Cell

Leading Edge
Essay

Immortal Strands? Give Me a Break

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DOI 10.1016/j.cell.2007.06.017

The "immortal strand" hypothesis proposes that asymmetrically dividing stem cells selectively retain chromosomes containing "old" DNA to prevent accumulation of mutations. As I describe in this Essay, such a possibility seems unlikely. An alternative explanation is that asymmetric cell divisions and cell fate are codirected by epigenetic differences between sister chromatids.

Introduction

In 1975, John Cairns proposed the immortal strand hypothesis as a mechanism by which adult stem cells minimize accumulation of mutations (Cairns, 1975). The basic assumption of the immortal strand hypothesis is that by selectively retaining template DNA, adult stem cells avoid acquiring mutations arising from errors in DNA replication that could lead to cancer (see also the Essay by T.A. Rando on page 1239 of this issue). The immortal strand hypothesis has been remarkably resistant to challenge, in part because retention of immortal strands has only been observed in a very limited number of cell types and is not easily confirmed experimentally. Recent reports have provided further data that appear to support the immortal strand hypothesis (Conboy et al., 2007; Karpowicz et al., 2005; Potten et al., 2002; Shinin et al., 2006; Smith, 2005). However, the immortal strand hypothesis is difficult to reconcile with current data on genome organization, DNA repair, and stem cell turnover. In this Essay, various objections to the immortal strand hypothesis are summarized, and limitations of experimental approaches to test this hypothesis are discussed. Despite these objections and reservations, it seems probable that chromosomes are segregated asymmetrically in certain cells but perhaps not to avoid acquisition of mutations. One possibility is that asymmetric cell divisions and cell fate are codirected by epigenetic differences between sister chromatids. If correct, the "silent sister" hypothesis that I propose here has widespread implications for studies of normal development and of stem cells in normal and malignant tissues.

Immortal Strands: Do They Exist?

Although the immortal strand hypothesis has been around for more than 30 years, the hypothesis is not widely accepted. Surprisingly, the immortal strand hypothesis has only been tested in mammalian systems in which stem cell identity remains uncertain. As a result, it is not clear whether the apparent asymmetric segregation events take place in stem cells or in other cells. Even in the systems where it has been concluded that asymmetric division does occur, it is not clear what fraction of cell divisions involve such asymmetric segregation. In some of the studies that reported evidence in support of the immortal strand hypothesis, there are potential technical artifacts as discussed below. No molecular mechanism for the selective retention of immortal template strands in stem cells has been proposed, and the immortal strand hypothesis is difficult to reconcile with the following facts and observations.

- DNA in every cell is subject to anywhere from a few thousand to millions of chemical modifications every day (Lindahl, 1993). Such DNA lesions occur on both DNA strands and the large majority of them are efficiently repaired. Mutations result when error-free repair fails or when errors are introduced by DNA polymerases or by insertion or deletion of DNA. Thus, replication errors are not the sole source of mutations in stem cells.
- Both strands of genomic DNA encode genes. In fact, there are more examples where genes overlap on opposing strands than would be expected by chance (Veeramachaneni et al., 2004). If immortal strands did indeed exist in order to protect against accumulation of replication errors, genes on newly copied strands (copied from "immortal" templates) are predicted to show more mutations than those on the template itself. Thus, the immortal strand hypothesis predicts that some genes are better protected against mutations than others, a notion with limited appeal.
- Some DNA-repair pathways are known to involve exchanges between sister chromatids. Sister chromatid exchange (SCE) interferes with maintenance of immortal strands. To circumvent this dilemma it has been proposed that SCE does not occur in stem cells with immortal strands because such cells prefer to die by "altruistic suicide" upon damage (Cairns, 2002; Potten et al., 2002). No actual studies of SCE in stem cells showing immortal strand segregation have been performed and the proposed altruistic suicide requires a DNA-damage response that exists neither in the precursors nor in the progeny of stem cells. Furthermore,

replenishment of stem cells from a reservoir of "pre-stem cells" raises issues about how stem cells are defined and how many times stem cells can divide.

• It has been suggested that stem cells that show immortal strand segregation can divide more times than other cells because telomere shortening is prevented (Karpowicz et al., 2005; Potten et al., 2002). However, in theory, only the 3′ end of template strands is protected against replicative shortening in cells that retain DNA template strands. This is because the 5′ end of DNA template strands must be processed following replication in order to create a single strand 3′ overhang required for telomere function (Lingner et al., 1995). Thus telomere losses are predicted to occur in stem cells whether or not template strands are retained. Sporadic and strand-independent losses of telomere repeats are expected to further limit the replicative potential of stem cells (Lansdorp, 2005).

