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Epigenetic differences between sister chromatids?

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Abstract

Semi-conservative replication ensures that the DNA sequence of sister chromatids is identical except for replication errors and variation in the length of telomere repeats resulting from replicative losses and variable end processing. What happens with the various epigenetic marks during DNA replication is less clear. Many chromatin marks are likely to be copied onto both sister chromatids in conjunction with DNA replication, whereas others could be distributed randomly between sister chromatids. Epigenetic differences between sister chromatids could also emerge in a more predictable manner for example following processes that are associated with lagging strand DNA replication. The resulting epigenetic differences between sister chromatids could result in different gene expression patterns in daughter cells. This possibility has been difficult to test because techniques to distinguish between parental sister chromatids require analysis of single cells and are not obvious. Here we briefly review the topic of sister chromatid epigenetics and discuss how the identification of sister chromatids in cells could change the way we think about asymmetric cell divisions and “stochastic” variation in gene expression between cells in general and paired daughter cells in particular.

Keywords

DNA replication; chromatin; epigenetic marks; sister chromatids

While asymmetric inheritance of proteins and RNA are known to contribute to differences in daughter cell fate, the possibility that epigenetic differences between sister chromatids also contribute to differences in cell fate has been proposed [1-5] but has been difficult to study. Indeed, most studies assume that sister chromatids, being genetically identical, are also functionally identical and that segregation to daughter cells proceeds randomly. However, given the plethora of epigenetic and chromatin modifications that are known to play a role in regulating gene expression (Figure 1), this assumption is perhaps an oversimplification for some genes (Figure 2). Many epigenetic marks are very dynamic during the cell cycle and some of these marks are known to be “erased” prior to mitosis. For example, heterochromatic protein 1 (HP1) binding to trimethylated lysine at position 9 of histone H3[6] is disrupted by the phosphorylation of serine 10 of histone H3 by Aurora B kinase prior to mitosis [7]. Conversely, at least some epigenetic marks including methylated

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cytosines and certain histone modifications (reviewed in [8], transcription factors such as CTCF [9] and Polycomb proteins [10] remain associated or bound to DNA during DNA replication and are present on mitotic chromosomes. Most likely such epigenetic marks serve to nucleate chromatin and higher order nuclear structures following mitosis and thereby function as the epigenetic “memory” that enables setting up similar gene expression patterns in parental cells and daughter cells. Therefore, the regulation of epigenetic marks during DNA replication to alter or preserve gene expression at a given locus could be an additional mechanism for differentiation or self-renewal fate decisions.

Several studies have shown that sister chromatids containing “old” template DNA are selectively retained in certain stem or progenitor cells [11-13]. These previous observations have typically been explained by the “Immortal Strand” hypothesis, which proposes that stem cells retain template DNA in order to protect against the accumulation of mutations resulting from DNA replication [14]. Based on published data and theoretical reasoning [4], we recently proposed that, if previous observations were not the result of technical artifacts, the primary function of selective segregation of DNA template strands could be the regulation of cell fate via epigenetic differences between sister chromatids (Figure 3, the “Silent Sister” hypothesis [4]). Given the dynamic expression of some genes during the cell cycle [15] and the fidelity in which epigenetic marks appear to be copied [16], epigenetic differences between sister chromatids could be limited to a select number of genes for example following processes restricted to lagging strand DNA replication [17]. Differences between sister chromatids are known to regulate the expression of the mating type-locus in fission yeast [3] and it was recently shown that segregation of sister chromatids in *E.coli* is not a random process [18]. Furthermore, another recent study showed that components of the yeast kinetochore, the protein complex that anchors chromosomes to the mitotic spindle, divide asymmetrically in a single postmeiotic lineage, suggesting a mechanism for the selective segregation of sister centromeres to daughter cells to establish different cell lineages or cell fates [19].

In order to study sister chromatid segregation in relation to gene expression and cell fate, molecular features that can be used to tell sister chromatids apart are essential. Suitable features to distinguish sister chromatids are not obvious since both sisters are the product of semi-conservative DNA replication and therefore are expected to have exactly the same DNA sequence. However, we recently found that DNA template strands that are present *prior* to cell division provide a means to reproducibly distinguish and identify sister chromatids in daughter cells using chromosome orientation fluorescence *in situ* hybridization (CO-FISH) [20]. Specifically, we found that murine chromosomes have an invariant orientation of pericentric major satellite DNA with respect to chromosome ends (Figure 4). We exploited this polarity to differentially label and follow sister chromatid segregation in post-mitotic cell pairs with probes specific for major satellite DNA. Importantly, we found that segregation of sister chromatids is non-random in a subset of murine colon cells [20]. Cell pairs exhibiting marked template strand asymmetry were found at different positions in the colon crypt, including at positions outside the crypt bottom where stem cells have been proposed to reside [21] indicating that asymmetric segregation is not restricted to stem cells. Neither the mechanism nor the function of the observed asymmetric chromatid segregation is currently known. In order to allow non-random segregation, we presume that centrosomes and centromeres have properties that enable specific kinetochore connections (Figure 5). Epigenetic differences between sister chromatids could be directly involved in the regulation of cell fate if microtubules originating from “mother” centrosomes [22] were to prefer kinetochores present on one of the two sister chromatids of specific chromosomes (Figure 5).

