suggestive of active replication of the virus in macrophages. These macrophages also had high levels of expression of genes encoding chemoattractant proteins such as CCL4 and CXCL10 that can recruit T cells and monocytes. The authors therefore reasoned that macrophage infection by SARS-CoV-2 triggers the infected cells to release these chemoattractants.

To determine possible roles for T cells that are recruited to the lungs, the authors assessed gene expression at the level of single cells. Grant and colleagues found that the T cells expressed RNA encoding the protein interferon-γ (IFN-γ), which is known to stimulate macrophages and monocytes to produce inflammatory molecules[6]. Indeed, the authors found that macrophages from people with COVID-19 expressed interferon-responsive genes at higher levels than did macrophages from people who didn't have COVID-19.

One of the unique characteristics of severe COVID-19 pneumonia is its particularly long duration, compared with the typical duration of pneumonia associated with other viral infections. Severe lung inflammation and prolonged respiratory failure persist in COVID-19 even after the viral infection is no longer detectable[7]. However, the mechanisms that drive this sustained inflammatory response have not been fully determined. One possibility is a positive feedback loop, as proposed by Grant and colleagues, in which macrophage infection by SARS-CoV-2 leads to the production of chemoattractants that recruit monocytes and T cells to the lungs (Fig. 1). The newly recruited monocytes might mature to form macrophages, and if the T cells produce IFN-γ that stimulates macrophages to produce more chemoattractants, this would perpetuate a cycle of inflammation.

Although the data supporting the authors' model are based almost exclusively on gene-expression profiling of immune cells, this scheme offers a unifying and plausible explanatory framework. To prove that this model is correct, each proposed step would need to be validated in functional studies, and the cause-and-effect relationship of each suggested link in this pathway should be tested. In addition, careful consideration should be paid to how the function of these immune cells of interest might integrate with the roles of other types of lung cell, such as epithelial cells, which are the main type of cell infected by SARS-CoV-2. Epithelial cells have been implicated previously[4] as being the primary driver of inflammation in COVID-19.

It is notable that the types of cell and molecule that comprise this proposed self-sustaining inflammatory circuit in COVID-19 are also present to some degree[8] in cells isolated from people with pneumonia arising from other types of bacterial or viral infection. This begs the question of whether the inflammatory pathway uncovered by the authors is specific to COVID-19, or whether it also operates in other forms of severe pneumonia. Confirmation of either possibility would represent a major advance. This is because such a discovery might lead to therapeutic targeting of infected macrophages, inflammatory T cells or specific inflammatory molecules as a way to block self-sustaining inflammatory circuits, and thereby offer a way to prevent persistent lung injury.

Kara J. Mould and **William J. Janssen** are in the Department of Medicine, National Jewish Health, Denver, Colorado 80206, USA.
e-mails: mouldk@njhealth.org; janssenw@njhealth.org

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DNA repair

Loops that mend the genome

Leonid A. Mirny

A study reveals that a process called loop extrusion, which is central to the folding and function of chromosomes, also seems to play a key part in the repair of double-strand DNA breaks. **See p.660**

One of the challenges that all organisms face is how to compress long DNA molecules — two metres long in the case of human DNA — into the cell nucleus in an ordered and tidy fashion. The DNA must also be protected from damage. On page 660, Arnould *et al.*[1] highlight the ingenuity with which cells achieve these aims: one of the mechanisms involved in packing and folding DNA, namely loop extrusion, is also involved in repairing damage.

Cells neatly compact their spaghetti of DNA in several ways. In cells with a nucleus, the DNA is first wrapped around cores of histone proteins to make structures called nucleosomes, which together form a chromatin fibre that looks like beads on a string. Loop extrusion is the subsequent compaction process, whereby a molecular motor binds a chromatin fibre and reels it in from the sides, forcing out a progressively larger loop in between (Fig. 1a).

