Nonrandom template segregation: A way to break the symmetry of stem cells

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Whether DNA segregates in a biased way has been a subject of intense controversy and debate. Although highly provocative in its biological implications, if true, technical problems have limited researchers from drawing firm conclusions from the data. Elabd et al. (2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201307110/DC1) now show a high frequency of nonrandom template segregation during differentiation of embryonic stem cells using rigorous experimentation and implicate the methyltransferase Dnmt3 as a key regulator of this process.

Nonrandom template segregation (NRTS) is a phenomenon in which two sister chromatids, supposedly the identical copies of each other produced by a precise DNA replication process, are distinguished and segregated nonrandomly to daughter cells. It has been more than a few decades since the first idea of NRTS was proposed (Cairns, 1975; Potten et al., 1978), and NRTS has been reported in organisms ranging from bacteria to plants (Rando, 2007; Tajbakhsh and Gonzalez, 2009; Lark, 2012; Lopez-Vernaza and Leach, 2013). Yet, speculation on the biological meaning of this phenomenon has remained controversial (Lansdorp, 2007; Rando, 2007; Tajbakhsh and Gonzalez, 2009). A major model, the immortal DNA strand hypothesis, suggested that stem cells or other long-living cells avoid replication-induced mutations by inheriting the old template strands. An alternative model suggested that NRTS carries distinct epigenetic information to daughter cells to allow them to adapt different fates. In addition to these different models in its biological meaning, some studies showed that all the chromosomes follow NRTS, whereas other studies showed that only a subset of the chromosomes follow NRTS. It remains unclear whether these distinct forms of NRTS are mechanistically and/or functionally related. Technical difficulties (described next) have added to the controversy.

The scheme of testing NRTS of all of the chromosomes is very simple: cells incorporate BrdU (or other nucleotide analogues) during an S phase. Two divisions later, all BrdU should go to one cell, whereas the other cell becomes completely BrdU negative, if cells follow NRTS (Fig. 1 A). Although addressing NRTS with this scheme seems simple, there are incredible numbers of pitfalls. Did the cells really undergo just one, but not more than one, round of DNA replication with the BrdU? Did they undergo just two rounds of cell division, but not one or more than two, by the time of observation? Did BrdU really label all chromosomes within the cell at a time of incorporation? Furthermore, were the cell pairs used to judge the BrdU inheritance really twin daughters from a single cell division? Do those cells that are observed to segregate BrdU asymmetrically really divide asymmetrically in terms of their fates? What is the frequency of asymmetric cell division of a given cell population, and are those asymmetrically dividing cells really the ones that show NRTS? To really understand this phenomenon, one must consider all of these questions at the same time, and answering all of them conclusively has been quite a mission impossible.

Elabd et al. (in this issue) have now addressed this question using extremely careful approaches. Starting from embryonic stem cell culture, they followed NRTS during embryoid body differentiation. Several lines of technical advancement and the application of rigorous standards by this group led to observations with striking consistency and frequency, thereby providing convincing evidence that NRTS indeed occurs during embryoid body differentiation. First, carefully adjusting the timing of BrdU administration and fixation, aided by live observation, made it possible to exclusively score daughter cell pairs generated by a cell division that incorporated BrdU with the right timing. This yielded a strikingly high frequency (~50%) of NRTS occurrence, making it unlikely that observed NRTS is caused by experimental artifacts. Furthermore, the inheritance pattern of labeled chromosomes showed tight correlation with the cell fate: BrdU segregation (i.e., newly synthesized DNA) highly correlated with the inheritance of differentiation markers, such as Bry and Gata4, strongly suggesting that it is a biologically relevant, regulated phenomenon (Fig. 1 B).

It is of note that the authors found that the "artificial occurrence of NRTS" counts up to ~5%. Such artificial NRTS occurred when unrelated cells migrate to come in contact, leading to apparent asymmetry in BrdU labeling between two cells. It has been often argued that the probability of labeled strands of all 46 (in humans) or 40 (in mice) chromosomes being segregated to one cell would be extremely low (2^{-46} or 2^{-40}) and that any observation of an asymmetric DNA labeling pattern even at a

