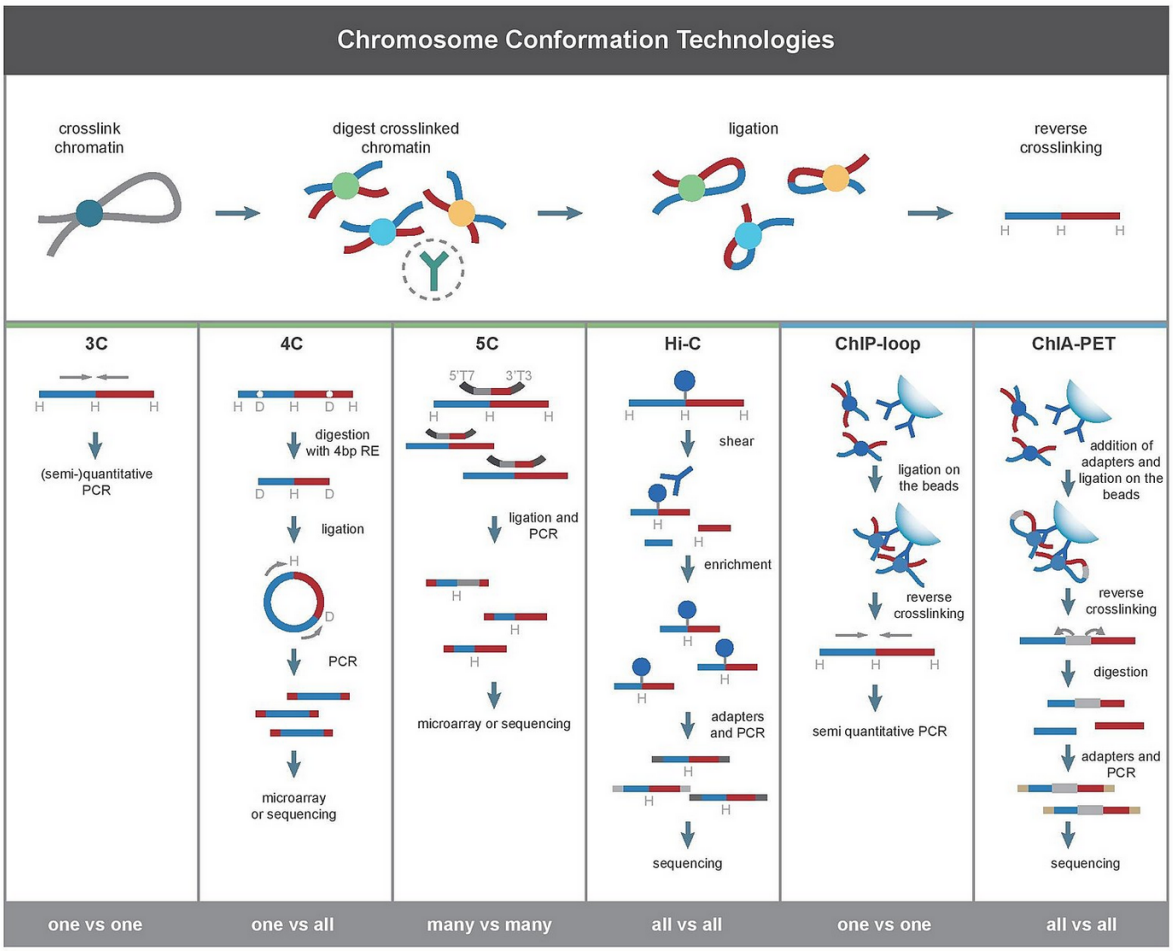
Conformation of sister chromatids in the replicated human genome

Background

- **Chromosome conformation capture**: a set of molecular biology methods used to analyze the spatial organization of chromatin in a cell.
  - Done by quantifying the number of interactions between genomic loci that are nearby in space. These interactions are often actually separated by many nucleotides, which makes understanding interactions hard when looking at the genome linearly.
  - There are various types of methods used to quantify the interactions, but this paper uses a specialized method of chromosome conformation capture.



