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DECLARATION OF INTEREST

The author declares no competing interests.

REFERENCES

Alberti, S., and Hyman, A.A. (2021). Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. Nat. Rev. Mol. Cell Biol. *22*, 196–213. https://doi.org/10.1038/s41580-020-00326-6.

Bartok, E., and Hartmann, G. (2020). Immune Sensing Mechanisms that Discriminate Self from Altered Self and Foreign Nucleic Acids. Immunity *53*, 54–77. https://doi.org/10.1016/j.immuni.2020.06.014.

Crow, Y.J., and Stetson, D.B. (2022). The type I interferonopathies: 10 years on. Nat. Rev. Immunol. *22*, 471–483. https://doi.org/10.1038/s41577-021-00633-9.

Hallegger, M., Chakrabarti, A.M., Lee, F.C., Lee, B.L., Amalietti, A.G., Odeh, H.M., Copley, K.E., Rubien, J.D., Portz, B., Kuret, K., et al. (2021). TDP-43 condensation properties specify its RNA-binding and regulatory repertoire. Cell *184*, 4680–4696.e22. https://doi.org/10.1016/j.cell.2021.07.018.

Harapas, C.R., Idiiatullina, E., Al-Azab, M., Hrovat-Schaale, K., Reygaerts, T., Steiner, A., Laohamonthonkul, P., Davidson, S., Yu, C.-H., Booty, L., and Masters, S.L. (2022). Organellar homeostasis and innate immune sensing. Nat. Rev. Immunol. *22*, 535–549. https://doi.org/10.1038/s41577-022-00682-8.

Hrecka, K., Hao, C., Gierszewska, M., Swanson, S.K., Kesik-Brodacka, M., Srivastava, S., Florens, L., Washburn, M.P., and Skowronski, J. (2011). Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature *474*, 658–661. https://doi.org/10.1038/nature10195.

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Ségéral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011). SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature *474*, 654–657. https://doi.org/10.1038/nature10117.

Maharana, S., Kretschmer, S., Hunger, S., Yan, X., Kuster, D., Traikov, S., Zillinger, T., Gentzel, M., Elangovan, S., Dasgupta, P., et al. (2022). SAMHD1 controls innate immunity by regulating condensation of immunogenic self RNA. Mol Cell *S1097–2765*. 00851–6. https://doi.org/10.1016/j.molcel.2022.08.031.

Nevers, Q., Albertini, A.A., Lagaudrière-Gesbert, C., and Gaudin, Y. (2020). Negri bodies and other virus membrane-less replication compartments. Biochim. Biophys. Acta Mol. Cell Res. *1867*, 118831. https://doi.org/10.1016/j.bbamcr.2020.118831.

Schlee, M., and Hartmann, G. (2016). Discriminating self from non-self in nucleic acid sensing. Nat. Rev. Immunol. *16*, 566–580. https://doi.org/10.1038/nri.2016.78.

Xiao, Q., McAtee, C.K., and Su, X. (2022). Phase separation in immune signalling. Nat. Rev. Immunol. *22*, 188–199. https://doi.org/10.1038/s41577-021-00572-5.

Right on target: Chromatin jets arise from targeted cohesin loading in wild-type cells

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Uncovering an informative feature of 3D genome structure, Guo et al. (2022) describe chromatin jets in quiescent murine thymocytes: 1–2 Mb structures formed by targeted cohesin loading at narrow accessible chromatin regions and visible as prominent off-diagonal stripes on contact maps.

