JCB Journal of Cell Biology

SPOTLIGHT

Dynamics of sister chromatids through the cell cycle: Together and apart

Motoko Takahashi and Toru Hirota

When and how sister chromatid resolution occurs after DNA replication is a fundamental question. Stanyte et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201801157) used CRISPR/Cas9 technology to label and track genomic loci in live cells throughout the cell cycle, shedding light on how replication is linked to mitotic sister chromatid organization.

The propagation of the genome depends on the transmission of rod-shaped cytological bodies called sister chromatids in mitosis. The formation of sister chromatids has two aims: to compact chromatin fibers and to resolve the cohesion that arises after replicating DNA molecules. Although both of these properties are microscopically discernible in metaphase chromosomes, when and how sister resolution occurs is a fundamental question in the chromosome field.

Previously, differential labeling of sister chromatids and their quantitative analysis of nonoverlapping volume indicated that the resolution between the sisters can be detected as soon as cells initiate mitosis, and it proceeds hand in hand with chromatin compaction through prophase (Nagasaka et al., 2016). Being based on volumetric analyses, however, this study was inapplicable for analyzing earlier cell cycle phases when chromosome mass is yet to be formed. Intriguingly, specific genomic sites probed by FISH often appear as doublets when cells are examined after DNA replication (Selig et al., 1992; Ono et al., 2013). This would imply that sister loci are well separated beyond the resolution of a light microscope, before the mitotic reorganization of sister chromatids takes place. However, the harsh pretreatments for FISH labeling could affect chromatin structure and the distance between paired dots, and thus additional approaches are needed to confirm this interpretation.

In CRISPR/Cas9 technology, catalytically inactive Cas9 (dCas9) can be tethered to a genomic locus specified by a single guide RNA (sgRNA), and methods have been developed to use fluorescence-labeled dCas9 to probe specific loci (Chen et al., 2013). In this issue, Stanyte et al. take advantage of CRISPR technology to examine in live cells where sister chromatids are physically linked in the genome and when they are resolved in the cell cycle. The beauty of live-cell imaging is that it shows at which time points sister loci become detectably separated and how the distance might change with regard to cell cycle progression.

Steadily detecting signals in long-term imaging requires sufficient intensity of fluorescent bodies under the condition where the phototoxicity limits the amount of excitation lights in imaging multiple sections for whole-cell thickness. Therefore, the first key experimental design in the study was to design suitable probes that generate sufficiently intense fluorescent signals. To achieve this, Stanyte et al. (2018) targeted genomic loci with tandem repetitive sequences, which recruit multiple fluorescent proteins, yield sufficient levels of signal, and represent discrete nuclear foci. Stanyte et al. (2018) created a collection of cell lines that stably express specific sgRNAs with fluorescently labeled dCas9 and chose as many as 16 lines to study the behavior of genomic loci on different chromosomes and in different genomic contexts. In many cases, sister locus separation could be successfully detected as discrete doublets in live cells. Spatial resolution at subpixel levels can theoretically be obtained by measuring the distance between the Gaussian-fitted centroids of two dots. Stanyte et al. (2018) verified the accuracy of these measured distances by comparing them with simulated distances and found that measurements <300 nm were less reliable. Accordingly, a threshold was set to 300 nm to discriminate doublets from singlets, which would disregard smaller separations as well as movements in the z axis direction (z slice interval was 500 nm). Under these best achievable conditions, Stanyte et al. (2018) could detect sister separation within a few hours after their replication in S phase, i.e., a long time before cells enter mitosis.

To analyze "synchronized" cell populations, mechanically shaken-off mitotic cells were imaged throughout the cell cycle, and their phases were defined retrospectively by the times preceding the second mitosis, e.g., 0.6–2.4 h before mitosis as G2 phase, etc. Analyses of individual allele trajectories revealed that doublets and singlets alternate back and forth over time, reflecting the highly mobile nature of chromatin fibers. Remarkably, both the incidence of doublets and the mean distance between

Division of Experimental Pathology, Cancer Institute of the Japanese Foundation for Cancer Research, Tokyo, Japan.

