends the origins of feathers back to long before birds evolved, and sheds light on their role. **See p.684**

In a simple world, an easy classification rule is that birds have feathers, mammals have hair and reptiles have scales, as did dinosaurs. However, the world is not necessarily simple, and it has been known for more than 25 years that at least some dinosaurs had feathers[1]. Previously, it was suggested[2] that flying creatures called pterosaurs – extinct distant relatives of birds and dinosaurs – also had feathers, but that idea was controversial[3]. On page 684, Cincotta *et al.*[4] confirm that pterosaurs possessed feathers, and also report that these feathers and their surrounding skin were coloured, perhaps providing signalling cues to other individuals of their kind.

Pterosaurs include more than 100 species of leathery-winged flying reptile. They are close relatives of, but clearly distinct from, dinosaurs. Pterosaurs and dinosaurs are preserved in the fossil record from roughly 230 million or 220 million years ago to 66 million years ago, a time frame that spans from part of the Late Triassic epoch to the mass extinction at the end of the Cretaceous period. Pterosaurs had large heads (Fig. 1) with sharp snouts, long necks, small bodies, tails of varying lengths and large wings made from a skin membrane that extended behind a 'leading edge' comprising the arm bones and a very long fourth finger. From the time of the early discoveries about these creatures, palaeontologists learnt that pterosaurs' bodies were covered in a 'fuzz' of short, whisker-like structures, which almost certainly provided heat insulation[5]. But was this pterosaur fluff composed of feathers?

Cincotta and colleagues describe the presence of diverse monofilaments (simple single-stranded whiskers) and branching feathers in the pterosaur *Tupandactylus*, in a fossil found in the Early Cretaceous period of Brazil (113 million years ago). Proposed fossil feathers have sometimes been rejected as being instead pieces of shredded skin or other tissue, overlapping monofilaments or degraded structures of some kind[3]. However, the detail of the regular branching in these structures inserted in the skin provides support for their identification as feathers.

Moreover, the feathers contain structures called melanosomes – capsules in feathers or hairs in which the pigment melanin resides. In modern birds and mammals, many of the dominant colours of feathers and hair come from a limited range of chemically distinct forms of melanin, mainly comprising eumelanin, which gives rise to black, brown, grey and blond colours, and phaeomelanin, which produces ginger colours. Melanin also occurs in the skin and in many internal organs, so it is important to be sure that the melanosomes are truly inside the feathers, not next to them. In this case, the feather melanosomes are definitely impressed into the tissues that represent the original keratin structural component of the feather.

Cincotta *et al.* report tissue-specific melanosome geometries – distinctive shapes of individual melanosomes and characteristic packing arrangements – in both feathers and skin tissues of the fossils, and these geometries indicate patchy distributions of colour. *Tupandactylus* was a large animal, with an estimated wingspan of 5 metres. It had a lightweight but huge head, with toothless jaws and two long, slender, bony rods, like the sail-supporting masts and spars of a sailing ship: one extending straight back and the other forming a near-vertical leading edge. In life, these spars supported a skin membrane that was covered with patchy coloured skin that, in turn, bore a short fuzz of coloured feathers.

The various species of *Tupandactylus* and their relatives had differently shaped crests (the structures built from skin stretched over the bony spars), each bearing irregular, large spots of colour – these crests are generally interpreted[6] to have been for signalling between individuals. Perhaps they were used

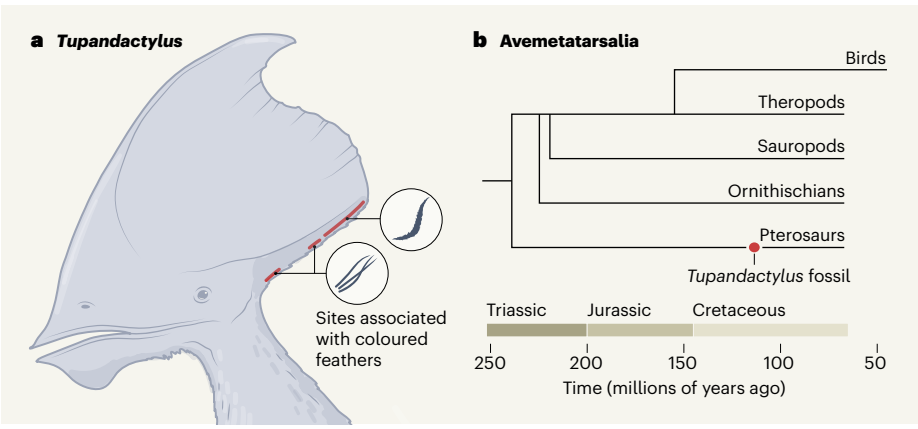

Figure 1 | Coloured feathers evolved early. a, Cincotta *et al.*[4] analysed a 113-million-year-old fossil of *Tupandactylus* – a species of flying vertebrate called a pterosaur. Pterosaurs and dinosaurs (theropods, sauropods and ornithischians) became extinct at the end of the Cretaceous period (66 million years ago). The authors report that the animal's head had coloured feathers – including both whisker-like monofilaments and branched feathers. The feathers contained capsules called melanosomes (not shown), which harbour the pigment melanin. The specific feather colour(s) are unknown. The colouring might have aided signalling between pterosaurs during mating displays. A complete head is shown, although the fossil is of a partial head. **b**, This discovery, along with other findings, might mean that coloured feathers evolved in the Triassic epoch, when the clade Avemetatarsalia, which includes pterosaurs, birds and dinosaurs, originated around 250 million years ago[10].