generation of gastric pouches by epithelial folding—relate to bilaterian segmentation? The simple mode of epithelial folding observed in cnidarians has inspired morphologists for more than 150 years, who saw similarities with mesodermal segmentation in bilaterians. Bilaterian mesodermal somites often emerge as outpocketings from the gut. The enterocoel theory proposed that both cnidarian pouches and bilaterian somites are derived from such outpocketings (*7*, *13*). The findings of He *et al*. support this theory.

One possible caveat is that the sequentially emerging *N. vectensis* folds are not generated from a posterior growth zone. This might represent a secondary simplification of cnidarian development, given that in the tube anemones (Cerianthida), which represent an early diverging branch of Cnidaria, additional folds are pushed forwards from a terminal growth zone (*9*). Examining the expression of *Hox* genes and of growth zone markers in these cnidarians could be especially rewarding, as it might establish a similar link between *Hox* spatial and temporal collinearity and the generation of body segments from a growth zone as is observed in vertebrates (*4*, *5*). Another note of caution concerns the unsolved axial relationships between cnidarians and bilaterians (*14*), which led to conflicting views about the nature of the cnidarian *Hox* axis (*15*). However, the data of He *et al*. seem to firmly settle this issue.

Twenty-five years after the revolutionary zootype concept, which considered the *Hox* genes a shared feature of all animals (*2*), we can now refine this idea and propose that the *Hox* gene cluster evolved with the emergence of segmental epithelial folds and pouches in the cnidarian-bilaterian ancestor. These persisted as gastric pouches in today's cnidarians and gave rise to mesodermal somites in the bilaterians. ■

REFERENCES

1. S. He *et al*., *Science* **361**, 1377 (2018).
2. J. M. W. Slack, P. W. H. Holland, C. F. Graham, *Nature* **361**, 490 (1993).
3. M. Akam, *Cell* **57**, 347 (1989).
4. D. Duboule, *Development* **134**, 2549 (2007).
5. J. Deschamps, D. Duboule, *Genes Dev.* **31**, 1406 (2017).
6. R. Mooi, B. David, *Annu. Rev. Ecol. Evol. Syst.* **39**, 43 (2008).
7. D. Tautz, *Dev. Cell* **7**, 301 (2004).
8. D. E. K. Ferrier, in *Hox Genes: Studies from the 20th to the 21st Century*, J. S. Deutsch, Ed. (Landes Bioscience and Springer Science, 2010), pp. 91–100.
9. D. Arendt *et al*., *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20150286 (2015).
10. G. Genikhovich *et al*., *Cell Rep.* **10**, 1646 (2015).
11. D. Arendt, M. A. Tosches, H. Marlow, *Nat. Rev. Neurosci.* **17**, 61 (2016).
12. T. Alexander, C. Nolte, R. Krumlauf, *Annu. Rev. Cell Dev. Biol.* **25**, 431 (2009).
13. A. Sedgwick, *Q. J. Microsc. Sci.* **24**, 43 (1884).
14. C. Nielsen, T. Brunet, D. Arendt, *Nat. Ecol. Evol.* **2**, 1358 (2018).
15. T. Q. DuBuc *et al*., *Nat. Commun.* **9**, 2007 (2018).

10.1126/science.aav0692

DNA REPLICATION

No strand left behind

Histone chaperones direct how epigenetic information is inherited in dividing cells

By **Kami Ahmad**[1] *and* **Steven Henikoff**[1,2]

Semiconservative replication of DNA faithfully transmits genetic information, but the copying of epigenetic information, which distinguishes cell identity and is embedded in chromatin, is more complicated: Unwinding and separating the parental double-stranded DNA for replication displaces all chromatin proteins, including the histones that package eukaryotic DNA into nucleosomes. This necessitates that chromatin organization be reestablished after every round of DNA replication. Furthermore, bound chromatin proteins must double after DNA replication, as one parental DNA chromatid becomes two daughters. The rules governing the distribution of histones to two daughter chromatids have long been the subject of speculation. However, on pages 1389 and 1386 of this issue, Petryk *et al*. (*1*) and Yu *et al*. (*2*), respectively, show that histone distribution is inherently asymmetric, but they identify mechanisms of chromatin regulation to achieve nearly equal distribution of parental nucleosomes to daughter chromatids. These findings have implications for how epigenetic information is propagated through cell divisions.

"Why are chaperones required to achieve the seemingly simple outcome of random symmetric parental-histone partitioning...?"