• The immortal strand hypothesis proposes that selective segregation of DNA strands is important to prevent accumulation of mutations and, indirectly, cancer. However, the coding sequence of the genome is not the only determinant in normal development or tumor formation. Nuclear transfer experiments have shown that mammalian offspring can be derived from the nucleus of a differentiated somatic cell (Wilmut et al., 1997) or even a tumor cell (Hochedlinger et al., 2004). These experiments underscore the importance of epigenetic events in normal development and tumor formation (Feinberg et al., 2006).

Are Observations Supporting This Hypothesis Valid?

The number of studies with data supporting the immortal strand hypothesis is limited. All existing reports describe indirect approaches for studying the segregation of DNA strands that suffer from various limitations. The challenges are to obtain true "single" cells for experiments and to avoid artifacts resulting from the fixation and processing steps required for analysis of the DNA strands that incorporated radioactive label or a nucleotide analog such as bromodeoxyuridine (BrdU). It seems possible that some of the cells that appeared to retain label could have been postmitotic differentiated cells rather than asymmetrically dividing stem cells. Evidence for the asymmetric segregation of label in culture could have come from two unrelated cells that happened to be stuck together when added to the culture or that happened to sit next to each other in the culture at the time of analysis. It is also important to note that most of the published data are illustrations showing qualitative pictures rather than quantitative data. At a minimum, raw image data of the published studies should be made available to allow analysis of the intensity and distribution of various labels in cells. The actual label distribution between daughter cells provides important information for models and the interpretation of results. For example, an 80:20 distribution of incorporated label seems compatible with sister chromatid exchange events unlike a 99:1 distribution. In principle, it should be possible to study immortal strand segregation directly using fluorescence in situ hybridization techniques in cells stripped of newly formed DNA containing BrdU (Bailey et al., 2004). Such studies may or may not validate previous results and could possibly also be used to address important additional questions about the nature of cells (and perhaps chromosomes) that show immortal strand segregation.

Only Some Cells Show Asymmetric Strand Segregation

There are no reports indicating that cells of the germline and cells of the early embryo show asymmetric segregation of DNA strands. Assuming such cells segregate chromatids randomly, one wonders why specialized stem cells or progenitor cells of the "mortal" soma need more protection against the accumulation of replication errors than cells of the "immortal" germline or cells of the early embryo, especially given that the latter are expected to divide more frequently than the stem cells of adult tissues. The notion that stem cells of the gut divide more than a thousand-fold over a lifetime (Cairns, 2002; Potten et al., 2002) is furthermore problematic. A recent ^{14}C carbon dating study of various human tissues has shown that the turnover of cells in hematopoietic tissues is much higher than in the gut (Spalding et al., 2005). Yet hematopoietic stem cells are estimated to divide less than a hundred times over a lifetime (Lansdorp, 1997). Thousands of cell divisions in any cell type are incompatible with these observations and estimates. Most likely, some of the rapidly dividing cells that show evidence of immortal strand segregation are not stem cells but progenitor cells that themselves are derived from a pool of stem cells with a much lower turnover.

Is There Another Explanation?

As outlined above, the immortal strand hypothesis faces many objections and raises many questions. The validity of at least some of the data supporting the hypothesis is also in question. Given these uncertainties, one could argue that future studies should primarily focus on whether asymmetric chromosome segregation does indeed occur, and, if so, in which cells, when, and where. However, one could also argue that accumulated data strongly suggest that asymmetric segregation of DNA template strands does occur in some cells. If one accepts this paradigm, a relevant question is could there be another explanation for the observations that appear to support the immortal strand hypothesis?

One alternative explanation is that, following DNA replication, sister chromatids in tissue-specific stem or progenitor cells carry distinct epigenetic marks at centromeric DNA as well as at specific genomic sites. Epigenetic differences between sister chromatid centromeres are required to direct nonrandom segregation of sister chromatids during mitosis, and epigenetic differences at certain genes could regulate the expression

