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Unbiased about chromosome segregation: give me a mechanism and I will make you "immortal"

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Abbreviations

BrdU	5-Bromo-deoxyuridine
ES cells	Embryonic stem cells
CO-FISH	Chromosome orientation fluorescence in situ hybridization
mtDNA	Mitochondrial DNA

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In the middle 1960s, several works reported the surprising finding that some cells, from bacteria and plants to mammalian systems, segregate their chromosomes in a nonrandom fashion (Lark and Bird 1965; Walen 1965; Feldman 1966; Lark et al. 1966; Lark 1967, 2012). Even more surprising, during chromosome segregation, these cells somehow distinguished the age of the DNA strands associated with each sister chromatid. It took about 10 years for someone to realize the implications of these results and propose a hypothesis about the purpose of nonrandom chromosome segregation. This became known as the "immortal strand" hypothesis in which John Cairns proposed that "*stem cells would be protected against errors of duplication if it were so arranged that the immortal daughter cell always receives the DNA molecules which have the older of the two parental strands and the mortal daughter always collects the molecules with the younger parental strand* (*that is*, *always collects the mistakes made in the previous generation*)" (Cairns 1975).

Since these early days, and revitalized by the recent hype for stem cell research, a field emerged to challenge this simple idea in different systems and until today this field is enflamed by highly controversial evidence that supports or denies it (Lansdorp 2007; Rando 2007). This is reflected in the reaction of John Cairns himself, after being

invited to write an Epilogue for this special issue, which I share below with his approval:

March 19, 2013

Dear Helder Maiato

The more I read the papers you have sent me, the more strongly do I realize that I should NOT add a short note to the end of your collection of papers. My simple thought about stem cells was published 38 years ago when most of your contributors were, no doubt, still in school (I remember on my first visit to America meeting someone who even then (more than half a century ago)) said he had believed that I was an old old man (at last, I am now 90 and feel about 100)… In short it is time for me to stop. But I have enjoyed reading your collection of papers and I do hope that you will send me the final printed version.

Best wishes

John Cairns

In the base of this controversy over the years lie a number of experimental limitations, such as the difficulty of unequivocally distinguishing and selecting stem cells, of correlating these cells with selective labeling of older or newer DNA strands, and of labeling DNA in a nontoxic manner. The debate is also motivated by the near absence of a clear working model for how the mitotic apparatus distinguishes and segregates sister chromatids, such as to separate them according to the age of their respective DNA strands. Finally, discussions have also concerned what the function of biased chromosome segregation might be, questioning its role in conserving sequence information but suggesting that it could play an important role in the segregation of epigenetic states. In any case, these discussions made clear that here, as in many other fields of cell biology, the demonstration of the molecular mechanisms underlying a given process is paramount to establishing its true nature and allowing discussions about its possible meaning.

With these considerations in mind, we felt that it was time to devote a special issue of Chromosome Research to establish the current state-of-the-art research around the immortal strand hypothesis and to identify the main challenges faced by the field. In order to do so, the first three reviews of this special issue present the current evidence in support for random chromosome segregation in several systems. Daniel Burke reviews the situation in the yeast *Saccharomyces cerevisiae*, Catherine Legraverend and Philippe Jay in mouse intestinal epithelial stem cells, and Sanjeev Waghmare and Tudorita Tumbar in mouse adult hair follicle stem cells. Next, Brendan Evano and Shahragim Tajbakhsh focus on discussing what is probably the best evidence so far for biased segregation of all template DNA strands in muscle stem cells. They also discuss possible mechanisms involved in the process. Supporting the demonstration of asymmetric chromosome segregation, Yukiko Yamashita discusses in the following contribution an intriguing example specifically affecting sex chromosomes in *Drosophila* male germline stem cells, discussing how and why only sex chromosomes. Finally, Xin Chen and colleagues discuss recent evidence of biased distribution of histones also in *Drosophila* male germline stem cells, and propose a model to explain the segregation and inheritance of epigenetic modifications during stem cell division.

One cannot envision biased DNA strand segregation without a mechanism involving interaction with the mitotic apparatus. As not much is known for chromosomes themselves, we envisioned that it may be useful to interrogate what we currently know about other asymmetries within the mitotic apparatus itself and how these are coordinated with cell fate determinants during stem cell division. This problem is discussed by Nasser Rusan and colleagues, focusing on biased segregation of centrosomes in several systems and the role of centrosome asymmetry in the asymmetric segregation of other organelles and mRNA. In this regard, mitochondrial and chloroplast DNA are classic examples of uniparental inheritance (Birky 2008). For instance, maternal inheritance of mtDNA in animals is nearly absolute and a possible way to minimize mutations transmitted to the progeny. This is thoroughly discussed by Arnold Bendich in a new theory in which uniparental inheritance of organelle DNA is seen as a mechanism of DNA abandonment in organelles that have been exposed to extensive DNA damaging agents, such as reactive oxygen species.

Last, but not least important, three new research articles focusing on the methodology to selectively track sister chromatid segregation patterns are presented. The first article by Amar Klar and Michael Bonaduce builds on the phenomenon of mating/cell type switching in the

fission yeast *Schizosaccharomyces pombe* as a purely (and elegant) genetic tool to determine that the strand segregation pattern of chromosome 2 is random. In a second article, Amar Klar and colleagues present a modified CO-FISH assay to refine previous experiments and interpretation related with the determination of the segregation pattern of chromosome 7 in mouse embryonic stem (ES) cells (Armakolas and Klar 2006). Based on this approach, the authors now concluded that segregation of chromosome 7 in mouse ES cells is random and draw attention to the profound cytotoxic effects of BrdU incorporation experiments. Finally, Maiato and colleagues present a new method for the selective and unequivocal tracking of single chromatids containing template DNA strands at high spatial and temporal resolution in *Drosophila*-cultured cells and discuss its possible application as a complementary strategy for the dissection of the molecular mechanism accounting for biased/asymmetric DNA strand segregation during (stem) cell division.

Our conclusion from the state-of-the-art in-depth analysis is that there is now compelling evidence that chromosome segregation is not random, at least in some systems. The obvious next step should then be concentrated in dissecting the molecular mechanisms facilitating the biased segregation of sister chromatids. Our understanding of the mechanisms will be the ultimate test to immortalize a simple idea. Clearly, this will require that the hard core cell division and stem cell communities join forces.

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