- **Hi-C (all-vs-all)**: a method of chromosome conformation capture that uses high-throughput sequencing to find the nucleotide sequence of fragments by using paired end sequencing (shotgun sequencing), which retrieves a short sequence from each end of a ligated fragment.
  - Generally, H-C allows for two sequences that should represent different restriction fragments to be ligated together in the proximity based ligation step.

    - The pairs of sequences are then individually aligned to the genome, allowing for the determination of fragments involved in the ligation event. This allows for all possible pairwise interactions between fragments to be tested.
- **Topologically associating domain (TAD)**: a self-interacting genomic region, where particular DNA sequences physically interact with each other more frequently than sequences outside the TAD. These regions often influence gene expression via interactions with enhancer-promotor interactions.
    - Boundaries at both sides of the TADs are often conserved between mammalian cell types, with highly enriched CCCTC-binding factor (CTCF) and cohesin (a protein complex that mediates several functions, including DNA looping in TADs).
    - Influence over these TADs allow for various chromosomal organization and conformation changes, leading to contributions to regulation of *trans*cription, development disorders, cancer, and DNA repair.
    - However, it's not known how cohesive linkages distribute on the genome to support these functions, and how they are coordinated with dynamic loop formation in TADs.
- Normal Hi-C technology cannot be used to explore topological interactions between sister chromatids of replicated chromosomes, since the identical DNA sequences in replicated chromosomes makes it impossible to distinguish between intramolecular and intermolecular contacts. This was obstacle that authors of this paper aimed to overcome by creating a specialized Hi-C method.