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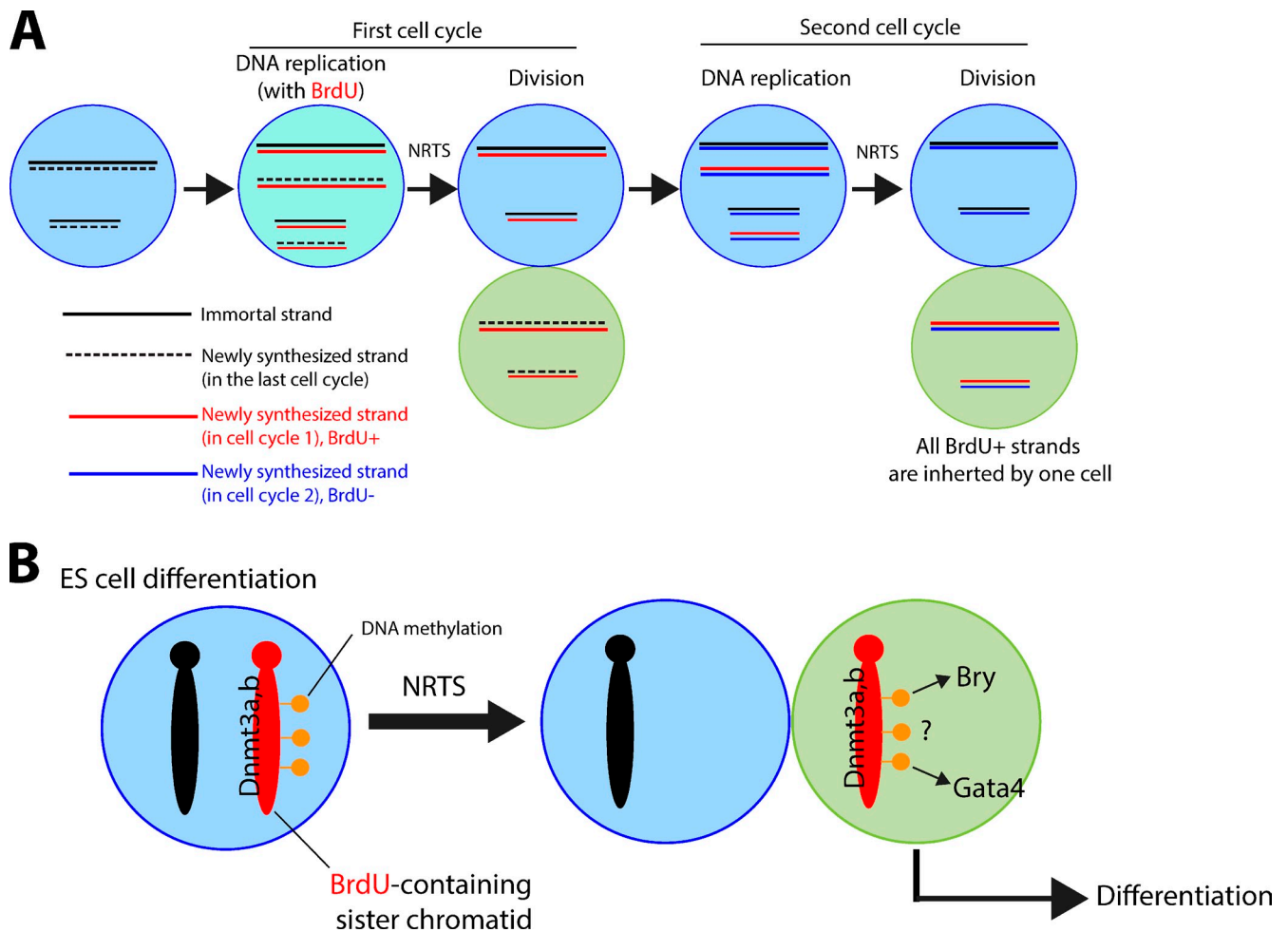www.jcb.org/cgi/doi/10.1083/jcb.201308110

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Figure 1. **New evidence for nonrandom template segregation.** (A) Assessment of NRTS by pulse–chase BrdU labeling. After BrdU incorporation during an S phase, BrdU will be exclusively segregated into one cell, but not the other, in two cell cycles. (B) NRTS observed during embryonic stem (ES) cell differentiation. The new work by Elabd et al. (2013) suggests that asymmetric BrdU segregation shows striking correlation with the inheritance of Dnmt3a and 3b and expression of differentiation genes, such as Bry and Gata4. Dnmt3a and 3b that are loaded onto the new template-containing sister chromatid (i.e., BrdU⁺) might lead to sister chromatid–specific DNA methylation, leading to lineage commitment.

low frequency would support NRTS. However, the new work by Elabd et al. (2013) clearly indicates that additional factors, such as crawling of cells, can cause an artificial NRTS-like outcome at a considerable frequency. Considering this, a new, more rigorous standard must be applied to future studies concerning NRTS.

