Prominent experimental examples of quantum control include preparing and stabilizing quantum states of a microwave signal that bounces between a pair of mirrors known as an optical cavity[3], and controlling the state of a superconducting quantum bit of information[4]. Progress in quantum control has been rapid during the past few decades, and researchers have been extending these techniques to other physical systems to exploit the advantages that different systems provide.

One such area under development is cavity quantum optomechanics, in which laser light inside an optical cavity is used to control the motion of a mechanical oscillator. Central to this field of research is the radiation–pressure interaction, whereby the reflection of light from an object modifies the object's momentum and, concurrently, causes the light to acquire a phase shift — a shift in the crests and troughs of the light's electric field — that depends on the object's position. Using this interaction, physicists can both precisely measure and control mechanical motion.

A key goal in optomechanics research has been to bring mechanical motion close to its ground state — the state that describes the tiny amount of jiggling that is imposed by quantum mechanics, even at absolute zero temperature. Realizing this state is a convenient starting point for future quantum experiments that would otherwise be unfeasible because of random heat-induced fluctuations of the mechanical motion.

A common route to achieving this goal is sideband cooling — a technique that uses light to reduce mechanical fluctuations and that was previously applied to trapped ions. The method requires the light in the optical cavity to have a lifetime that is much longer than the period of the mechanical motion. This configuration of experimental parameters is known as the resolved-sideband regime, and precludes fast measurements of the mechanical motion because the cavity accumulates a signal of such motion over a relatively long timescale.

Rossi and colleagues developed an optomechanical experiment that operates well outside the resolved-sideband regime. The authors placed a millimetre-sized mechanical membrane inside an optical cavity that was continuously supplied with light from a laser (Fig. 1). They monitored the resulting phase shifts in the light using a device known as a homodyne detector, which enabled the membrane's position to be measured continuously.

The authors then passed the signal from the detector through a filter, which essentially converted the information about the membrane's position into information about its momentum, and used this new signal to control the intensity of a second laser. The light from the second laser applied a feedback force to the membrane that greatly suppressed the membrane's motion. Using this approach, the team achieved a mean thermal occupation of approximately 0.3, which means that the oscillator was in the ground state for more than 75% of the time.

Rossi and co-workers' achievement can be viewed as the culmination of decades of research in engineering and quantum physics, and it builds on the work of several other groups around the globe that are too numerous to list here. The use of laser light to both monitor mechanical motion and apply a feedback force was first studied theoretically[5] in the late 1990s, and a proof-of-concept experiment was carried out shortly thereafter[6]. Since then, improvements in optomechanical experiments have enabled researchers[7] to achieve a thermal occupation of about 5.3, which is equivalent to a ground-state probability of 16%. The technique has also been used to stabilize the mirrors in gravitational-wave detectors[8].

Key to the present work's success was the fact that the speed with which the experiment precisely measured the position of the membrane was much faster than the rate at which the membrane returns to thermal equilibrium. Such a regime is said to have high 'quantum cooperativity', and allowed the physics of the Heisenberg uncertainty principle to be clearly visible in Rossi and colleagues' experimental results.

The authors' work not only demonstrates the utility of quantum measurement and feedback, but also highlights the richness of optomechanical experiments that operate well outside the resolved-sideband regime. Among many applications, working in this regime allows optomechanical interactions to be carried out that, when combined with the authors' control method, offer a route towards producing 'quantum-superposition' states of mechanical motion[9]. Such states would be useful to both develop quantum technologies and probe the foundations of physics. ■

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MICROBIOLOGY

Chromatin clues to a parasite's coat switch

The parasite *Trypanosoma brucei* causes sleeping sickness. It evades human defences by changing the version of a protein that coats its surface. Analysis of its genome and nuclear structure clarifies this variation process. SEE LETTER P.121

STEVE KELLY & MARK CARRINGTON

Most infections don't usually cause prolonged illness in humans because the body's immune system recognizes the presence of a molecular fragment made by the pathogen, termed an antigen, as alien, and triggers a defence response that eliminates the pathogen. However, pathogens use a range of strategies to evade such destruction. One approach is called antigenic variation, whereby a pathogen population keeps changing the antigens that are expressed. If antigenic variation occurs more rapidly than the host can respond to a newly expressed antigen, infection can persist. Müller *et al.*[1] report on page 121 that in the parasite *Trypanosoma brucei*, the structure of the DNA–protein complex known as chromatin has a role in how antigenic variation occurs in this organism.