Sister-chromatid-sensitive Hi-C

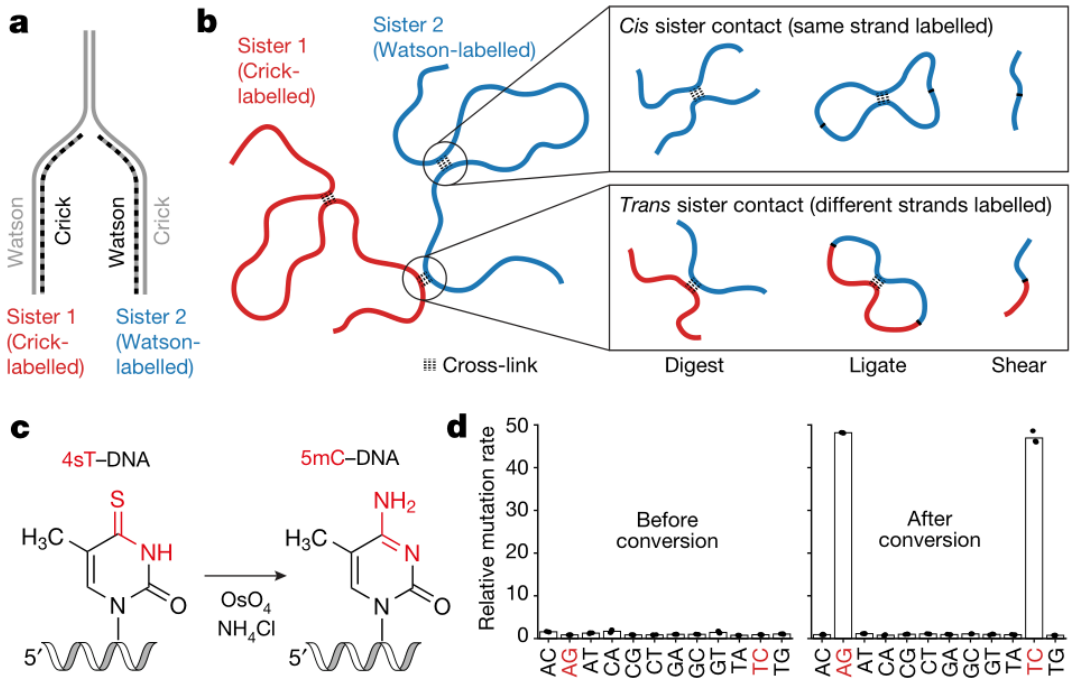
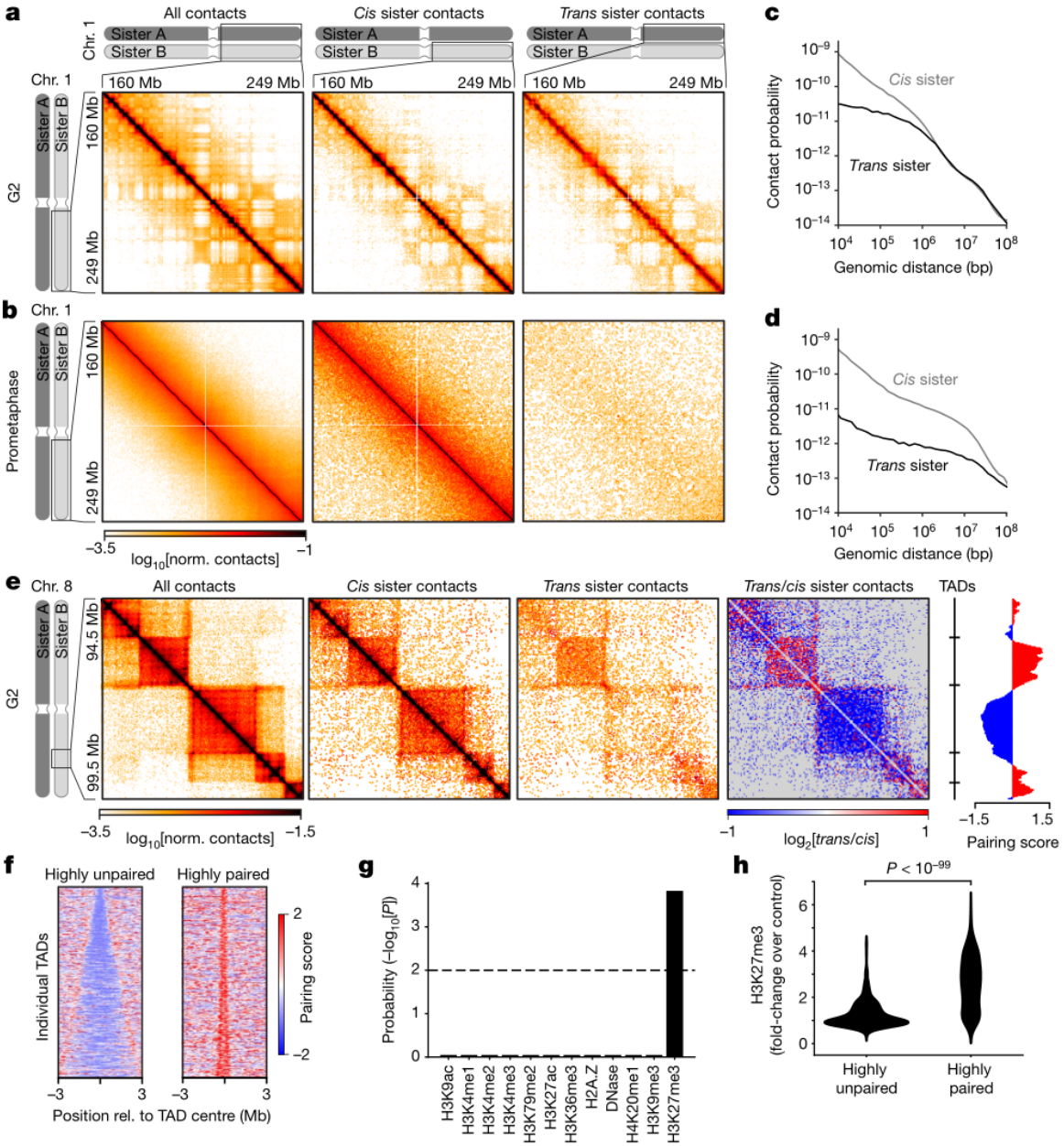


