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Letters

"The Immortal DNA Strand": Difficult to Digest?

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The "immortal DNA strand" hypothesis was originally formulated by Cairns in 1975 and proposed as a mechanism to protect the genome of tissues with high turnover, such as the intestinal epithelium and the skin, from accumulating mutations occurring during DNA replication. Cairns proposed that, at one point during development, past the phase of expansion of the stem cell population, stem cells switch from a symmetric to an asymmetric mode of cell division. During each subsequent asymmetric division, one of the two template DNA strands of each chromosome (the "stem" template) is selectively transmitted to the "stem" daughter cell, whereas mutations accruing during replication will be passed on to the short-lived "non-stem" daughter cell, together with the "non-stem" template (Cairns, 1975). Over the past decade, a lot of effort has been put into addressing the fundamental tenet of the Cairns hypothesis, i.e., whether or not asymmetric segregation of all chromosomes (ASAC) occurs in tissues with high turnover. The most frequently used experimental approach involves labeling the "stem" template DNA strand with [^{3}H] thymidine or BrdU when conditions are conducive to symmetric stem cell divisions (yielding two stem cells), such as during periods of rapid growth or injury-induced repair, and then monitoring for evidence of selective retention of a labeled parent strand by long-lived daughter cells. Despite indirect evidence in support of the Cairns hypothesis obtained in a variety of tissues using this type of approach (reviewed in Lansdorp, 2007; Rando, 2007), the idea has remained controversial.

We therefore read with interest in a recent issue of *Cell Stem Cell* the study from Quyn et al. (2010), who used a labeling approach as part of their investigation of the orientation of cell division in the intestinal epithelium. The intestinal epithelium is probably the best candidate site for ASAC for several reasons: It is the most rapidly renewing tissue in the body; it is regenerated by long-lived multipotent stem cells (Barker et al., 2007; Sangiorgi and Capecchi, 2008); and no quiescent intestinal stem cells, which could play a role of "guardian of the genome," have been identified so far. Using DNA labeling with a nucleotide analog during postirradiation crypt regeneration, as in previous studies, Quyn et al. (2010) reported a distribution of label-retaining cells (LRCs), along the mouse small-intestine crypt axis, similar to that previously published by Potten et al. (2002), thus providing support for the Cairns hypothesis. Of note is the discrepancy in the frequency of LRCs segregating chromosomes asymmetrically reported by Potten et al. and Quyn et al. Using a second BrdU labeling assay to monitor loss of the newly synthesized DNA strands from LRCs (label-loss-at-the-second- division assay), Potten et al. reported that nearly all LRCs segregate chromosomes asymmetrically, whereas at least 40% of mitotic LRCs did not in the Quyn study.

To what extent do the studies by Potten et al. (2002) and Quyn et al. (2010) validate the "immortal strand" concept? In our view, outstanding questions remain. Most notably, the possibility exists that the results of both studies might have been affected by the injury protocol used in their experiments and the cellular response to injury. However, this point has been at least partly addressed by a recent study from Falconer et al. (2010) in which DNA strand distribution between stem and nonstem daughter cells in mouse colon sections was analyzed without prior irradiation. These authors observed a higher frequency of daughter cell pairs with extreme asymmetry than would be predicted by simulated random segregation, which they interpreted as evidence for nonrandom segregation of chromatids. However, in our view, the fact that 100% asymmetry (ASAC) was never observed may in fact argue against the Cairns hypothesis.

Could the long-term label retention in the stem cell compartment observed by Potten et al. and Quyn et al., and the asymmetric segregation seen by Falconer et al., reflect the asymmetric segregation of a unique subset of chromosomes? If so, Cairns's original underlying hypothesis (1975) about protection against the consequences of accumulating mutations would no longer hold, and the physiological role of such asymmetry would be entirely unclear. One way of investigating this possibility might be to combine chromosome orientation fluorescent in situ hybridization (CO-FISH) with composites of chromosome-specific probes. Alternative experimental approaches could perhaps also avoid the concerns about prelesioning of the tissue that are inherent to the standard label retention assay. For example, an inevitable consequence of ASAC after labeling of cells with thymidine analogs is the generation of unlabelled cells after a chase period corresponding to two cell divisions (label loss at the second division). In view of the reportedly high proportion of intestinal epithelial stem cells transiting through the S phase under steady state conditions (Barker et al., 2007), monitoring the proportion of cells with label loss at the second divison might be an attractive alternative to the label retention assay.

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Response to Legraverend et al.

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I thank Drs. Legraverend, Escobar, and Jay for their interest in our recent manuscript, in particular our data showing asymmetric segregation of label-retaining DNA in dividing cells in the stem cell compartment of the intestine. I welcome the opportunity to respond to the specific questions raised in the letter about our study (Quyn et al., 2010) and about whether our data support Cairns' hypothesis or alternative interpretations are possible.

As Legraverend et al. (2010) discuss, asymmetric DNA segregation was previously observed in mouse gut epithelium by Potten et al. (2002) using a labeling protocol related to ours and 2D, sectioned tissue material. In our view, our data are entirely consistent with and build on the findings described in the previous analysis, and any numerical discrepancy is likely a result of the difference in protocols used. Importantly, we used 3D imaging of whole tissue, which permits examination of entire mitotic figures in the context of whole tissue from all angles, and thus excludes potential sectioning artifacts. We use this type of analysis to count the number of mitotic cells that unambiguously segregate their labeled DNA asymmetrically and also record differences in stem cell versus non-stem cell compartments. This type of quantitation was not performed previously.

Legraverend et al. also raise the possibility that the long-term label retention in the stem cell compartment observed in Quyn et al. (2010), by Potten et al. (2002), and by Falconer et al. (2010) in their recent related paper reflects the asymmetric segregation of a unique subset of chromosomes. However, the EdU label in our dividing cells is very clearly restricted to only the basal side of dividing cells and in these cases, all the DNA on the basal side is labeled with EdU as shown by perfect overlay with DAPI. These data seem inconsistent with the idea that only a subset of chromosomes is labeled. Please note that in symmetrically dividing, label-retaining cells we commonly observed patchy EdU distribution, suggesting the we can detect subsets of chromosomes with this method.

Lastly, Legraverend et al. raise questions about whether the asymmetry we observed was induced by the radiation used for eliminating stem cells and might reflect a cellular response to injury. In our opinion, this is unlikely for a number of reasons: (1) Tissue is analyzed 11 days after the radiation event (3 days of labeling plus 8 days of recovery). At this stage the tissue is completely normal in appearance and function. (2) Asymmetric segregation correlates perfectly with asymmetric alignment of mitotic spindles, which is detected in nonirradiated tissue. (3) Division in the non-stem cell compartment (above position +4) rarely showed asymmetric segregation, and (4) tissue from $Apc^{Min+/-}$ mice did not show asymmetric segregation, suggesting that it is not a general consequence of radiation treatment. Moreover, as Legraverend et al. discuss, Falconer et al. used an entirely different approach that did not involve injury and yet also observed nonsymmetric DNA segregation.

We completely agree that the underlying mechanism for asymmetric segregation/division, the biological relevance for cancer, and the relationship to stem cell maintenance are key questions to tackle in future research and that a combination of tools that take into consideration the issues raised by this discussion are required to address these issues.

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