In addition to validating the presence of these microbes in tumours and blood in cancer, it will be important to gain insights into their distribution and function. Poore *et al.* and others[6] identified microbial signatures in tumours on the basis of nucleic-acid sequences; however it is not known where these microbes are located (within or around tumour cells, immune cells or in connective tissue known as the stroma) and whether or not they are alive. And more work will be needed to determine whether the microbes are driving cancer or are merely passengers in an altered tumour microenvironment. There are clear examples of how microbes in tumours might contribute to cancer development and to resistance to cancer therapy[3,10]. However, other data suggest that the presence of microbes in tumours is associated with better long-term outcomes[11].

Finally, further mechanistic insights into how microbes enter and persist in cancerous tissue are needed, as well as research into how best to target them for treatment and even cancer prevention. Such strategies will need to be nuanced, and must take into account the potential effect on all microbial niches, because many of the body's resident microbes have a crucial role in overall physiology. Although some preclinical studies suggest that co-targeting microbes and tumour cells with antibiotics and chemotherapy is associated with delayed tumour outgrowth[10,12], other work suggests that treatment with broad-spectrum antibiotics[13] can worsen the outcomes of people receiving immunotherapy, probably owing to disruption of the gut microbiome. Thus, there is context dependence, which must be taken into consideration. Nonetheless, the opportunities for both clinical advances and basic insights that are presented by an ability to monitor and manipulate the microbiome are tantalizing.

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Molecular biology

A reaction chamber for chromatin modification

Nick Gilbert & Fred van Leeuwen

Chromatin, the complex of DNA and protein in cell nuclei, can be modified by ubiquitin molecules. It emerges that this modification occurs in a molecular reaction chamber formed from an enzyme and a scaffold protein. **See p.592**

DNA is packaged around histone proteins to form a macromolecular structure called chromatin. Histones are often modified by the attachment of small molecules, to guide gene activity and genome stability[1,2]. For example, ubiquitin modifications on histone H2B (termed H2Bub) are associated with domains of active gene expression[3,4]. But how these modified domains arise is far from understood. Previous work[4] has indicated a simple model for the formation of H2Bub domains: an enzymatic complex that is needed to add ubiquitin to H2B is recruited when the enzyme that catalyses gene transcription, RNA polymerase II, passes along chromatin. On page 592, Gallego *et al.*[5] provide an alternative model, in which the enzymatic complex forms a liquid-like, 'phase-separated' reaction chamber that adds ubiquitin to H2B, independently of RNA polymerase II.

In yeast, ubiquitin attachment to H2B (a process called ubiquitination) is executed by the enzymes Bre1 and Rad6. A third protein, Lge1, is also required for H2B ubiquitination in yeast ; Lge1 physically binds to Bre1, but its molecular role in ubiquitination has been a mystery[6]. Gallego *et al.* revisited Lge1 because its amino-acid composition indicates that it has an intrinsically disordered region (IDR) containing a 'sticker' sequence at the amino terminus, enriched in arginine, tyrosine and glycine amino-acid residues. Other proteins that contain IDRs have been shown to weakly interact and undergo liquid–liquid phase separation (LLPS) — a process in which proteins self-associate into liquid-like condensates or droplets, similar to membraneless organelles[7]. LLPS is gaining prominence as a concept that can explain key aspects of chromatin structure and function[8–12].

Gallego *et al.* showed that, in a reaction carried out in a test tube, Lge1 undergoes LLPS to form condensates. This process is driven by the protein's IDR — specifically, by tyrosine residues in the sticker region. The authors observed an odd phenomenon when they added Bre1 to the test tube: Lge1 acted as a scaffold around which Bre1 formed a shell, limiting growth of the condensate. The presence of Bre1 also led to the transient accumulation of Rad6 in the shell, along with arrays of nucleosomes (structural units of chromatin consisting of DNA coiled around eight histones). Rad6 and the nucleosomes subsequently spread evenly throughout the condensate. Thus, Lge1 and Bre1 form 'core–shell condensates' that act as reaction chambers, capturing the ubiquitination machinery and its substrate, H2B in nucleosomes.

"The authors' work provides an exciting model to describe the environment in which nucleosomes are modified."

One of the challenges of studying LLPS is testing ideas generated *in vitro* and through modelling, in living cells[13,14]. This is mainly because proteins that have IDRs are not amenable to structural studies, and condensates might be too small and dynamic to be visualized easily under physiological conditions. Using complementary approaches *in vitro* and in yeast cells, Gallego and colleagues provided evidence for the existence of the reaction chamber *in vivo*.

