News & views

Epigenetics

How to silence an X chromosome

Jackson B. Trotman & J. Mauro Calabrese

The non-coding RNA *Xist* has been shown to enlist the SPEN protein to recruit a team of protein complexes — initiating the process that prevents transcription of one of the two X chromosomes found in female mammalian cells. **See p.455**

Female mammals have two X chromosomes, whereas males have only one. A remarkable solution has therefore evolved to prevent a gross imbalance in gene expression occurring between the sexes: in every cell that has two X chromosomes, one entire X chromosome is 'silenced' to prevent RNA from being transcribed from it. This process is called X-chromosome inactivation (XCI) and initiates early in the development of female embryos. Once complete, XCI is essentially stable for life[1] — thus, by extension, a human X chromosome can be propagated in the silenced state for more than 100 years.

XCI has become a paradigm for epigenetic processes — those in which DNA and associated proteins are modified to alter gene expression — and has been intensively studied for decades. For the past 25 years, much of this research has centred on a long non-coding RNA (lncRNA) called *Xist*, which is needed to orchestrate XCI. However, the details of *Xist*'s silencing mechanism have been elusive. Dossin *et al.*[2] report a stunning series of experiments on page 455 that reveal how *Xist* silences genes by partnering with a protein called SPEN.

Xist is expressed exclusively from the X chromosome that will be inactivated, where it spreads locally and silences nearly every gene on the chromosome by associating with an array of proteins. For example, *Xist* engages the Polycomb protein complexes (which modify the histone proteins that package DNA into a condensed form called chromatin) to maintain gene silencing on the inactivated X chromosome[3,4]. Although this maintenance function is well documented, how *Xist* silences active genes in the first place has remained a mystery — in part because the majority of *Xist*'s protein partners were unknown. But in 2015, a series of studies[5–9] revealed a comprehensive set of proteins involved in XCI. These screens all identified SPEN as a *Xist*-binding protein that is essential for XCI.

SPEN belongs to an evolutionarily conserved family of RNA-binding proteins that have been implicated in transcriptional silencing and, curiously, RNA processing in both animals and plants[10]. To interrogate SPEN's role in XCI, Dossin *et al.* first used a biological system known as an auxin-inducible degron to rapidly degrade SPEN in mouse embryonic stem cells. Consistent with a 2019 report[11], the authors observed that *Xist* is almost completely unable to silence genes along the X chromosome in the absence of SPEN. In an important first, the authors demonstrated that SPEN is required for successful XCI *in vivo* in mice. They also found that SPEN was needed to dampen expression of 'escapees' — genes on the silenced X chromosome that partially evade XCI.

By observing fluorescently labelled molecules in living cells, Dossin *et al.* found that SPEN is recruited to the X chromosome as soon as *Xist* expression begins at the onset of XCI. SPEN contains four RNA-binding domains (called RRMs) at its amino-terminal end and an evolutionarily conserved SPOC domain at its carboxy-terminal end. The authors found that, although RRMs 2–4 are required to bind *Xist*, the SPOC domain is the essential mediator of gene silencing. As suggested by previously reported experiments[12], forcing an interaction between *Xist* and the SPOC domain alone was enough to restore XCI in cells that lack SPEN.

It has been proposed[7,13] that SPEN confers gene-silencing capabilities on *Xist* by recruiting and/or locally activating the enzyme HDAC3, which removes gene-activating acetyl groups from histones. However, HDAC3 accounts for only part of the gene silencing that occurs during the early stages of XCI[13]. To find other mechanisms by which SPEN might bring about silencing, Dossin *et al.* used a mass spectrometry technique to identify proteins that interact with the SPOC domain.

