generated mice in which sympathetic neurons could be acutely activated, and found that overactivation of the SNS in these mice caused greying in the absence of stress. Together, these results indicate that noradrenaline released from active sympathetic neurons triggers MeSC depletion (Fig. 1b). Interestingly, Zhang *et al.* found that the propensity of an area to turn grey correlates with its level of sympathetic innervation.

Exactly how does sympathetic activity cause depletion of MeSCs from hair follicles? Normally, these stem cells are maintained in a dormant state until hair regrowth is required. However, when the researchers tracked MeSCs labelled with a fluorescent protein, they discovered that MeSC proliferation and differentiation increase markedly under extreme stress or exposure to a high level of noradrenaline. This results in mass migration of melanocytes away from the bulge, and leaves no remaining stem cells. To further confirm this result, the researchers suppressed MeSC proliferation pharmacologically and genetically. When proliferation was dampened, the effects of stress on MeSC proliferation, differentiation and migration were blocked.

Zhang and colleagues' work raises several questions. For instance, is the mechanism underlying MeSC depletion in response to stress the same as that which causes greying during ageing? Future experiments modulating SNS activity over a longer period would determine whether age-related greying can be slowed or hastened. Perhaps, in the absence of sympathetic signals, MeSCs have the capacity for unlimited replenishment, pointing to a way to delay age-related greying.

Are other pools of stem cells similarly susceptible to stem-cell depletion in response to stress, if they or the cells that make up their niche express β_2-adrenergic receptors? In support of this idea, haematopoietic stem and progenitor cells (HSPCs), which give rise to blood and immune lineages, reside in a bone-marrow niche that contains stromal cells, and stimulation of those cells by the SNS causes HSPCs to leave their niche[11,12]. Perhaps, like MeSCs, stress depletes HSPCs — which could partially explain why immune function is impaired in response to chronic stress[13,14]. Whether this type of relationship extends beyond MeSCs and HSPCs is an open question.

It is fascinating to consider what possible evolutionary advantage might be conferred by stress-induced greying. Because grey hair is most often linked to age, it could be associated with experience, leadership and trust[15]. For example, adult male silverback mountain gorillas (*Gorilla beringei beringei*), which get grey hair on their backs after reaching full maturity, can go on to lead a gorilla troop[16]. Perhaps an animal that has endured enough stress to 'earn' grey hair has a higher place in the social order than would ordinarily be conferred by that individual's age.

Connecting the dots between stress, fight or flight, stem-cell depletion and premature greying opens up several avenues for future research. Beyond developing anti-greying therapies, Zhang and colleagues' work promises to usher in a better understanding of how stress influences other stem-cell pools and their niches.

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Cardiovascular biology

Suspect that modulates the heartbeat is ensnared

Xiaohan Wang & Richard W. Tsien

The activity of calcium channels in the heart increases during what is called the fight-or-flight response. An investigation into the 50-year-old mystery of how this occurs has captured a previously overlooked suspect. **See p.695**

In the Sherlock Holmes tale *The Adventure of the Dancing Men*, the detective runs a heart-pounding race to try to save his client's life. The thumping of the sleuth's heart — a literary example of the 'fight-or-flight' effect[1] — reflects the changes that occur when the entry of calcium ions into the heart rises[2]. On page 695, Liu *et al.*[3] provide a solution to the long-standing riddle of how this occurs, through deductions worthy of Sherlock Holmes.

Some aspects of how calcium enters the heart during a fight-or-flight response are known. The process is mediated by the hormone adrenaline acting on β-adrenergic receptors — proteins that reside in the surface membrane of heart cells called cardiomyocytes. Receptor activation leads to an increase in the opening of what is called an L-type voltage-gated calcium channel. This occurs through a mechanism that involves the molecule cyclic AMP (cAMP)[4,5] and an enzyme called protein kinase A (PKA) that requires cAMP for its function[6]. Similar types of PKA-mediated processes are found in other contexts. For example, some neurons use cAMP and PKA to enhance calcium entry through L-type calcium channels[7].

Exactly how the stimulation of β-adrenergic receptors modulates calcium-ion influx has been debated since the 1970s. Researchers have uncovered tantalizing clues to the identity of the target molecule that PKA modifies by the addition of a phosphate group (phosphorylation). However, proposals for specific candidate targets, phosphorylation sites[8–10] and modulatory mechanisms have been repeatedly called into question by further tests, including some in painstakingly constructed mouse models[11–13]. Liu *et al.* use a powerful technique called proximity proteomics[14] to implicate a previously under-appreciated suspect[15] and to establish its role.