Fig. 1 | scsHi-C methodology based on nascent DNA labelling in live cells. **a**, Sister-chromatid-specific labelling using synthetic nucleotides. During DNA replication, a synthetic nucleotide analogue incorporates into different strands (Watson or Crick) within each sister chromatid. Dashed line, labelled DNA; solid line, unlabelled DNA. **b**, Strategy to distinguish *cis* from *trans* sister contacts in a Hi-C experiment based on 4sT-mediated DNA labelling. After progression through S phase in the presence of 4sT, each sister-chromatid contains one labelled DNA strand of opposing strandedness (**a**). Chromatin is crosslinked in cells and Hi-C samples are prepared using standard procedures, followed by chemical conversion to induce 4sT signature mutations and Illumina-based sequencing. Half-reads are classified as labelled if at least two signature mutations are present. If a ligation junction contains two labelled half-reads that map to the same strand, it is classified as a *cis* sister contact; if it contains two labelled halves that map to opposing strands, it is classified as a *trans* sister contact (Extended Data Fig. 4a). **c**, Signature point mutations are induced by conversion of 4sT to 5mC by treating DNA with OsO_4 and NH_4Cl at elevated temperatures. Functional groups that are changed in the course of the reaction are highlighted in red. **d**, Point-mutation rates of genomic DNA from HeLa cells grown in medium containing 4sT relative to control DNA from cells grown without 4sT, before and after OsO_4/NH_4Cl-mediated conversion. Bar graphs indicate the mean of $n = 3$ biologically independent experiments.

- **Sister-chromatid-sensitive Hi-C (scsHi-C)**: specialized DNA labeling of sister-chromatids in order to distinguish between strands for Hi-C method, allowing for the genome-wide analysis of sister-chromatid interactions in human cells.