Confirming earlier work[14], the authors found that SPEN's SPOC domain interacts not only with HDAC3, but also with the associated co-repressor proteins NCOR1 and NCOR2 (also called SMRT), and with components of the nucleosome remodelling and deacetylase (NuRD) complex, all of which are epigenetic

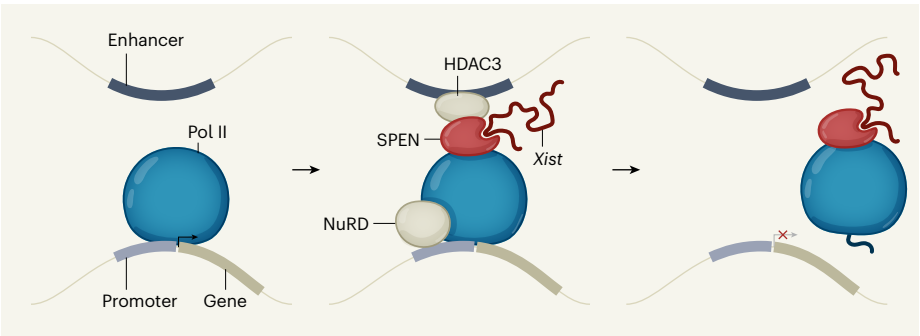

Figure 1 | Mechanism of gene silencing by SPEN. The long non-coding RNA *Xist* and its protein cofactor, SPEN, suppress (silence) gene expression in one of the two X chromosomes found in female mammalian cells. This is an essential process that prevents a gross imbalance in gene expression between males and females. Dossin and colleagues' experiments[2] suggest that SPEN initiates this silencing mechanism by binding to active gene promoters (DNA sequences that initiate transcription) and enhancers (sequences that increase the likelihood of transcription). SPEN recognizes active promoters in part by interacting with constituents of the machinery used for gene transcription, including RNA polymerase II (Pol II, the enzyme that catalyses transcription). SPEN also recruits and/or locally activates the gene-inactivating protein HDAC3, and gene-silencing protein complexes such as the nucleosome remodelling and deacetylase (NuRD) complex. Once a gene has been silenced, SPEN disengages from its binding site, possibly displacing Pol II in the process.

silencers. Moreover, the authors observed that the SPOC domain interacts with parts of the machinery used for transcription and splicing (the process by which newly made RNA transcripts are turned into messenger RNA), including RNA polymerase II, the enzyme that catalyses transcription. Dossin and colleagues identified interactions with components of the N^6-methyladenosine (m^6A) methyltransferase complex, several of which have been linked to XCI[6,11,15]. Accordingly, SPEN and its array of associated proteins might function like a molecular multi-tool to silence genes in various genomic contexts. Although much of SPEN's silencing function might derive from its interactions with known epigenetic silencers, its association with transcription and RNA-processing machineries leaves open the possibility that SPEN can also silence genes through another, as-yet-undefined mechanism.

Perhaps most strikingly, Dossin *et al.* adapted a technique called CUT&RUN to map the location of SPEN on an X chromosome that was being inactivated. This revealed that, shortly after *Xist* starts to be expressed, SPEN associates with active gene promoters and enhancers (DNA regions that initiate and increase the likelihood of transcription, respectively), but then disengages from these sites after it has silenced transcription. These discoveries imply that SPEN is part of a system that recruits silencing machinery specifically to transcriptionally active regulatory elements at the onset of XCI (Fig. 1). Whether this mechanism also requires chromatin modifications, RNA polymerase II, actively transcribed RNA or other factors should be addressed in the future. Another issue that should be investigated is why *Xist* isn't silenced by SPEN, given that a large amount of SPEN accumulates over the *Xist* gene.

SPEN binds to a region of *Xist* RNA called Repeat A, which is required to initiate gene silencing[5,8,16]. Because deleting the *Spen* gene largely mirrors the effects of deleting Repeat A (ref. 11), SPEN seems to be responsible for most of Repeat A's silencing ability. However, Repeat A also binds to other proteins, including those that normally promote splicing, as well as to RBM15 and RBM15B, SPEN's SPOC-domain-containing cousins[5,15,17]. Therefore, it is now crucial to determine how these proteins might compete or cooperate with SPEN to initiate gene silencing. Moreover, deletion of Repeat A drastically reduces levels of the *Xist* RNA itself[18], and, in certain contexts, deletion of SPEN similarly reduces levels of *Xist*[11]. How Repeat A is required for the production of *Xist*, and how its role in *Xist* production relates to its ability to initiate silencing, are key questions for the future.

For decades, *Xist* has served as a leading example of RNA's role in regulating gene expression. Most notably, *Xist* was one of the first mammalian RNAs shown to be involved in Polycomb-mediated silencing[3,4]. It therefore seems appropriate that, by studying this RNA, Dossin *et al.* might have uncovered a new and fundamental aspect of gene regulation — the transient recruitment of SPEN to regulatory elements by RNAs, or even by proteins, which could be a general mechanism for silencing transcription throughout the mammalian genome.