Chromatin assembly in the wake of the replication fork relies on two distinct processes: first, the transfer of parental histones, and second, the deposition of new histones. Experiments with bulk chromatin demonstrated the retention of parental histones on daughter strands and enabled isolation of histone chaperones, which promote the efficient deposition of new histones in the wake of the replication fork (*3*), but distinguishing how exactly this happens has been limited by methods to track histones on daughter chromatids. This is critical because all chromatin features (including histone modifications and nucleosome positioning relative to DNA sequence) must be copied onto both daughter chromatids to be propagated from one cell generation to the next. The two daughter chromatids differ in how they are replicated (see the figure), constrained by the requirement of DNA polymerases (Pols) to synthesize DNA in a 5′-to-3′ direction: the top "leading" DNA strand is replicated rapidly and processively by DNA Pol ε, whereas the bottom "lagging" DNA strand is replicated in interspersed segments of RNA primers and new DNA fragments by DNA Pols α and δ. These Okazaki fragments are further processed to displace the RNA primers and fill in the gaps to complete the double-stranded daughter chromatid. These complicated events on the lagging strand delay its completion, resulting in the generation of an extensive single-stranded DNA loop of the lagging strand behind the replicative helicase [the minichromosome maintenance (MCM) complex consists of MCM2 to MCM7].

How are these two strands packaged in the wake of the replication fork? The bulk of parental nucleosomes are distributed to daughter chromatids with tetramers of histone H3 and histone H4 [referred to as $(H3\text{-}H4)_2$] intact, and two H2A-H2B dimers are added to complete the nucleosome (*3*). Early experiments with viral circularized DNA (minicircles) concluded that parental histones were equally partitioned between the two daughter strands, with the remaining gaps filled by new histone deposition (*4*). This conclusion was further supported by genome-wide mapping of DNA replication forks (*5*, *6*).

The studies of Petryk *et al*. and Yu *et al*. reveal that this partitioning is carefully orchestrated by the replicative machinery with the help of specific histone chaperones that are well positioned to capture histones displaced from the parental chromatid. Yu *et al*. used genome-wide mapping to follow old and new nucleosome assembly on the leading and lagging strands in budding

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yeast, detecting a slight lagging-strand preference for deposition of parental histone $(H3\text{-}H4)_2$ tetramers. Deletion of the two histone-binding proteins, Dpb3 and Dpb4, which are subunits of DNA Pol ε, increased lagging-strand preference, revealing a role for histone chaperones in regulating parental histone-assembly preference. In mouse embryonic stem cells, Petryk *et al.* mapped Okazaki fragments to determine replication fork directionality and to map parental and new histone deposition across the genome. They detected a slight preference of parental histones for the leading strand. By mutating histone-binding residues within a histone-binding domain of MCM2, a replicative helicase subunit, they observed a dramatic loss of symmetric assembly, whereby parental histones were predominantly deposited on the leading strand and new histones on the lagging strand. This implies that deposition of parental $(H3\text{-}H4)_2$ tetramers on the leading strand is the default state but that deposition is delayed by binding to a chaperone domain of the replicative helicase.

Why are chaperones required to achieve the seemingly simple outcome of random symmetric parental-histone partitioning into daughter chromatids? DNA replication in eukaryotic cells displaces ~10 nucleosomes each minute, and displaced histones must be quickly captured. The leading daughter strand is available for capturing displaced histones first, because it is produced much faster than the lagging one. If binding to replicative chaperones delays histone transfer, this would allow replication of the lagging strand to be completed and thereby promote equal partitioning.

The finding that nucleosome partitioning is carefully controlled has important implications. Gene expression requires the precise localization of chromatin features, including the localization of histone modifications to promoters and regulatory elements, and the binding of transcription factors to their cognate DNA sites. The relatively slow reassembly of chromatin in the wake of the replication fork probably provides a window of opportunity for displaced transcription factors to rebind DNA, maintaining gene expression programs (*7*). The equal partitioning of histones allows equal transcription factor–binding on both daughter chromatids, ensuring that daughter cells maintain the gene expression program of their lineage.