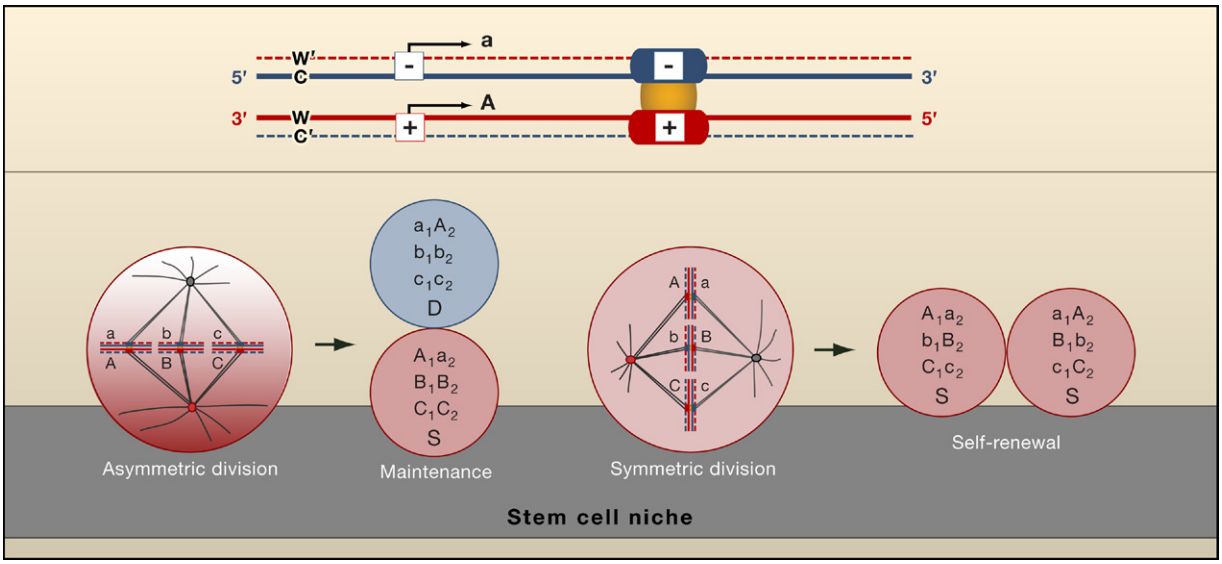

Figure 1. The "Silent Sister" Hypothesis

(Top panel) According to this hypothesis, the two sister chromatids in metaphase chromosomes carry distinct epigenetic marks (indicated by + and – signs) at centromeric DNA and at certain stem cell genes (shown is a single gene: A, expressed; a, silent). Epigenetic differences at centromeres between sister chromatids are proposed to enable chromatid-specific segregation of chromosomes during mitosis. Such nonrandom partition of chromatids is proposed to regulate the expression of certain genes present on those chromatids in the daughter cells following mitosis.

(Bottom panel, left) Selective attachment of microtubules (MT) coming from the "mother" centrosome (Yamashita et al., 2007) (indicated by the red dot close to the stem cell niche) to sister chromatids containing the Watson (W) template strand (here defined as the 3′ to 5′ template strand indicated by the solid red line) during a polar asymmetric stem cell division. Shown are three metaphase chromosomes (e.g., out of the 46 chromosomes in a human cell). The preference for one of the chromatids could result from a gradient of MT-guiding proteins (red) that regulate preferential MT binding to the kinetochore at the sister chromatid containing the Watson template strand. Selective partition of sister chromatids results in one stem cell (S) with two active copies of self-renewal genes (the two parental copies of these genes are indicated by $_1$ and $_2$) ready to be transcribed (A, B, and C) and one cell committed to differentiate (D); expression of the two copies of the stem cell genes is suppressed (a, b, and c). Note that an occasional sister chromatid exchange event between a centromere and a relevant stem cell gene (illustrated as a_2 in cell S and A_2 in cell D) is not incompatible with the silent sister hypothesis. Expression of silenced stem cell genes in stem cells (S) is predicted to be restored (bottom right panel) and aberrant expression of a single stem cell gene in a differentiating cell (A_2 in cell D) is not predicted to prevent differentiation altogether. However, inappropriate expression of multiple self-renewal genes (perhaps in combination with inappropriate suppression of tumor suppressor genes) in differentiating cells could result in abnormal cell proliferation. Such defects could result from high levels of sister chromatid exchange, defects in cell polarity, failure to establish or recognize epigenetic marks at centromeres, or other factors involved in the proposed strand-specific chromatid segregation pathways.