Although the process of loop extrusion was hypothesized decades ago[2–5], only in the past few years has it become clear that it is a universal mechanism that organizes DNA in organisms from bacteria to humans. In 2016, computational models showed that extrusion can compact DNA, turning a hairball of chromatin into detangled yet tightly packed chromosomes[6]. Simulations also indicated that, when extrusion is stalled by barriers on the chromatin (a normal part of the process), it produces chromosomal domains seen in data from Hi-C — a technique used to characterize chromosome structure[7].

These studies[6,7] further suggested that 'structural maintenance of chromosomes' (SMC) complexes — once thought to be passive rings or staples — are actually loop-extruding motors. Moreover, proteins known as CCCTC-binding factors (CTCFs), which attach to specific DNA sequences, were proposed to be barriers that catch and stall SMC motors called cohesins[7] (Fig. 1b). All in all, a range of experimental evidence — from *in vivo* depletion of SMCs and CTCFs, to direct visualization of single molecules — now supports the existence of loop extrusion[8].

Cellular processes often multitask. So, could this ubiquitous mechanism for weaving the genome have other functions? During cell division[9,10], the formation of loops is key to compacting chromosomes to enable accurate passing of genetic material into daughter cells. But the role of extrusion during interphase — the period during which cells duplicate their DNA and grow in preparation for the next division — is yet to be understood.

There are several possibilities. One of these is in regulating gene expression: extrusion can bring together distant genomic elements (such as enhancers and promoters, which regulate transcription) that are not separated by a CTCF barrier. Such barriers can also turn extrusion into genomic tracking. To explain, when a cohesin protein stalls on one CTCF, it

can continue reeling DNA in on the other side, thereby tracking over long genomic regions[7,11] (Fig. 1b). This mechanism has been implicated in the stochastic expression of certain cell-adhesion proteins[12] and the random rearrangement of antibody gene segments, resulting in striking antibody diversity[13]. Arnould *et al.* add to this list of possibilities, suggesting that loop extrusion safeguards the genome by supporting the repair of double-strand DNA breaks (DSBs).

To repair severed DNA, cells must first establish a large (roughly one million base pair) region of modified H2AX histones flanking the break[14]. Phosphate groups are added to the histones by certain enzymes, including one called ATM, producing a region of what are known as γH2AX histones[15]. These histones signal the presence of the break to repair enzymes.

Arnould *et al.* confirm observations[16,17] that γH2AX regions closely resemble chromosomal domains that are established by loop extrusion and demarcated by CTCFs. They also find that the ATM that establishes the modifications remains bound at the DSB (Fig. 1c). How, then, can this ATM reach histones located a million base pairs away? The group provides evidence that genome folding is key. The asymmetry of the modified regions relative to the DSB, with limited spreading of histone modifications beyond CTCF barriers, indicates that the spreading is mediated by a 'directional' mechanism that tracks along the genome and stops at CTCFs, rather than by spatial contacts made between disparate chromosome regions or a phase separation that would establish contact symmetrically and wouldn't obey CTCF barriers. Extrusion readily provides such a directional mechanism.

Furthermore, the temporal dynamics of γH2AX spreading support a directional process. A previous study[18] showed that the modifications established by ATM spread directionally, rather than by spatial contacts or the more random process of diffusion. Another study[19] found that γH2AX spreads directionally at a rate of 150,000 bases per minute. This speed is consistent with the speed of loop extrusion measured *in vitro* and in bacteria[8].

One possibility for the directional spreading of γH2AX, then, is that DSB-bound ATM uses loop-extruding SMCs on either side of the break to scan flanking DNA. To achieve this, a break or ATM would stall each SMC on one side, similar to CTCF, while still allowing it to reel chromatin in on the other side, progressively pulling more-distant regions to be modified by the stationary ATM. Such stalling should generate a characteristic pattern of stripes on a Hi-C map – as Arnould *et al.* and others[20] found.