Correspondence to Toru Hirota: thirota@jfcr.or.jp.

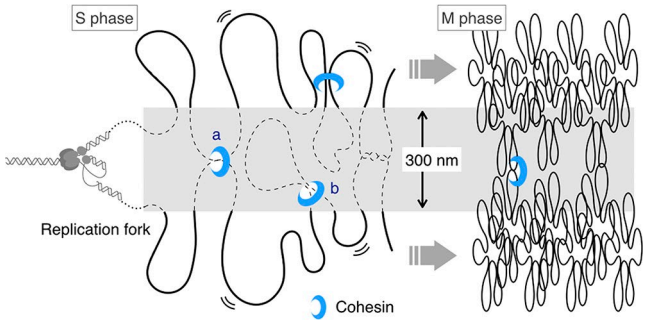

Figure 1. **Dynamic organization of sister chromatids after replication.** Replicated chromatin fibers are highly mobile and readily dissociate from each other for >300 nm, beyond the achievable resolution by light microscopy. Cohesin, which mediates interchromatid (a) and intrachromatid (b) tethering, possibly confers a correct size for sister chromatids in S and G2 phase, forming a prospective dynamic structure that relates to organizing compacted chromosomes in subsequent mitosis.

the doublets remained largely constant from S to G2. Based on these results and the accompanying modeling simulations, Stanyte et al. (2018) concluded that once sister loci separate, they quickly reach their relative position and behave as dynamic chromatin polymers (Fig. 1).

The kinetic properties of sister loci that Stanyte et al. (2018) have found provide a framework that allows us to imagine how sister chromatids are organized after replication, which was difficult before their work. First, they indicate that sister chromatids are not constantly connected along their entire lengths; instead, sisters readily dissociate from each other >300 nm apart. What promotes sister loci resolution after they have been replicated? Possible scenarios are proposed based on the dynamic reorganization of cohesin (Fig. 9 in Stanyte et al., 2018). It will be interesting to know how condensin II might fit into these models as it has been implicated in promoting sister resolution (Ono et al., 2013).

It is noteworthy to find that sister loci were resolved with high incidence even when the probes were positioned close to cohesin enrichment sites found in chromatin immunoprecipitation (ChIP) sequencing (Ladurner et al., 2016). Rather counterintuitively, loci proximal to the cohesin-enriched site appeared in doublets more frequently than those distal. Moreover, the incidence of doublets was not reduced at loci close to peaks for both cohesin and sororin, a protein that stabilizes cohesin binding on chromatin. These unexpected observations challenge the widely assumed idea that cohesin enrichment sites are persistent cohesive sites of sisters in which cohesin mediates interchromatid tethering. A plausible explanation is that a fraction of cohesin-mediating sister chromatid cohesion (cohesive cohesin) dynamically localizes along chromatin such that it cannot be mapped by ChIP sequencing, as depicted in Fig. 9 in Stanyte et al. (2018). Alternatively, given that cohesin also mediates intrachromatid interaction and organizes domains called topologically associated domains (TADs; e.g., Rao et al., 2017), the mass effects that TADs have might facilitate resolution of sister loci.

A second remarkable property of sister loci is that they stay within their relative position and do not diffuse further away. Stanyte et al. (2018) found that depletion of sororin allowed sisters to move swiftly and farther apart and indicated that cohesive cohesin is required to tether sister loci in proximity, consistent with previous observations (Nishiyama et al., 2010; Ono et al., 2013). What might be the underlying mechanism or mechanisms that constrain chromatin polymers at their relative positions? A tempting hypothesis is that the robust reorganization of compacted chromosomes occurring at the onset of mitosis may require a template "structure" that has been prepared during replication, and such structure may have an appropriate chromatid size. The finding that the mean sister locus distance in mitosis largely takes over from S and G2 phase seems to support this idea. Transaction of cohesin and condensin II on chromatin along with replication would be instrumental to organizing the dynamic chromatid structure and to mechanistically link these two essential events in the cell cycle. In line with this notion, perturbation of replication fork progression, for example by inactivating the SMC5/6 complex, radically damages chromosome structure in mitosis (Gallego-Paez et al., 2014).