Conversely, it has been shown that DNA template strands are not retained in hematopoietic stem cells [23], suggesting that asymmetric strand segregation is also not a general property of stem cells. It is possible that chromatin differences between sister chromatids do not exist in such cells, bypassing the need to selectively segregate sister chromatids to daughters. Alternatively, epigenetic differences between sister chromatids in these stem cells could be functionally insignificant or lead to “stochastic” differences in the expression of genes between daughter cells.

By combining sister chromatid information with gene expression data it should be possible to test the hypothesis that epigenetic differences between sister chromatids contribute to differences in gene expression. If the silent sister hypothesis is supported by further evidence, current models explaining “stochastic” variation in gene expression between cells and models of cell fate determinants in asymmetric cell divisions will have to be adjusted. In this case broad implications are foreseen. If the silent sister hypothesis is disproven, an important contribution to our understanding of biology will nevertheless have been made. Until recently, it was not possible to test the silent sister hypothesis because molecular features to reliably distinguish between sister chromatids had not been described. With our recently published data showing that sister chromatids can be identified using DNA template strand sequences [20] it should be possible to test the “silent sister” hypothesis in the next few years.

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Figure 1.

A plethora of histone modifications and other epigenetic marks such as (hydroxy-) methylation of cytosine and binding of specific RNA molecules are implicated in the regulation of gene expression

Figure 2.

Given the challenge to faithfully copy the plethora of possible chromatin marks and epigenetic modifications (Figure 1) onto both sister chromatids it seems unlikely that sister chromatids can always be considered functionally equivalent.

Figure 3.

The silent sister hypothesis. Prior to replication (top panel) the DNA in a chromosome has two complementary strands: “Crick” (5′ to 3′, top strand, blue line) and “Watson” (3′ to 5′, bottom strand, red line). A single gene “A” is shown which is expressed as a result of specific chromatin marks (+ sign in figure). Following DNA replication (middle panels) “Watson” and “Crick” DNA template strands are copied to yield two sister chromatids with identical DNA sequence. The silent sister hypothesis proposes that not all chromatin marks are copied onto both sister chromatids during or following DNA replication. As a result only one sister chromatid will inherit the active chromatin mark (+) and the other sister chromatid (the “silent sister”) will not (−). In the figure active chromatin marks (+) follow the “Watson” DNA template strand and the sister chromatid with the original “Crick” DNA template strand does not have this chromatin mark and therefore does not support expression of gene A (indicated by a small a). Following random segregation of sister chromatids (bottom left panel), the two daughter cells will show “stochastic” variation in the expression of gene A (A or a) which is predicted to follow the parental DNA template strand that was inherited. Note that failure to copy suppressive chromatin marks will result in similar “stochastic” variation in gene expression. If sister chromatids of specific chromosomes are furthermore specifically retained in one of the daughter cells (e.g. via specific chromatin marks at sister chromatid centromeres connecting microtubules to “mother” centrosomes [22], sister chromatid asymmetry in chromatin marks at specific genes could directly regulate gene expression and cell fate as shown (bottom right panel).

Figure 4.

Highly conserved uni-directional orientation of major satellite DNA in murine chromosomes revealed by four color CO-FISH. **a.** Schematic diagram of the CO-FISH procedure. **b.** Pseudo-color CO-FISH image of murine chromosomes. The orientation of T-rich and A-rich major satellite sequences relative to the 5' and 3' end of chromosome ends is preserved in all chromosomes except the Y chromosome (arrow, no major satellite DNA). **c.** Magnification of the boxed chromosome shown in b. **d.** Definition of DNA template strands based on the uniform orientation of repetitive DNA. The top strand containing T-rich major satellite DNA is designated as the "Crick" strand corresponds to the plus (+), 5' to 3', forward, sense strand in genome sequence databases. Figure 3 Asymmetry at centrosomes [22] could result from differences in the timing or number of microtubules nucleating from each pole or from proteins enriched at a specific pole that can travel along microtubules (Figure 6a). An example of the latter could be the Adenomatous Polyposis Coli (APC) tumor suppressor protein, given its involvement in chromosome segregation and spindle assembly [24-28]. Distinguishing sister chromatids by a cell likely depends on differences in centromeric or pericentric chromatin. Asymmetry of kinetochore proteins was recently described in yeast [19] and replication by either leading or lagging strand synthesis of (peri-)centric DNA could lead to asymmetric loading of chromatin proteins at centromeres [17]. Reprinted by permission from Macmillan Publishers Reference [20], copyright 2009.

Figure 5.

Models for asymmetric segregation of sister chromatids. Only the template strand of double stranded DNA in sister chromatids prior to mitosis is shown (green and red uninterrupted lines). a. The unidirectional orientation of repetitive DNA at centromeres could result in uneven distribution of epigenetic marks (M) between sister chromatids that foster nucleation or capture of microtubules coming from the “dominant” centrosome. b. Differences in higher-order chromatin structure could alter the elastic properties of (peri-)centric chromatin allowing selection of sister chromatids via microtubules from the “dominant” centrosome. c. Regulation of cell fate predicted by the “Silent Sister” hypothesis. Alternatively, strand-specific methylation [29] or strand-specific transcription of centromeric DNA [30-32] could help establish chromatin asymmetry at sister chromatid centromeres. Centromeric RNA has been implicated in centromere assembly [33] and opposite strands of major satellite DNA are differentially transcribed in specific cell types during murine development [34]. Such chromatin differences could be recognized by factors from asymmetric spindles or favor selective attachment to microtubules via differences in elastic properties (Figure 5b) [35]. Reprinted by permission from Macmillan Publishers Reference [20], copyright 2009.