Eukaryotic genomes are organized into complex 3D structures across a range of length scales. Understanding the formation and regulation of these structures is crucial, as they regulate diverse processes including gene expression, DNA repair, recombination, and replication. Chromatin conformation capture methods, such as Hi-C, have been instrumental in uncovering patterns of 3D genome organization, including A/B compartments, topologically associating domains (TADs), and loops. A/B compartments arise from active and inactive chromatin regions associating primarily with other regions of the same type both intra- and inter-chromosomally and are visible on contact maps as a checkerboard pattern (Figure 1). TADs are local chromatin domains characterized by increased contact within the region compared with outside the region; they are visible on contact maps as squares (Figure 1) (Fudenberg et al., 2017). TADs often exhibit strong stripes/flames and corner peaks on contact maps, which are indicative of cohesin-mediated loop extrusion, wherein the SMC (Structural Maintenance of Chromosomes) complex cohesin loads onto DNA and extrudes bidirectionally until encountering a block or being unloaded (Davidson et al., 2019; Kim et al., 2019; Fudenberg et al., 2017; Gabriele et al., 2022). However, many aspects of the loop extrusion process remain poorly understood, including the factors that regulate cohesin loading

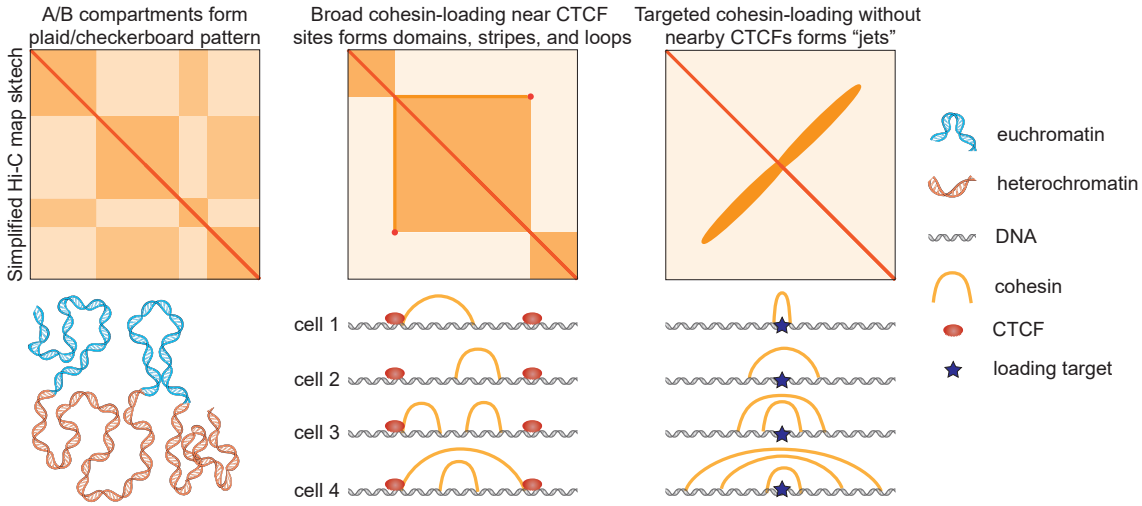

Figure 1. Hi-C contact maps provide insights into the mechanisms of 3D genome structure regulation
Top: simplified sketches of Hi-C contact maps showing A/B compartments (left); TADs, loops, and stripes (middle); and "jets" (right), with likely mechanisms shown below. A/B compartments form because of self-segregation of euchromatin and heterochromatin. TADs, stripes, and loops arise from cohesin looping in broad regions bounded by extrusion barriers such as CTCF boundaries. Jets result from focal cohesin loading in a narrow region and subsequent bidirectional extrusion without nearby extrusion barriers.

onto chromatin and whether specific targeted loading sites exist in eukaryotes. A key signature of targeted loading in Hi-C maps is a stripe perpendicular to the diagonal from the site of targeted loading. Such targeted loading of loop-extruding SMC complexes resulting in an off-diagonal stripe has previously been observed in bacteria (Marbouty et al., 2015; Wang et al., 2017) and very recently in perturbed mammalian cells (Liu et al., 2021) but had not yet been seen in unperturbed mammalian cells.