The process of antigenic variation has evolved independently in many organisms[2–5]. It has certain common features, such as the presence of a reservoir of many versions of a particular gene, and hence the possibility that many different antigens can be expressed that correspond to that gene or gene family. Another aspect central to infection persistence is the presence of mechanisms to ensure that only one version of such a gene is expressed at a time, with all the other versions existing in a silenced state that might later be reversed[6].

Antigenic variation has been studied intensively in *T. brucei*, which causes African trypanosomiasis, historically known as sleeping sickness, in humans, and a range of diseases in livestock. The disease can be fatal if trypanosomes enter the brain, causing a range of neurological symptoms that including the disturbance of sleep patterns[7]. Although the incidence of the human disease is in decline[8], the animal illness remains a major cause of poverty among farmers in sub-Saharan Africa[9].

The surface of a *T. brucei* trypanosome is

covered with closely packed molecules of a glycoprotein termed VSG (Fig. 1). During an infection, switching events occur that result in a different version of the VSG being expressed from a reservoir of thousands of VSG genes, most of which are substantially different from each other[10,11]. This switching process enables the parasite to evade immune-mediated destruction, and the infection can thus persist for decades[12].

Most of the parasite's VSG-encoding gene repertoire occurs in tandem arrays close to DNA sequences called telomeres, which are found at the ends of chromosomes[13]; these arrays are known as subtelomeric arrays. In addition, at any given time, approximately 15 other VSG-encoding genes — including the one being expressed[6] — are present in expression sites. These are regions of chromosomes next to telomeres that are specialized for the expression of VSG-encoding genes. Only one expression site is active, and it is located in a nuclear structure termed the expression-site body[14]. The other expression sites are inactive, and all the genes are said to be silent. Antigenic variation can occur either by a change in the sequence of the VSG gene in the active expression site through a DNA-mediated process called recombination[5], or by the replacement of one expression site with another in the expression-site body[5].

The processes involved in gene silencing must operate on all copies of the VSG-encoding gene apart from the one being expressed. Müller and colleagues' study addressed three questions about this process. How are the subtelomeric arrays of VSG-encoding genes kept silent? Is the same mechanism used for all the silenced expression sites? And how is this silencing reversed?

Müller and co-workers report a newly generated assembly of the *T. brucei* genome that adds substantially to the one previously reported[15]. The authors reconstructed 33 subtelomeric arrays of VSG-encoding genes, and determined on which of the chromosomes 27 of these were located. This advance in our understanding of the trypanosome genome reveals that approximately half of the parasite's DNA is devoted to VSG-encoding genes.

Their genome assembly allowed the authors to investigate the silencing of VSG-encoding genes. They first confirmed by RNA sequencing that the subtelomeric arrays of VSG-encoding genes are not expressed. Second, using a DNA-crosslinking technique called Hi-C to monitor the physical proximity of DNA sequences to each other in the nucleus, the authors report that there is a greater compaction of subtelomeric arrays than of other regions of the chromosomes. Such compaction is characteristic of silenced chromatin in which genes are not expressed. The telomeres of *T. brucei* are located near the outermost region of the nucleus[16], termed the nuclear periphery, and it is probable that the silent VSG-encoding arrays are located there, too.

