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X-chromosome inactivation: the gift that keeps on giving

Understanding the underlying molecular mechanisms of dosage compensation and how cells equalize gene expression from the sex chromosomes has interested scientists for more than six decades. However, with so many questions still unanswered, the field continues to capture the attention of researchers.

Mammals have evolved a wide range of strategies to achieve sex-chromosome dosage compensation. In 1961, Mary F. Lyon first formulated the X-chromosome inactivation (XCI) hypothesis[1]. Writing in *Nature*, she proposed that one of the X chromosomes in female mammals is inactivated, which effectively silences gene expression from this chromosome[2].

Since that impressively astute hypothesis and those original observations, XCI has been established, extensively exemplified and meticulously studied[3]. In turn, studies elucidating the mechanisms of dosage compensation and XCI have provided essential insights and unifying themes across diverse fields. For example, studies of the Xist RNA have substantially advanced the understanding of the functions of long non-coding RNAs[4,5]. Furthermore, investigations into how the inactive X chromosome (Xi) is silenced have revolutionized the understanding of heterochromatin formation and maintenance[6], while further investigation of XCI has provided important insights into three-dimensional chromatin organization in general[7]. Common themes between XCI and imprinting have brought to the fore how diverse epigenetic mechanisms have evolved to silence specific parental alleles[8,9]. Finally, the mechanisms underlying XCI are also relevant to understanding the mechanisms of diseases caused by X-linked mutations[10].

Even though the understanding of XCI has grown during the last decades, many questions remain. In this issue of *Nature Structural & Molecular Biology*, we present work that provides answers to lingering questions in the field. Collombet et al. demonstrate that during the initiation of XCI, Xist-driven compartmentalization of the Xi in heterochromatin does not seem to sequester transcribing RNA polymerase II away from its targets. Instead, the authors from the Heard and Darzacq labs show that RNA polymerase II is still present on chromatin within the X chromosome, but its levels are markedly reduced. In the same issue, another study from Poonperm et al. advances the molecular understanding of how the Xi is reorganized to quickly and uniformly replicate at late S phase, concomitantly with other heterochromatic areas. The authors from the Hiratani lab reveal how SmcHD1, an Xi-binding protein, ensures Xi chromatin homogeneity, and thus uniform late replication timing. SmcHD1 does so by packing more-labile DNA domains, which would otherwise stick out from the main heterochromatin core, and thus would have been more likely to form contacts and be transcriptionally activated. In an accompanying piece, Valsecchi and colleagues contextualize the findings of these two articles in an insightful News & Views.

An unintended consequence of XCI is potential imbalance in the expression of genes from either the single active X chromosome (Xa) in female cells or the one X chromosome in male cells, versus those in their autosomal counterparts. Here is where dosage compensation comes into play, although how it is regulated post-transcriptionally has begged additional studies. Ruckle et al. provide evidence that adenosine methylation (m^6A) may be a key regulator of the process. Authors from the König lab show that X-derived transcripts seem to be less decorated by m^6A and are thus more stable than autosomal mRNAs. This increased stability partially mitigates their numerical deficit. In an accompanying News & Views, Jachowicz examines these findings, considering the bigger picture and posing interesting questions about potential follow-up studies.

To highlight these studies, on this month's cover we feature the tri-color calico cat, the 'poster child' of the XCI field. Calico cats have two colors of fur on an otherwise white background and are almost always female. Lyon originally proposed that this coat color pattern arises from the inactivation of one or the other of the two equivalent X chromosomes in females, during early embryogenesis. In the orange patches, the X chromosome with the allele that results in brown color is inactivated, while in the brown patches, the X chromosome with the allele that results in orange color is inactivated. Inactivation of the X chromosome, also known as lyonization, occurs randomly, resulting in a unique mosaic of fur coat colors.

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