First, by analysing protein sedimentation in cell extracts, the group showed that Lge1 forms a large complex that can capture Bre1. Subsequently, they tagged Lge1 and Bre1 with fluorescent protein fragments to visualize interactions between the two proteins in

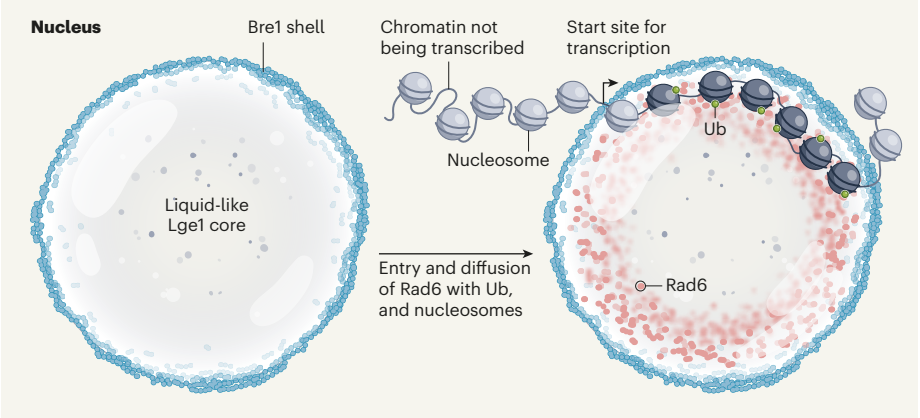

Figure 1 | The core–shell structure. DNA in cell nuclei is wrapped around histone proteins to form units called nucleosomes. The enzymes Rad6 and Bre1, along with the protein Lge1, add ubiquitin (Ub) molecules to histones in actively transcribed regions of the genome (a process called ubiquitination). Gallego *et al.*[5] report that Lge1 condenses into liquid-like droplets, acting as a core scaffold around which Bre1 forms a shell. Nucleosomes at active genes are recruited to and then diffuse into the droplets, along with Rad6, temporarily bound to ubiquitin (binding not shown). The authors find that this core–shell structure promotes ubiquitination.

cells using microscopy. The proteins formed concentrated clusters, consistent with LLPS. Finally, the authors provided evidence that the core–shell structure enhances the efficiency of H2B ubiquitination *in vitro* and in cells, where it mainly affected gene sequences that were undergoing active transcription. Their work provides an exciting model to describe the environment in which nucleosomes are modified.

What can this study teach us about the fundamental concepts of chromatin organization and regulation? The Lge1–Bre1 condensate is unlike known LLPS condensates because it involves not only a phase-separated structure, but also an enzymatically active protein shell. The protein composition of the LLPS reaction chamber can regulate its own size and can control the rate at which Bre1, Rad6 and nucleosomes enter the condensate. When this reaction chamber captures nucleosome arrays, it could well facilitate modification of multiple nucleosomes in succession, producing H2Bub domains (Fig. 1).

The WAC protein is the counterpart of Lge1 in humans. Mutations in WAC have been linked to neurodevelopmental disorders[15,16], and there is emerging evidence that alterations in LLPS are associated with human diseases[7]. Gallego and colleagues showed that WAC also has an IDR and that it can partially perform the role of Lge1 in yeast. Their data indicate that abnormal core–shell compartmentalization might have a role in disease.

The idea that proteins can condense into reaction chambers raises several questions. For example, how do Lge1–Bre1 condensates affect other mechanisms of chromatin organization and modification, and how are they targeted to regions of the genome undergoing active transcription? Perhaps nucleosomes in the main body of a gene — which have different patterns of nucleosome packing and chromatin modification from those of other chromosomal regions — are preferred substrates for the condensates. Another possibility is that the transcription machinery promotes targeting of these gene-body nucleosomes to the core–shell condensate; this is because RNA polymerase II has a repetitive carboxy-terminal domain that can also undergo LLPS (ref. 17). Similarly, researchers will now be asking whether reaction chambers decondense nucleosome arrays to enable access for other chromatin-modifying enzymes that are not part of the condensate, and whether these chambers dissolve once chromatin is ubiquitinated.

Another important consideration is how other macromolecules might co-assemble with the Lge1–Bre1 condensate in the crowded environment of the nucleus. H2B ubiquitination promotes the activity of the enzymes Dot1, Set1 and Set2, which add methyl groups to histones[4,18] — does this cross-talk also occur within a condensate? The N terminus of Dot1 is predicted to be an IDR and can promote H2B ubiquitination when overexpressed[19]. This is compatible with a model in which Dot1 co-assembles with Lge1–Bre1 condensates to coordinate cascades of nucleosome modification. Alternatively, perhaps Dot1 forms a distinct condensate. In this scenario, nucleosome arrays might be handed off between adjacent condensates, or the condensates might interact through fusion events.

The idea of phase-separated condensates is not new. Scientists have been investigating their molecular properties for decades, but it now seems that these structures provide a mechanism for regulating processes inside cells, in particular for chromatin organization. This probably reflects the fact that many reactions that occur in the nucleus involve disordered proteins acting in conjunction with electrically charged strings of nucleic acids, providing an optimal environment for LLPS[20]. Gallego *et al.* add to this body of work, and highlight the fact that simple linear models for enzymatic reactions on chromatin are probably over-simplifications. Instead, reactions occur in 3D, with layered condensates forming enzymatic microenvironments that promote and regulate the reactions, and that could simultaneously unfold chromatin[11] — all in the milieu of a local reaction chamber.

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