The evidence that cohesin itself is the primary loop extruder remains modest, however. Arnould and colleagues found that loss of one cohesin subunit (Scc1) led to loss of extrusion stripes near DSBs on Hi-C maps, but that depleting or stabilizing cohesin itself had only slight effects on γH2AX profiles. It's possible that other SMC complexes, such as SMC5/6 or MRX, or an Scc1-independent cohesin have the predominant role here. Data from another study[20] hint at this: extrusion stripes occur near DSBs in dividing yeast cells, but diminish following depletion of MRX (rather than cohesin). There might well be a complex web of loop-extrusion activities, mediated by different motors.

The findings could have implications well beyond DNA repair. Histone modifications are key to development and cellular identity, but the mechanisms that establish and maintain them are poorly understood. The suggestion of a role for the SMC–CTCF system in spreading histone modifications s radically different from the known mechanism underlying this process. Until now, extrusion- and modification-dependent folding mechanisms were considered to be entirely separate[11].

It is also possible that the roles of cohesin and other SMCs in repairing breaks go beyond γH2AX spreading. Cohesin has long been implicated in DNA repair, but was thought to be a passive ring, keeping together duplicated chromosomes for repair. The findings suggest more-active roles, for example in enabling the search for a region of DNA that can act as a template for repair[19]. What's more, the recruitment of SMCs to breaks, and the central role of cohesin in other processes that deal with DSBs (such as a type of division called meiosis and immunoglobulin-gene regulation), suggests that these motors might be important in detecting and managing broken DNA during many normal cellular processes. So, SMC complexes – master weavers of the genome – might be in charge not only of making loops, but also of finding and tying together broken fibres.

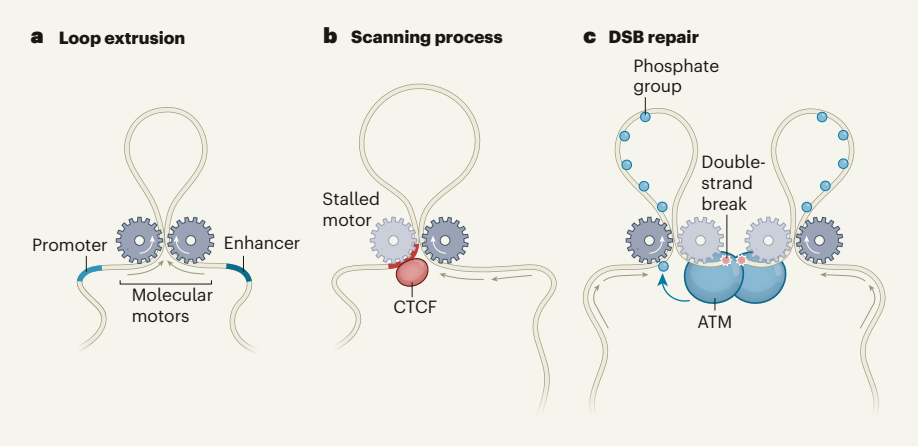

Figure 1 | Roles for DNA loop extrusion. a, To regulate gene expression, molecular motors (here depicted as cogwheels) reel in DNA from both sides, pushing out a loop in the process. This loop extrusion can bring together sequences that control gene expression, such as enhancers and promoters. **b**, When a motor reels in a section of DNA that is bound to a CTCF protein, it stalls. The other motor can continue reeling, allowing the DNA to be scanned for regulatory sequences. **c**, Arnould *et al.*[1] have found that loop extrusion is also involved in repairing DNA double-strand breaks (DSBs). Such breaks trigger the recruitment of the ATM protein, which adds phosphate groups to histone proteins (not shown). The authors show that molecular motors flank either side of the break and scan DNA past the ATM enzyme, allowing histone phosphorylation across large regions and so signalling to repair proteins (not shown).

Leonid A. Mirny is at the Institute for Medical Engineering and Science and the Department of Physics, Massachusetts Institute of Technology, Massachusetts 02139, USA.
e-mail: leonid@mit.edu

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