L-type voltage-gated channels provide a route by which calcium ions enter cardiomyocytes to help trigger a heartbeat. If channel opening is boosted, this results in a stronger and faster heartbeat. Previous investigations of how PKA might modulate channel opening focused mainly on amino-acid residues in the channel that occur in structural motifs possibly phosphorylated by PKA. But when Liu and colleagues performed a tour-de-force experiment in mice in which the channel was engineered so that all candidate

phosphorylation sites were converted to an amino acid (alanine) that can't be phosphorylated, and when this channel was studied alone, PKA-mediated enhancement of L-type channels nevertheless persisted. The authors therefore looked elsewhere for the elusive mediator of PKA's ability to regulate the fight-or-flight effect.

Reasoning that some unknown factor must come into close proximity to the calcium channel during this regulatory process, the authors conducted a systematic search. Using proximity proteomics, Liu and colleagues engineered channel subunits to contain an enzyme that adds a tag called biotin to any protein within a radius of approximately 20 nanometres[15]. Tagged proteins were then identified by mass spectrometry. Hundreds of proteins in proximity to the calcium channel were analysed, and the authors found that the protein Rad was enriched in the channel microenvironment under resting conditions, but was noticeably depleted during stimulation of the β-adrenergic receptor. This dovetailed with an earlier clue — Rad is known to inhibit L-type voltage-gated calcium channels[15], and, in mice, deletion of the gene that encodes Rad mimics the effect of β-adrenergic stimulation and eliminates further adrenaline-mediated enhancement of the activity of L-type channels[16].

Liu *et al.* investigated whether PKA could prevent Rad-mediated channel inhibition. The authors tested whether phosphorylation of amino-acid residues on Rad would enable it to move away from the vicinity of the calcium channel. They narrowed the candidate residues down to four serines (in some experiments, just two), which, if replaced by alanine, abolished PKA-mediated regulation of calcium entry.

The calcium channel's β-subunit was the prime suspect as the target of Rad inhibition. Ablation of the interaction between the calcium channel's α_{1C}-subunits and its β-subunits fully eliminates PKA-mediated modulation of channel activity[17]. Indeed, the authors' measurements, using a technique called fluorescence resonance energy transfer, showed that the interaction between Rad and the calcium-channel β-subunit was inhibited by PKA phosphorylation of the key serines in Rad that the authors had identified. Further tightening the noose around Rad's metaphorical neck, electrical recordings demonstrated that all of the biophysical fingerprints of modulation by β-adrenergic signalling — such as the activity of previously inactive calcium channels and a shift in the voltage dependence of their activation[18] — were prevented by eliminating Rad phosphorylation.

The results make a compelling case for the following scenario (Fig. 1). Adrenaline binds and activates the β-adrenergic receptor. This, in turn, results in the activation of an enzyme that produces cAMP, which activates PKA. PKA phosphorylates Rad and causes it to leave the vicinity of the calcium channel, thereby preventing it from inhibiting the channel.