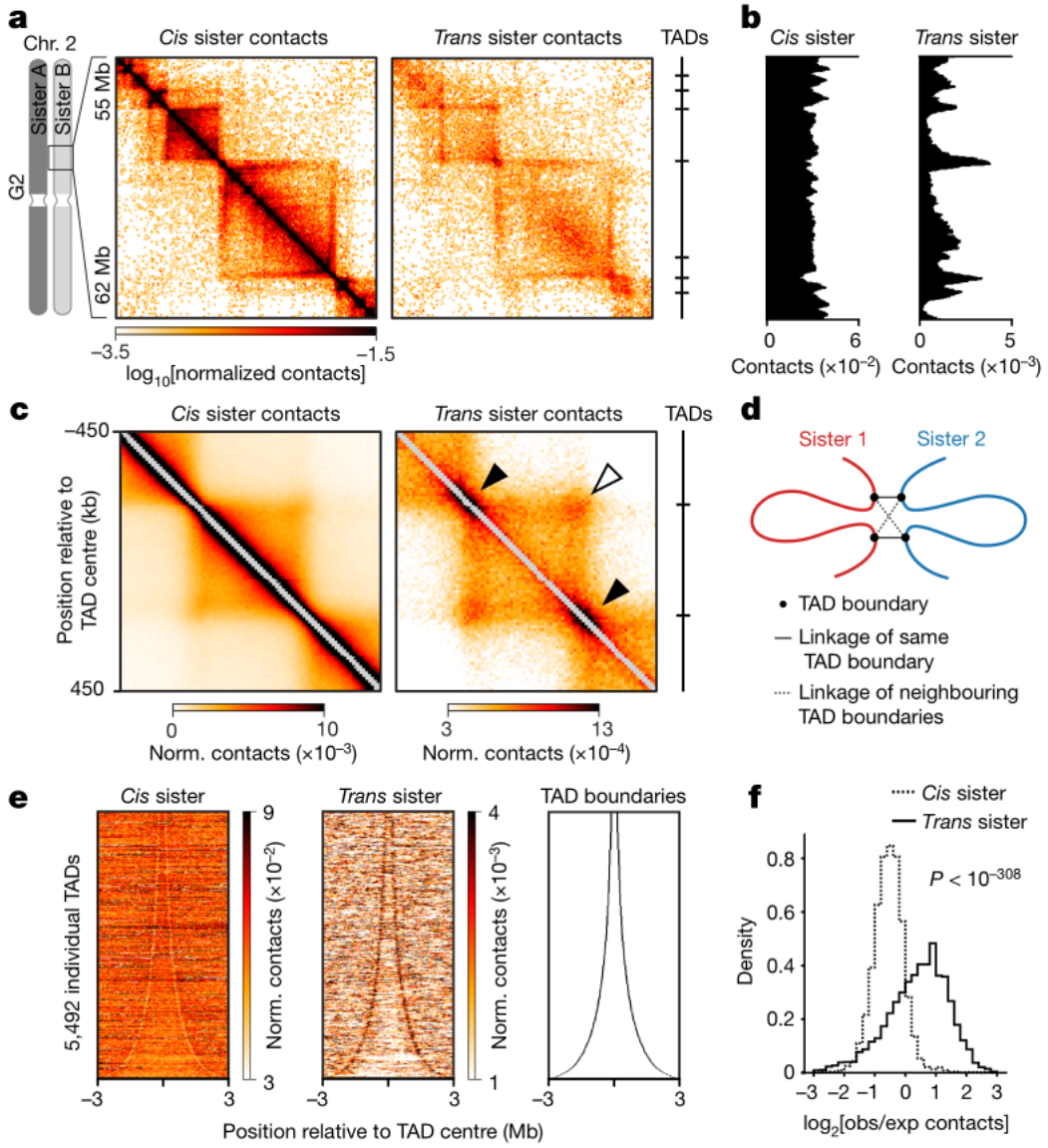
Conformation of replicated chromosomes

- Using scsHi-C, the authors were able to investigate and measure the extent of sister-chromatid resolution during mitosis by constructing genome-wide scsHi-C maps of cells synchronized to G2 or mitotic prometaphse.
- I wasn't exactly how they were using the data generated in figure two below, but what is appeared to indicate was that TADs set the limits of discrete domains with variable degrees of sister-chromatid pairing, with the overall degree of sister-chromatid pairing within TADs defined by characteristic chromatin modifications.