Now, Guo et al. (2022) have used *in situ* Hi-C in quiescent murine thymocytes to reveal off-diagonal stripes, which they term "jets." These structures, which are also visible in resting B cells, manifest on contact maps as strong stripes perpendicular to the main diagonal (Figure 1). Jets are observed at a subset of small open chromatin regions flanked by large heterochromatin domains. Merging Hi-C data with ChIP sequencing and ATAC sequencing (ATAC-seq), the authors find that the regions producing jets are especially enriched in ATAC-seq signals (indicative of accessible chromatin), occupancy by RAD21 (a cohesin subunit) and NIPBL (commonly held to be a cohesin loader), and H3K27ac marks (indicative of active regulatory regions). Partial depletion of cohesin strongly reduces jet strength across all sites, indicating that jets are cohesin-dependent structures; depletion of CTCF (CCCTC-binding factor; a cohesin block) was not required to observe jets but did alter the strength and shape of a subset of jets. Polymer simulations reproduce jet-like structures when cohesin complexes are restricted to loading in narrow regions; by contrast, uniform cohesin loading across a broad region reproduced TAD-like structures, consistent with previous findings (Fudenberg et al., 2017).

The properties of jets shed new light on several aspects of loop extrusion. Jets most closely resemble the plumes previously described by Liu et al. (2021) in that they extend perpendicular to the diagonal of a Hi-C map and appear to originate at isolated areas of accessible chromatin flanked by large heterochromatin regions (Liu et al., 2021). Unlike plumes, which require depletion of both CTCF and WAPL (a cohesin unloader) to form and can only be observed for 24 h post-WAPL/CTCF depletion, jets can be observed in unperturbed wild-type cells without depleting CTCF or WAPL. Jets are also much longer than plumes, extending up to 1–2 Mb from their origin. Because jets do not require perturbation to be observed, this distance provides a reasonable estimate of the physiological extrusion range of cohesin in non-cycling cells—an important insight that can help constrain loop-extrusion models. Additionally, jets provide further support to the hypothesis that cohesin can continue to extrude unidirectionally even when blocked in one direction, previously put forth in modeling studies and substantiated by experimental observations of chromatin stripes/flares (Fudenberg et al., 2017). A subset of jets located near CTCF sites is deflected away from the perpendicular angle of other jets in unperturbed cells. When CTCF is depleted, these jets return to a perpendicular angle. Guo et al. (2022) identify that this indicates unidirectional blockage of cohesin at the CTCF site in wild-type cells.

Guo et al. (2022) also provide an answer to the long-standing question of whether cohesin loading is uniform or preferentially occurs at specific sites. They clearly show that cohesin does show preference for specific sites rather than binding uniformly across the genome. The key next step is to uncover the factors driving this site preference. Guo et al. identify high NIPBL binding and H3K27ac marks as distinguishing features of jet sites, but it is unclear whether those features are sufficient for targeted cohesin loading or even causative of it. In particular, though

NIPBL is commonly believed to act as a cohesin loader, it is also part of the translocating cohesin complex and may thus be present as a result of increased cohesin occupancy in the region rather than a cause (Rhodes et al., 2017; Davidson et al., 2019; Kim et al., 2019). It also remains to be determined why jets can be observed in quiescent immune cells but have not been seen in Hi-C and Micro-C data from dozens of other mouse and human cell types. It is possible that there is something unique about the chromatin environment of these non-cycling cells that gives rise to jets, akin to the flares observed in zebrafish sperm (Wike et al., 2021). As jets/plumes manifest in other cycling cell types only once CTCF and WAPL are depleted (Liu et al., 2021), another possibility is that quiescent thymocytes and B cells have unusually long-lived cohesin and less-abundant CTCF, allowing cohesin to extrude long jets without dissociating or being blocked by CTCF. Interestingly, this suggests that cells may achieve cell-type-specific regulation of 3D genome structure by regulating the dynamics of CTCF and cohesin, which may help to facilitate cell-type-specific regulation of gene expression.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Davidson, I.F., Bauer, B., Goetz, D., Tang, W., Wutz, G., and Peters, J.M. (2019). DNA loop extrusion by human cohesin. Science *366*, 1338–1345. https://doi.org/10.1126/science.aaz3418.

Fudenberg, G., Abdennur, N., Imakaev, M., Goloborodko, A., and Mirny, L.A. (2017, January). Emerging evidence of chromosome folding by loop extrusion. Cold Spring Harbor Symp. Quant. Biol. *82*, 45–55.