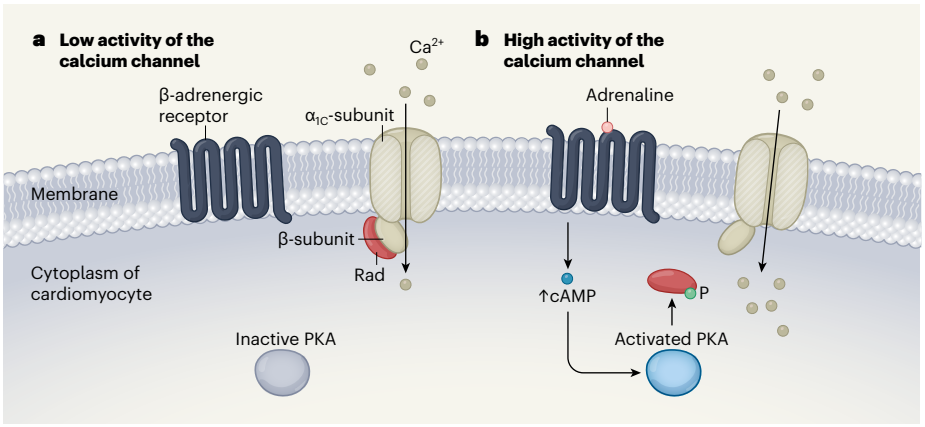

Figure 1 | Modulation of the cardiac calcium channel. In heart cells called cardiomyocytes, the activity of calcium-ion channels increases during what is called the fight-or-flight response. Activation of the enzyme protein kinase A (PKA) is required for this process, and, in mouse studies, Liu *et al.*[3] reveal that its elusive target is the protein Rad. **a**, In the absence of a fight-or-flight response, the β-adrenergic receptor is not stimulated and PKA is inactive. Rad binds to a subunit of the calcium channel (beige; only the α_{1C}- and β-channel subunits are shown) and calcium-ion (Ca^{2+}) entry into cardiomyocytes is low. **b**, During the fight-or-flight response, the hormone adrenaline activates the β-adrenergic receptor. This leads to the production of cyclic AMP (cAMP) molecules, which activate PKA. Activated PKA adds a phosphate group (P) to Rad, causing Rad to dissociate from the channel and enabling channel activity to increase. This elevation of Ca^{2+} in the cytoplasm boosts the heartbeat.

The study puts Rad and other members of this family of proteins front and centre as players in calcium-channel modulation. Is Rad the entire missing chapter in the story of PKA's role in the heart, given Liu and colleagues' compelling arguments that other potential PKA targets are unnecessary? Sceptics will want further *in vivo* evidence from a type of mouse model termed a knock-in — animals whose original Rad sequence is replaced either with a version in which Rad's own PKA-phosphorylation sites are mutated or with a version in which the part of Rad needed for the interaction with the β-subunit is eliminated — to see whether any PKA-mediated modulation of the calcium channel still occurs. Hints of differences between channel regulation in the embryonic and adult heart[13] also warrant further study.

"The study puts Rad and other members of this family of proteins front and centre as players in calcium-channel modulation."

Might cardiac regulation by Rad be of clinical value? Heart failure in humans is associated with loss of regulation of calcium channels by β-adrenergic receptors. Rad levels fall during heart failure[19], perhaps providing a temporary increase in the strength of heart contraction[16]. However, this would also reduce the heart's ability to further increase its strength[20], what is known as its functional reserve, which would be a severe price for a person's heart to pay.

There will undoubtedly be debate about how PKA modulation of calcium channels operates in neurons, such as in PKA-responsive CA1 pyramidal cells in which Rad is essentially absent. In those neurons, the mutation of a particular serine (serine 1928) to alanine in the L-type channel eliminates channel modulation and L-type channel-dependent strengthening of inter-neuronal (synaptic) connections[7]. Here, PKA might be phosphorylating the calcium channel, after all.

Organ-specific pathways for regulation would make functional sense. Rad can completely inhibit calcium-channel activity, and so modifying such inhibition would give heart cells a wide range of regulatory capability[18], suitable for a brief flight-or-fight response. Perhaps other cell types needing a more sustained but subtler boost to their calcium-channel activity might operate better without Rad-mediated regulation and rely instead on milder, more direct modulation of a subunit of the calcium channel.

Liu and colleagues have set a high bar for future detective work on cellular signalling in the heart. Their work shows the power of a systematic round-up of suspects and relentless interrogation of their roles.

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Condensed-matter physics

A milestone in the hunt for metallic hydrogen

Serge Desgreniers

An optical study of cold solid hydrogen at extreme pressures indicates that electrons in the material are free to move like those in a metal. This suggests that the long-sought metallic phase of hydrogen might have been realized. **See p.631**

Hydrogen is the most abundant element in the Universe. Its molecular-gas state is simple, but its solid state has proved to be complex. In 1935, it was predicted that solid hydrogen should behave like an electrical conductor at elevated pressures, owing to its molecules being separated into their atomic constituents[1]. This prediction heralded a race to prove experimentally that solid hydrogen displays such metallic behaviour under ultrahigh compression. However, although there have been many claims of proof (for example, refs 2–4), these studies have been challenged. Now, on page 631, Loubeyre *et al.*[5] report that dense hydrogen shows a discontinuous and reversible change in optical reflectivity at extreme pressure and low temperature that can be attributed to a phase transition into a metallic state.