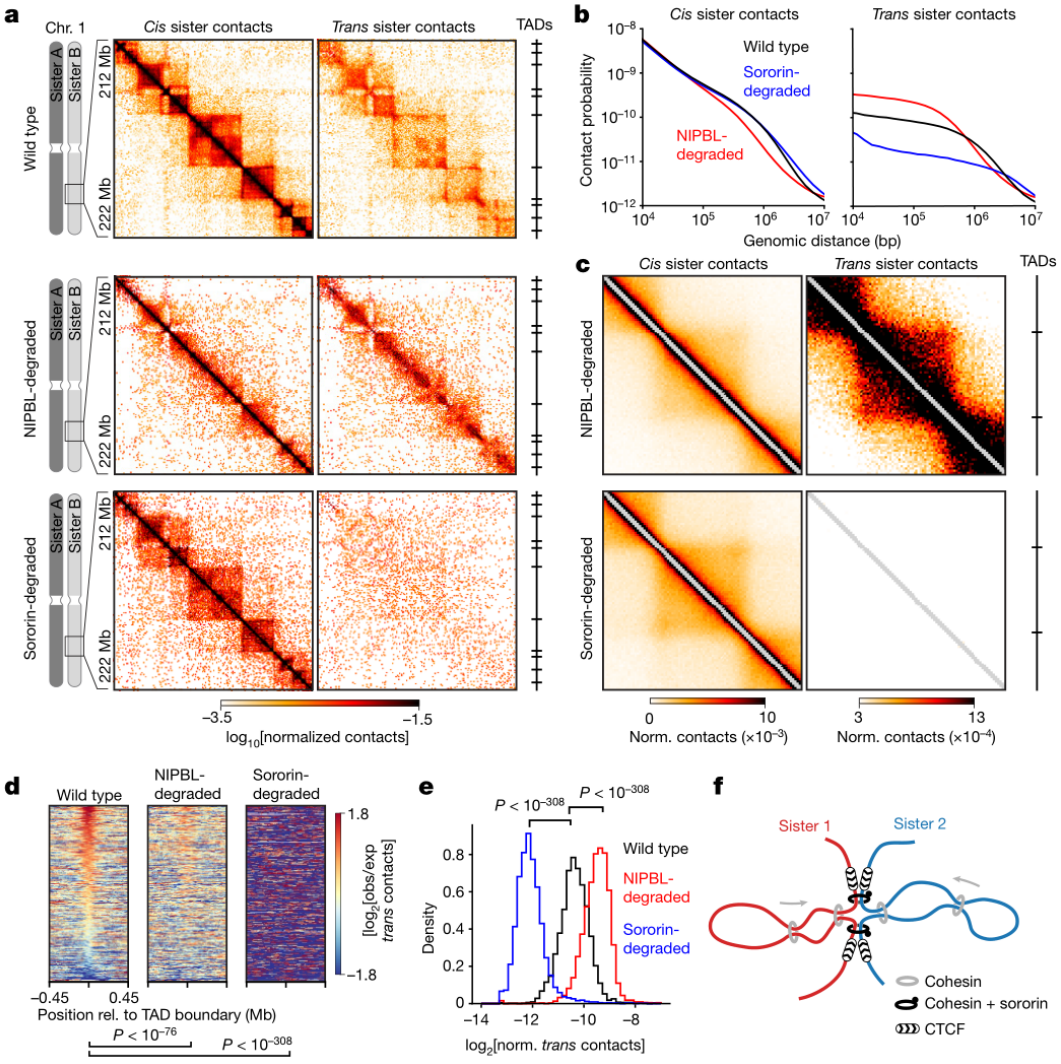
TADs in replicated chromosomes

- Next, the authors investigated how chromatin fibers fold within individual TADs.
- Cis were mostly found along the diagonal of TADs, indicating frequent short-range intramolecular contacts.
- Trans filled TAD areas without substantial accumulation along the diagonal, indicating that sister DNAs are not stritcly aligned in a “railroad” configuration within TADs.
- Trans contract maps (b) and aggregated contact probability maps of TADs (c) showed that *trans* sister contracts were enriched at TAD boundaries and at corner positions connecting neighboring boundaries.
- Overall, sister-chromatids are predominantly liked at TAD boundaries, whereas they separate extensively inside TADs.



Control of sister-chromatid topologies

- Durign G2 phase, about half of all chromatin-bound cohesin dynamically turns over to form *cis*-chromatid loops that shape TADs, whereas the other half binds soroin and persistently links sister chromatids.
- It is possible that *trans* sister contacts concentrate at TAD boundaries due to motor-driven loop extrusion, or through a mechanism that involves cohesin independently of DNA loops.
- Again, I'm not exactly sure how the data below was used to draw the conclusions, so I'm going to reframe from inaccurately trying to describe it. However, what they did find is that loop-forming cohesin is necessary to separate sister chromatids within TADs resulting in locally enriched sister-chromatid contacts at the boundaries, meaning that it contributes to the formation of discrete highly paired domains.
- Additionally, they found that the sororin-stabilized pool of cohesin is not required to form intra-chromatid loops or TADs in G2, but it is required to prevent the separation of sister chromatids and maintain their global alignment during the G2 phase.



Conclusion

- Their scsHi-C analysis showed that a pool of cohensin that mediates linkage between replicated DNA molecules minatians the blocal alignment of sister chromatids after DNA replication.
- Also, another pool of cohesin that dynamically forms loops locally separates sister chromatids within TADs during G2.
- An independent study in a fungus reproduced similar results.
- The scsHi-C method allows for investigation of the organization of sister chromatids and has implications for understanding the maintenance, expression, and mechanical transport of the genome.
- The global alignment of sister-chromatid arms by sororin-stabilized cohesin favors interactions between homologous genome regions, as required for error-free homology-directed DNA damage repair.
- How the other coordinated activities that allow sister-chromatid resolution and thus allows for cells to resolve whole chromosome arms when entering mitosis remains unknown ScsHi-C may allow for the investigation of such mechanisms and others, possibly such as how pairing and recombination of homologous chromosomes occur during meiosis.

References

Mitter, M., Gasser, C., Takacs, Z., Langer, C. C., Tang, W., Jessberger, G., … others (2020). Conformation of sister chromatids in the replicated human genome. *Nature*, *586*(7827), 139–144.