Gabriele, M., Brandão, H.B., Grosse-Holz, S., Jha, A., Dailey, G.M., Cattoglio, C., Hsieh, T.H.S., Mirny, L., Zechner, C., and Hansen, A.S. (2022). Dynamics of CTCF-and cohesin-mediated chromatin looping revealed by live-cell imaging. Science *376*, 496–501. https://doi.org/10.1126/science.abn6583.

Guo, Y., Al-Jibury, E., Garcia-Millan, R., Ntagiantas, K., King, J.W.D., Nash, A.J., Galjart, N., Lenhard, B., Rueckert, D., Fisher, A.G., et al. (2022). Chromatin jets define properties of cohesin-driven *in vivo* loop extrusion. Mol. Cell *82*.

Kim, Y., Shi, Z., Zhang, H., Finkelstein, I.J., and Yu, H. (2019). Human ohesion compacts DNA by loop extrusion. Science *366*, 1345–1349. https://doi.org/10.1126/science.aaz4475.

Liu, N.Q., Magnitov, M., Schijns, M., van Schaik, T., van der Weide, R.H., Teunissen, H., van Steensel, B., and de Wit, E. (2021). Rapid depletion of CTCF and cohesin proteins reveals dynamic features of chromosome architecture. bioRxiv. https://doi.org/10.1101/2021.08.27.457977.

Marbouty, M., Le Gall, A., Cattoni, D.I., Cournac, A., Koh, A., Fiche, J.B., Mozziconacci, J., Murray, H., Koszul, R., and Nollmann, M. (2015). Condensin- and replication-mediated bacterial chromosome folding and origin condensation revealed by Hi-C and super-resolution imaging. Mol. Cell *59*, 588–602. https://doi.org/10.1016/j.molcel.2015.07.020.

Rhodes, J., Mazza, D., Nasmyth, K., and Uphoff, S. (2017). Scc2/Nipbl hops between chromosomal cohesin rings after loading. eLife *6*, e30000. https://doi.org/10.7554/eLife.30000.

Wang, X., Brandão, H.B., Le, T.B.K., Laub, M.T., and Rudner, D.Z. (2017). Bacillus subtilis SMC complexes juxtapose chromosome arms as they travel from origin to terminus. Science *355*, 524–527. https://doi.org/10.1126/science.aai8982.

Wike, C.L., Guo, Y., Tan, M., Nakamura, R., Shaw, D.K., Diaz, N., Whittaker-Tademy, A.F., Durand, N.C., Aiden, E.L., Vaquerizas, J.M., et al. (2021). Chromatin architecture transitions from zebrafish sperm through early embryogenesis. Genome Res. *31*, 981–994. https://doi.org/10.1101/gr.269860.120.

BRCA1 protects against its own fragility

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Deshpande et al. (2022) demonstrate that BRCA1, a tumor suppressor tasked with protecting the genome, is encoded by a gene that is intrinsically fragile.

Roughly one in ten women get breast cancer within their lifetime (Howlader et al., 1975-2017). But for those who inherit a pathogenic *BRCA1* or *BRCA2* allele, the lifetime risk is greater than 50% (Kuchenbaecker et al., 2017). Of the *BRCA1* familial cancers, 80%–90% have loss of heterozygosity (Kuchenbaecker et al., 2017). Yet the *BRCA1* gene sequence is only 0.00004% of the human genome. Is the frequent loss of a second *BRCA1* allele merely the result of chance? Or does the loss of one *BRCA1* allele predispose cells to losing the second? In new research that directly answers these questions, Deshpande and colleagues show that in cells heterozygous for a detrimental *BRCA1* mutation, the *BRCA1* gene bears the hallmarks of a fragile site, breaking when subjected to replication stress (Deshpande et al., 2022). Furthermore, this fragility frequently leads to mutations that inactivate the second copy of the gene.

BRCA1 and BRCA2 are key tumor suppressors that preserve the integrity of the genome, minimizing oncogenic mutations. Both proteins play critical roles in homologous recombination repair of DNA double-strand breaks and protect stalled