One common feature of controversial fields is often the lack of a strong mechanistic basis from which to test predictions. This has certainly been the case of NRTS. Elabd et al. (2013) provide some of the first mechanistic insights into the process: they showed that the de novo methyltransferases Dnmt3a and 3b are required for efficient NRTS during embryoid body differentiation (Fig. 1 B). It is interesting that de novo DNA methylation, but not maintenance DNA methylation, seems to be required for NRTS. Interestingly, a higher concentration of Dnmt3a and 3b proteins were often cosegregated with BrdU-containing nuclei, suggesting that cells undergoing differentiation inherit the enzyme that allows de novo DNA methylation. This may indicate that cells undergoing differentiation need to methylate DNA to promote differentiation. However, it would not explain why de novo DNA methylation is required to allow

the distinction and asymmetric segregation of sister chromatids. A possible unifying theory may be that in a differentiating embryonic stem cell, a newly synthesized strand would be bound by de novo DNA methyltransferases, which may methylate DNA specifically on the newly synthesized strand, before or after the cell division, allowing the cell to molecularly distinguish older versus newer template DNA strands, hence enabling their asymmetric segregation. Upon cell division, DNA methylation in a differentiating cell may initiate the cascade of epigenetic events to program them into a particular cell fate/lineage. If this is the case, this may finally answer the question in epigenetics of whether the epigenome is acquired before fate determination or vice versa: epigenome changes are first triggered in the mother cell—before cell division and fate acquisition—and the daughter cell is born with a suitable epigenome that allows it to adopt a certain cell fate. These data seem to suggest that the meaning of NRTS is the transmission of epigenetic information, instead of protecting the genome stability through avoidance of DNA mutation.

Now, if the meaning of the NRTS is transmission of the epigenome, instead of protecting the genome stability through

avoidance of DNA mutation, one would wonder whether all the chromosomes should follow NRTS or whether NRTS of only a subset of chromosomes might suffice. It is possible that only a small number of genes must be regulated through NRTS: a few key fate-determining genes, which can trigger a cascade of downstream events, harbor differential epigenetic information between two sister chromatids, and the segregation of these key genes into two daughter cells breaks symmetry and leads to asymmetric cell fates. In particular, once cells settled into a certain lineage (e.g., adult stem cells), the number of fate-determining genes that needs to be regulated through NRTS might be small, leading to chromosome-specific NRTS. It is important to note that in this model, NRTS does not influence all of the genes on a chromosome: not all of the genes on a chromosome are epigenetically marked or have distinct epigenetic marks between two sister chromatids. Therefore, NRTS will influence only a few hypothetical genes whose epigenetic information is distinct between two sister chromatids, leading to asymmetric cell fate. As long as the epigenetic information of key marked genes is segregated nonrandomly, the rest of the genes on the chromosomes, which might be identical genetically and epigenetically to their sisters, do not influence the asymmetric outcome of a cell division. Chromosome-specific NRTS has been proposed previously for chromosome 7 in mice (Armakolas and Klar, 2006), although more recent studies suggested that the finding may be partly caused by the experimental method used in the original study (Falconer et al., 2012; Sauer et al., 2013). A recent observation that *Drosophila melanogaster* male germline stem cells show NRTS only with X and Y chromosomes, but not autosomes, might be a variation of NRTS, specific to lineage-committed adult stem cells (Yadlapalli and Yamashita, 2013). In contrast, during early stages of development, such as embryonic stem cell differentiation, the number of fate-determining genes that need to be regulated through NRTS might be larger, and those genes may be scattered on many chromosomes, leading to NRTS at the whole genome level. NRTS involving (almost) all of the chromosomes can be easily detected by BrdU pulse–chase experiments. Then, the underlying biological significance of NRTS at the whole genome level and single chromosome level may be the same after all. However, NRTS of a small number of the chromosomes cannot be assessed by pulse–chase labeling with BrdU and requires other methods such as chromosome orientation in situ hybridization at single-chromosome resolution. This method has not been used in many studies yet to address potential NRTS of a small subset of the chromosomes. Therefore, there might be many more cell types that perform NRTS that have yet to be discovered. In summary, the work by Elabd et al. (2013) provides a robust method and model system of NRTS that will allow investigation of its molecular mechanisms and biological meaning.

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