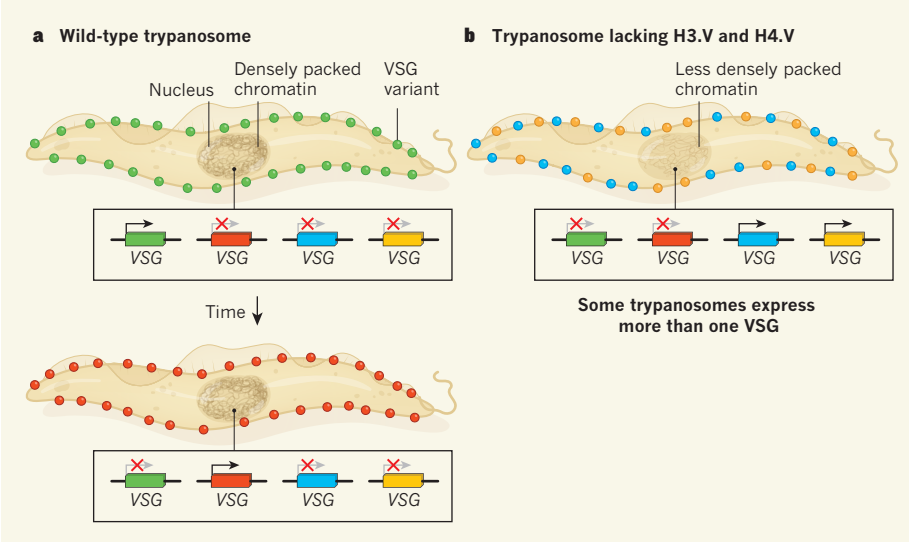

Figure 1 | Coat switching in the parasite *Trypanosoma brucei*. **a**, The trypanosome parasite, which causes sleeping sickness, evades destruction by the immune system by varying over time the version of a glycoprotein called VSG that coats its surface. The parasite usually expresses only one copy of its many versions of VSG-encoding genes at a time, enabling its surface coat to change more rapidly than its host can target a defence response against it. VSG-encoding genes can be found in the periphery of the nucleus in a densely packed region of chromatin (the complex of DNA and protein). **b**, Müller *et al.*[1] shed light on how the coat-switching process occurs, and report that if trypanosomes lack H3.V and H4.V, which are DNA-binding proteins called histones, the chromatin surrounding VSG-encoding genes exists in a conformation that is less densely packed than the conformation in wild-type trypanosomes. Such less densely packed chromatin favours gene expression, and some of the trypanosomes that lack both H3.V and H4.V can express more than one VSG at a time.

How might gene silencing and localization of silent arrays of VSG genes to the nuclear periphery be maintained? The answer is probably complex. Several factors influence the silencing of VSG-encoding genes[17,18]. Müller and co-workers report that two variant versions of histone proteins, called H3.V and H4.V, also have a role in this silencing process. These histones are a component of chromatin, and mark sites in the genome of *T. brucei* at which the synthesis of RNA transcripts by the enzyme RNA polymerase II is terminated[19].

The authors engineered *T. brucei* to lack either H3.V or H4.V, or both, and investigated how this affected the structure of the nucleus and chromatin and the silencing of VSG-encoding genes. Experiments using Hi-C and assessing the localization of telomeres in the nucleus indicated that the absence of H3.V, but not of H4.V, altered nuclear organization and resulted in increased clustering of telomeres at the nuclear periphery.

The authors next analysed chromatin structure using a technique called ATAC-seq, which assesses the ability of an enzyme to access specific sequences of DNA. If the enzyme can access a particular sequence, the DNA is probably in an uncompacted chromatin structure that might facilitate access for the components needed to drive gene expression. Müller *et al.* found that, if both H3.V and H4.V were absent, chromatin accessibility of VSG-encoding sequences in expression sites was increased compared with accessibility in the wild-type situation. To address how these changes affected the expression of VSG-encoding genes, the authors used single-cell RNA sequencing to determine the number of expression sites being expressed in individual cells. They found that, although most cells still expressed just a single VSG in the absence of H3.V and H4.V, some cells in the population expressed up to four different VSGs. It is not known why all the expression sites were not activated when the chromatin accessibility for these genes increased more than usual. But there are probably many levels of control to limit VSG expression to just one at a time, given that this capacity is a key element of pathogen survival.

Many questions remain to be answered. What happens to the expression-site body in the cells that lack H3.V and H4.V? Does its location change, and might the number of these structures increase? Perhaps the cell's ability to construct multiple expression-site bodies is restricted, which might therefore limit the number of active expression sites in parasites that lack H3.V and H4.V. Another interesting issue is whether changes in chromatin accessibility alter the ease with which expression-site sequences can move into an expression-site body. Answers to these questions might help to illuminate the intimate relationship between genome architecture and the mechanism of antigenic variation in one of the world's most puzzling, problematic and pugnacious pathogens. ■

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NEURODEVELOPMENT

Pruned to perfection

During development, some synaptic connections between neurons are removed by immune cells called microglia, and others are retained. The discovery of a 'don't eat me' signal that prevents excess pruning sheds light on this process.

SERGE RIVEST

The signals transmitted between neurons through synaptic connections are responsible for most, if not all, brain functions, from learning to decision-making. During brain development, synapses that are stimulated less often than others are eliminated through a process called pruning, whereas those that are highly stimulated are retained. This refines the brain's ability to respond to stimuli and environmental cues. Microglia, the brain's innate immune cells, have a key role in pruning — they engulf and digest synapses through a process called phagocytosis. But the mechanism that determines which synapses they avoid has been unclear. Writing in *Neuron*, Lehrman *et al.*[1] describe a 'don't eat me' signal, involving a protein called cluster of differentiation 47 (CD47), that prevents inappropriate synaptic pruning by microglia.