Jackson B. Trotman and **J. Mauro Calabrese** are in the Department of Pharmacology and the Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA.
e-mails: jbt@unc.edu; jmcalabr@med.unc.edu

1. Vallot, C., Ouimette, J.-F. & Rougeulle, C. *BioEssays* **38**, 869–880 (2016).
2. Dossin, F. *et al. Nature* **578**, 455–460 (2020).
3. Silva, J. *et al. Dev. Cell* **4**, 481–495 (2003).
4. Plath, K. *et al. Science* **300**, 131–135 (2003).
5. Chu, C. *et al. Cell* **161**, 404–416 (2015).
6. Moindrot, B. *et al. Cell Rep.* **12**, 562–572 (2015).
7. McHugh, C. A. *et al. Nature* **521**, 232–236 (2015).
8. Monfort, A. *et al. Cell Rep.* **12**, 554–561 (2015).
9. Minajigi, A. *et al. Science* **349**, aab2276 (2015).
10. Su, H., Liu, Y. & Zhao, X. *Cancer Transl. Med.* **1**, 21–25 (2015).
11. Nesterova, T. B. *et al. Nature Commun.* **10**, 3129 (2019).
12. Ha, N. *et al. iScience* **8**, 1–14 (2018).
13. Żylicz, J. J. *et al. Cell* **176**, 182–197 (2019).
14. Shi, Y. *et al. Genes Dev.* **15**, 1140–1151 (2001).
15. Patil, D. P. *et al. Nature* **537**, 369–373 (2016).
16. Wutz, A., Rasmussen, T. P. & Jaenisch, R. *Nature Genet.* **30**, 167–174 (2002).
17. Pintacuda, G. *et al. Mol. Cell* **68**, 955–969 (2017).
18. Hoki, Y. *et al. Development* **136**, 139–146 (2009).

This article was published online on 5 February 2020.

Materials science

Why surface roughness is similar at different scales

Astrid S. de Wijn

Most surfaces are rough at many length scales. Simulations show that this characteristic originates at the atomic level in metal-based materials when smooth blocks of these materials are compressed.

Almost all solid surfaces are rough. This roughness occurs at length scales that encompass 13 orders of magnitude — from the kilometre-scale peaks of mountains, down to atomic-scale bumps. Roughness seems to emerge regardless of what is done to a surface. Yet there is little understanding of how this roughness comes about, and especially why it is often self-affine, meaning that a surface looks similar on different length scales. Writing in *Science Advances*, Hinkle *et al.*[1] show that self-affine roughness has its origin at the atomic level.

As anyone who has ever slipped on a wet floor will have noticed, the roughness of surfaces can have a crucial role in practical situations. Smooth surfaces are slippery when wet, but are also easier to lubricate inside moving machinery than are rough surfaces. By contrast, we sand surfaces before painting them to make them rougher, and thereby to increase the adhesion of the paint. The effects of roughness are less straightforward in other situations: for example, the roughness of the surfaces of skis and snowboards affects their friction on snow differently depending on the temperature and humidity[2]. Engineers have therefore developed many techniques to control surface roughness, such as grinding, polishing and so on. Hinkle and colleagues' results help us to understand better how roughness emerges, and thus might provide new ideas for how to control it.

The authors carried out computational simulations of three materials: a single, perfect gold crystal, an alloy and a metallic glass. These materials have very different amounts and types of disorder, which means that roughness might be expected to develop through different mechanisms or to have different characteristics for each of them. Because the deformation of a material is likely to contribute to the formation of roughness, the researchers simulated the compression of flat blocks of these materials beyond their elastic limit — that is, at forces that cause irreversible (plastic) deformation. Because the length scales of the effects the researchers were looking for span several orders of magnitude, the simulations had to be quite large, containing tens of millions of atoms. Such simulations are computationally extremely expensive.

Hinkle and colleagues investigated how fluctuations in the roughness produced in the simulations change when the size of the area being observed is increased. They observed that the roughness profiles of all three materials seem to obey a power law — that is, they do indeed display self-affine scaling, over nearly two orders of magnitude (from about 1 nanometre up to the size of their