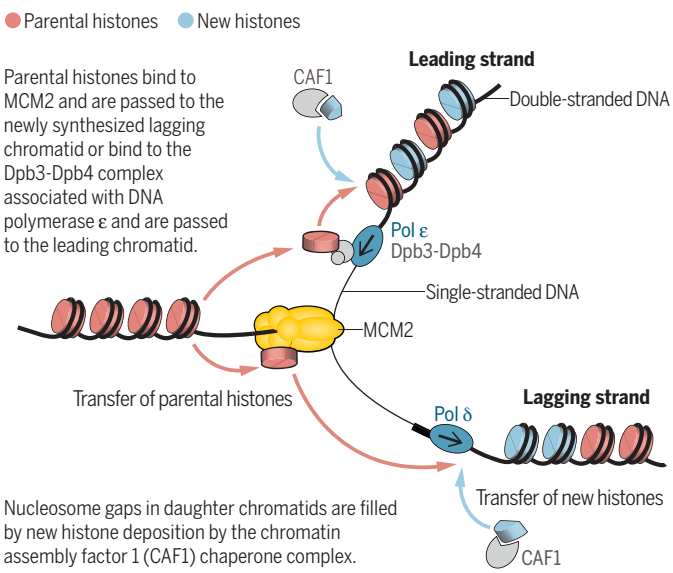
The symmetric partitioning of parental histones also creates a problem for copying patterns of histone variants and modifications. Each daughter chromatid only receives half the parental histones; thus, parental modification patterns are diluted. Further, precise nucleosome positions are not maintained because nucleosomes are displaced and then reassembled. This explains why chromatin features in eukaryotic genomes primarily rely on transcription factors that bind precisely at DNA sequences in the genome, recruiting histone modifying activities and chromatin remodelers to reestablish genomic landscapes. In limited cases, modified nucleosomes may recruit the cognate modifying enzyme, leading to modification of new histones and restoration of modification patterns (*8*). It is notable that many of the replicative histone chaperones are required for heterochromatic (densely packed chromatin) silencing of gene expression (*6*, *9*). Indeed, heterochromatic silencing of the yeast mating-type locus is defective in the absence of Dpb3 and Dpb4 (*2*), underlining the importance of delaying histone partitioning in regions where completion of the lagging strand is delayed.

Replicative histone chaperones also allow histone partitioning to be altered. Histone variants form specialized nucleosomes in certain regions of genomes. The H3.3 histone variant is enriched in active chromatin regions, and during replication, parental H3.3-containing nucleosomes are split (by an unknown chaperone) into two H3.3-H4 dimers. This allows "semiconservative" partitioning of an H3.3-H4 dimer to each daughter chromatid (*10*, *11*). These kinds of regional controls over partitioning can be imagined to affect chromatin accessibility and the dilution of histone modifications.

The regulated distribution of nucleosomes assures equal partitioning, but is unequal partitioning ever beneficial? Although, for most cells, gene expression programs in daughter cells should resemble those of the parent cell, stem cells are an exception, where one daughter cell retains stem cell identity and the other begins to differentiate. Notable differences in chromatin accompany this division in the fruitfly male germline, whereby the daughter stem cell retains the bulk of the parental histones and the differentiating daughter cell genome is packaged with new histones (*12*). This is a spectacular example of asymmetric partitioning between cells, and preliminary data show that this is the consequence of asymmetric histone partitioning in the wake of the replication fork (*13*). Asymmetric partitioning in this example results from greatly delaying the completion of the lagging strand; thus, only the leading strand is available to capture displaced histones. Asymmetric partitioning in the wake of the replication fork may promote initiation of a differentiation program because the daughter cells with lagging-strand chromatids lose all parental histone modifications and are thus open for new transcription factor binding. Understanding how asymmetric nucleosome assembly behind the replication fork may be coordinated with developmental events remains an exciting future challenge. ■

Histone partitioning at the replication fork

Chromatin-associated proteins, including parental histones, are displaced from DNA as the MCM helicase melts double-stranded DNA into single-stranded DNA.



REFERENCES

1. N. Petryk *et al.*, *Science* **361**, 1389 (2018).
2. C. Yu *et al.*, *Science* **361**, 1386 (2018).
3. A. T. Annunziato, *Genes (Basel)* **6**, 353 (2015).
4. M. E. Cusick *et al.*, *J. Mol. Biol.* **178**, 249 (1984).
5. D. J. Smith, I. Whitehouse, *Nature* **483**, 434 (2012).
6. P. Vasseur *et al.*, *Cell Rep.* **16**, 2651 (2016).
7. S. Ramachandran, S. Henikoff, *Cell* **165**, 580 (2016).
8. D. Reinberg, L. D. Vales, *Science* **361**, 33 (2018).
9. M. Foltman *et al.*, *Cell Rep.* **3**, 892 (2013).
10. M. Xu *et al.*, *Science* **328**, 94 (2010).
11. C. Huang *et al.*, *PLOS Genet.* **9**, 618 (2013).
12. V. Tran, C. Lim, J. Xie, X. Chen, *Science* **338**, 679 (2012).
13. M. Wooten *et al.*, bioRxiv 242768 [Preprint]. 23 August 2018.

10.1126/science.aav0871

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Science **361** (6409), 1311-1312.
DOI: 10.1126/science.aav0871