(Bottom panel, right) Random segregation of sister chromatids restores expression of "silent" stem cell genes and results in self-renewal of stem cells. Without polar distribution of guiding proteins (even red color) both daughter cells inherit a random mixture of active and inactive stem cell genes, which is predicted to typically restore the expression of the stem cell genes in both daughter cells if cells receive appropriate signals from the microenvironment (the stem cell "niche"). Following expression, epigenetic differences between sister chromatids are restored during DNA replication. Note that only one out of eight possible random expression patterns for the three genes in the two daughter cells is shown.

of those genes following mitosis (Figure 1). According to this hypothesis, two alternate fates are possible in dividing stem and progenitor cells. Random segregation of chromatids is predicted to result in maintenance of self-renewal properties in both daughter cells. In contrast, selective retention of chromatids with "active" stem cell genes is predicted to result in maintenance of self-renewal properties in that cell and loss of stem cell properties in the cell that inherits the opposite "silent" sister chromatids. Apart from specific epigenetic marks at centromeres and certain genes, the difference between random and selective chromatid segregation is predicted to depend on cell polarity and the orientation of the mitotic spindle (Figure 1, bottom panels).

Both the immortal strand as well as the silent sister hypotheses require distinction between sister chromatids at centromeres and sister chromatids at kinetochores to enable strand-specific segregation of chromatids. The existence and nature of such differences remain to be established. However, this is perhaps not surprising if the presence of epigenetic asymmetry at centromeres is indeed limited to rare primary cells that undergo asymmetric division in the context of a specific stem cell "niche." The role of epigenetic factors in centromere function is well established, and chromatid-specific epigenetic differences at centromeres could be related to the transcription or replication of centromeric sequences (Slotkin and Martienssen, 2007). Epigenetic asymmetry at centromeres and selected genes is predicted to be developmentally controlled and could vary between chromosomes and between cells. The pathways and proteins that preferentially connect the mitotic spindle to the "active" sister chromatid before connecting to the silent sister chromatid remain to be established.

The silent sister hypothesis predicts that expression of a subset of "stem cell" genes (A, B, and C

in Figure 1, bottom panels) is regulated by epigenetic marks resulting in one sister chromatid supporting and one sister chromatid (the silent sister) suppressing the expression of that gene in daughter cells following mitosis. The opposite asymmetry could suppress/support the expression of "differentiation" genes and other genes such as tumor suppressor genes. Although of interest in the context of asymmetric cell divisions and cell fate, such genes are not considered here. While the possibility that gene expression and immortal strand segregation could be related was suggested previously (Armakolas and Klar, 2006; Conboy et al., 2007; Karpowicz et al., 2005; Shinin et al., 2006), no models that allow clear predictions were proposed. According to the silent sister hypothesis the expression of specific genes in stem cells and stem cell fate is governed by epigenetic differences between sister chromatids and by symmetric (expansion) divisions versus asymmetric (maintenance) divisions. The genes that are regulated by the postulated chromatid-specific epigenetic marks are not known. Some of these genes are expected to encode factors required to erase the epigenetic marks involved in silencing stem cell genes (Figure 1, bottom right). Other candidates are the genes that in undifferentiated, self-renewing embryonic stem cells display "bivalent" chromatin features typically associated with silent as well as active chromatin (Bernstein et al., 2006). Such bivalent marks could reflect the proposed transition from silent to active chromatin at selected genes in cells that randomly segregate sister chromatids (Figure 1, bottom right).

Conclusions

This Essay has attempted to summarize why the immortal strand hypothesis is an unlikely proposition. It remains possible that nonrandom segregation of chromosomes simply does not occur. However, a growing body of evidence does appear to support nonrandom segregation of sister chromatids during mitosis, making it worthwhile to consider alternatives to the immortal strand hypothesis. One possibility is the silent sister hypothesis proposed here. The immortal strand hypothesis proposes that asymmetric divisions in certain stem cells protects against accumulation of DNA replication errors. In contrast, the silent sister hypothesis proposes that nonrandom segregation of sister chromatids is required to direct gene expression and cell fate in stem and progenitor cells. In a sense, the silent sister and immortal strand hypotheses are not mutually exclusive, as the segregation of immortal strands to a particular cell type during stem cell division could serve both as a determinant of cell fate and as a way to promote genomic integrity. However, the silent sister hypothesis predicts that the primary purpose of nonrandom chromatid segregation is to regulate cell fate. The possibility that sister chromatids show epigenetic differences provides a new framework to explore some of the major puzzles in stem cell biology: how do stem cells regulate self-renewal and how do stem cells switch between symmetric and asymmetric cell divisions? (Morrison and Kimble, 2006). If correct, the nature and regulation of the proposed epigenetic marks, the identification of all of the genes that are regulated by such marks, and the molecules and pathways involved in strand-specific chromatid segregation will be subject to intensive study over the next few years. Such studies could transform current ideas about normal development, stem cell biology, and tumor formation.

ACKNOWLEDGMENTS

I thank E. Falconer, R. Kay, E.-J. Uringa, L. Lefebvre, and the reviewers for helpful comments on this manuscript.

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