In summary, the elegant work by Stanyte et al. (2018) establishes that resolution of sister loci proceeds shortly after they are replicated. Although highly mobile, sister chromatids remain within proper ranges after reaching their maximum distance. What promotes the resolution and what binds sisters at the position are some of the key next questions to understanding how DNA replication is linked to mitotic chromosome assembly. Studying the functions of the evolutionarily conserved structural maintenance of chromosomes family of protein complexes could help address this question in the future.

Acknowledgments

We apologize to those colleagues whose work could not be cited because of length restrictions.

Research in T. Hirota's laboratory is funded by the Japan Society for the Promotion of Science (15H05977 and 15H02365), Japan Agency for Medical Research and Development (17cm0106123h0002), and the Vehicle Racing Commemorative Foundation.

The authors declare no competing financial interests.

References

Chen, B., L.A. Gilbert, B.A. Cimini, J. Schnitzbauer, W. Zhang, G.-W. Li, J. Park, E.H. Blackburn, J.S. Weissman, L.S. Qi, and B. Huang. 2013. Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. *Cell.* 155:1479–1491. https://doi.org/10.1016/j.cell.2013.12.001

Gallego-Paez, L.M., H. Tanaka, M. Bando, M. Takahashi, N. Nozaki, R. Nakato, K. Shirahige, and T. Hirota. 2014. Smc5/6-mediated regulation of replication progression contributes to chromosome assembly during mitosis in human cells. *Mol. Biol. Cell.* 25:302–317. https://doi.org/10.1091/mbc.e13-01-0020

Ladurner, R., E. Kreidl, M.P. Ivanov, H. Ekker, M.H. Idarraga-Amado, G.A. Busslinger, G. Wutz, D.A. Cisneros, and J.-M. Peters. 2016. Sororin actively maintains sister chromatid cohesion. *EMBO J.* 35:635–653. https://doi.org/10.15252/embj.201592532

Nagasaka, K., M.J. Hossain, M.J. Roberti, J. Ellenberg, and T. Hirota. 2016. Sister chromatid resolution is an intrinsic part of chromosome organization in prophase. *Nat. Cell Biol.* 18:692–699. https://doi.org/10.1038/ncb3353

Nishiyama, T., R. Ladurner, J. Schmitz, E. Kreidl, A. Schleiffer, V. Bhaskara, M. Bando, K. Shirahige, A.A. Hyman, K. Mechtler, and J.M. Peters. 2010.

Sororin mediates sister chromatid cohesion by antagonizing Wapl. *Cell*. 143:737–749. https://doi.org/10.1016/j.cell.2010.10.031211111234

Ono, T., D. Yamashita, and T. Hirano. 2013. Condensin II initiates sister chromatid resolution during S phase. *J. Cell Biol.* 200:429–441. https://doi.org/10.1083/jcb.201208008

Rao, S.S.P., S.-C. Huang, B. Glenn St Hilaire, J.M. Engreitz, E.M. Perez, K.-R. Kieffer-Kwon, A.L. Sanborn, S.E. Johnstone, G.D. Bascom, I.D. Bochkov, et al. 2017. Cohesin Loss Eliminates All Loop Domains. *Cell*. 171:305–320. e24. https://doi.org/10.1016/j.cell.2017.09.026

Selig, S., K. Okumura, D.C. Ward, and H. Cedar. 1992. Delineation of DNA replication time zones by fluorescence in situ hybridization. *EMBO J.* 11:1217–1225.

Stanyte, R., J. Nuebler, C. Blaukopf, R. Hoefler, R. Stocsits, J.M. Peters, and D.W. Gerlich. 2018. Dynamics of sister chromatid resolution during cell cycle progression. *J. Cell Biol.* https://doi.org/10.1083/jcb.201801157