It is common practice to use a device called a diamond anvil cell to achieve ultrahigh compression of a material and to study changes in the material's physical properties at high density. A diamond anvil cell squeezes a sample, which is confined to a microscopic chamber in a thin metal foil, between two diamond anvils (Fig. 1a). The device operates on a deceptively simple physical concept: pressure is inversely proportional to the area of a surface over which a force is applied. In the present case, this simplicity comes with an inherent drawback: reaching extreme pressures inevitably implies working with tiny sample volumes.

Conventional techniques have been the bottleneck in applying extreme pressures to highly compressible materials such as hydrogen. Over the past few decades, research groups around the world have pushed the boundaries of pressure generation. They have also refined the tools and methods needed to accurately estimate pressures applied to a microscopic sample of compressed gas. Nevertheless, debate continues over the accuracy of reported pressures and the interpretation of results drawn from measurements of physical properties.

Recognizing this long-standing problem, Loubeyre and colleagues' research group developed an innovative approach that involves the precise sculpting of diamond-anvil surfaces using a stream of massive ions[6] – a technique called focused ion-beam milling. A similar experimental development has also been reported[7]. The profiled anvils produce extreme pressures that can be reliably estimated, reaching more than 400 gigapascals (about 4 million times Earth's atmospheric pressure). Moreover, the shape of the anvils helps to confine dense hydrogen samples that are suitable for optical measurements.

Under increasingly extreme pressures, dense hydrogen becomes more and more opaque to visible light. For pressures in excess of about 300 GPa, solid hydrogen becomes penetrable only by electromagnetic radiation of lower energy than visible light[2–4,8], such as infrared radiation (Fig. 1b). Loubeyre *et al.* measured the optical transparency of solid hydrogen at pressures much higher than those reached previously, using the near-to-mid-infrared emission from a source of synchrotron radiation – electromagnetic radiation that is produced when charged particles are accelerated in a curved path.

The authors found that a compressed sample of hydrogen blocks all light and exhibits an abrupt increase in optical reflectivity when the pressure is raised above 425 GPa (Fig. 1c). Moreover, they discovered that this transition is reversible. The authors

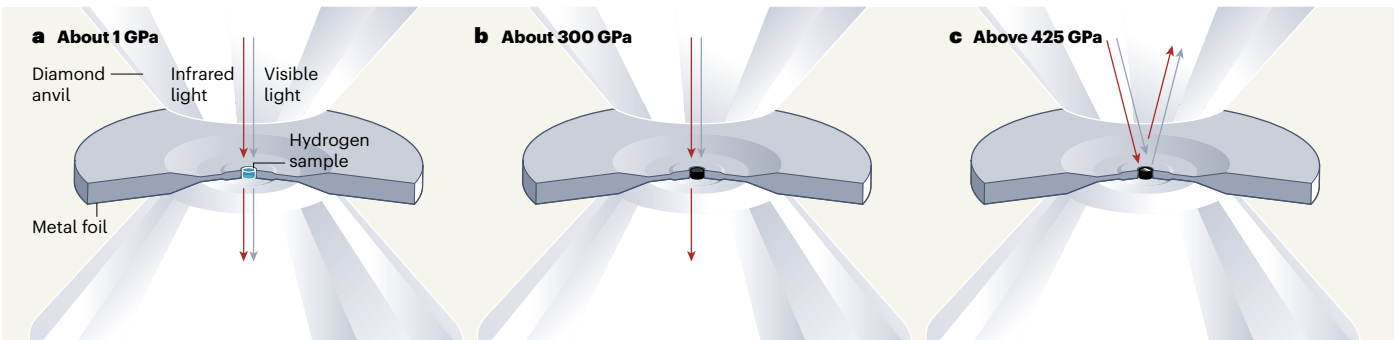

Figure 1 | Effect of increasing pressure on cold solid hydrogen. a, Loubeyre *et al.*[5] have studied solid hydrogen at extreme pressure and low temperature using a device known as a diamond anvil cell. This device compresses a sample of the material, which is confined to a microscopic chamber in a thin metal foil, between two diamond anvils. At first when the pressure is applied, the sample is transparent to both infrared and visible light (GPa, gigapascals). **b**, When the pressure is raised to roughly 300 GPa, the dense hydrogen loses its transparency to visible light. **c**, Finally, when the pressure is above 425 GPa, the sample becomes reflective to both infrared and visible light, indicating a shift into the long-sought metallic state of hydrogen.