About a decade ago, it was shown that synapses requiring elimination send an 'eat me' signal to microglia[2] (Fig. 1a). This signal involves the proteins C1q and CR3, which are part of the complement cascade — a complex series of interactions that is best known for activating cells of the innate immune system to eliminate disease-causing organisms and damaged cells. 'Don't eat me' signals act to limit the effects of 'eat me' signals in the immune system, but it was not known whether the same process occurs during synaptic pruning in the developing brain.

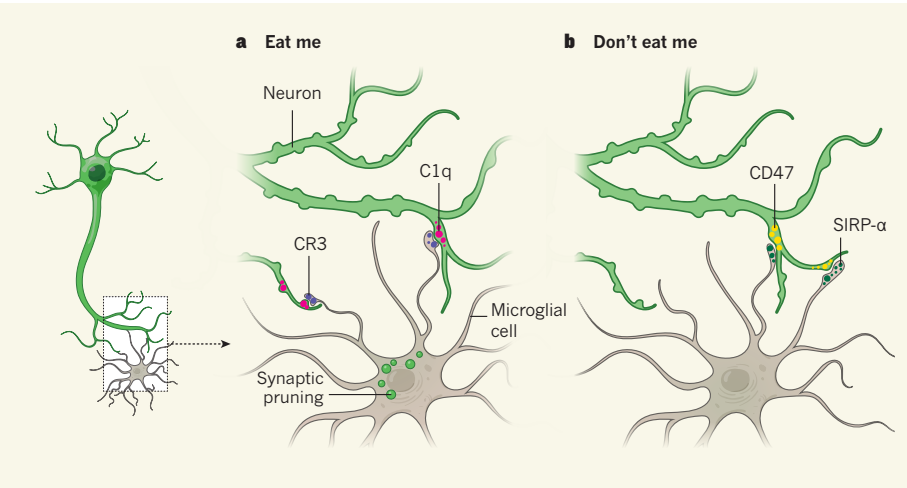

Figure 1 | Opposing signals in synaptic pruning. **a**, Unnecessary synaptic connections between neurons can be removed during brain development in a process called pruning, in which the termini of neurons leading into synapses are engulfed and digested by microglia — the brain's innate immune cells. Synapses destined for elimination release an 'eat me' signal, in which an immune protein called C1q signals to the protein CR3 on microglia to promote pruning. **b**, Lehrman *et al.*[1] report an opposing 'don't eat me' signal. The protein CD47 is expressed on active synaptic termini, and signals to its receptor SIRP-α on microglia, discouraging the immune cells from digesting the synaptic terminal.

CD47 is a cell-surface protein that has many immune functions, including acting as a 'don't eat me' signal for macrophages[3], microglia's sister cells, which exist outside the brain. Lehrman *et al.* analysed whether CD47 is expressed in the dorsal lateral geniculate nucleus (dLGN), a region of the brain involved in vision. This region receives inputs from neurons called retinal ganglion cells (RGCs) that originate in the retina. The authors demonstrated in mice that, at five days after birth, synapses from RGCs to other neurons in the dLGN are being pruned at high levels.

Lehrman and colleagues found that CD47 was expressed at higher levels in the dLGN than in other brain regions at this time. Moreover, the protein SIRP-α, which acts as a cell-surface receptor for CD47, was highly expressed by microglia at the same developmental stage. Using a super-resolution imaging technique, the researchers showed that CD47 was located in 25% of synapses in the mouse dLGN 5 days after birth.

Next, the group investigated whether CD47 functions as a 'don't eat me' signal in this context. First, they measured phagocytosis of synaptic material in mice genetically engineered to lack CD47. They found that microglia engulfed more RGC inputs in CD47-deficient mice than in their wild-type siblings. The mutant mice also displayed higher levels of pruning than did controls, and had fewer synapses in the dLGN by ten days after birth — a change that persisted into adulthood. The authors observed a similar phenomenon in mice lacking the gene that encodes SIRP-α, indicating a possible CD47–SIRP-α interaction on microglia.

The researchers used various *in vitro* approaches to test whether CD47–SIRP-α signalling could prevent the phagocytosis of isolated synaptic termini, called synaptosomes. These analyses revealed that microglia lacking SIRP-α engulfed synaptosomes more efficiently than did wild-type microglia, and that microglia preferentially engulfed synaptosomes lacking CD47 over wild-type ones. Together, these data indicate that CD47–SIRP-α signalling acts as a 'don't eat me' signal to protect against excessive microglia-mediated pruning and synapse loss (Fig. 1b).

Blocking or disrupting neuronal stimuli and environmental cues to neurons can alter synaptic pruning and refinement in