Discovering non-random segregation of sister chromatids: the naïve treatment of a premature discovery

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INTRODUCTION
In 1966, Richard Consigli, Harish Minocha and I published a paper in Science entitled “Segregation of sister chromatids in mammalian cells” (Lark et al., 1966). The first sentence of the abstract read as follows: “Segregation of sister chromatids in embryonic mouse cells in primary tissue culture is not random.” In so doing, we reported the unexpected existence of non-random mitotic segregation of eukaryotic chromosomes in stem cells, the focus of this book. Non-random segregation in mouse cells was discovered as a consequence of analyzing bacterial DNA replication and segregation during the next few years (1967–1969) we demonstrated the phenomenon in plant cells as well.

Our results are an example of the discovery of a phenomenon for which an appropriate hypothesis was lacking at the time; possibly an anomaly because it did not lend itself to any existing body of information in either a supportive or contradictory role. It was data driven science — and in some sense, premature (see Stent, 1972). This brief memoir traces the sequence of events leading to the discovery of non-random replication and describes the scientific context at that time: what we knew and what we did not know (or even suspect) may explain the pre-maturity that often becomes associated with data driven science.

THE DISCOVERY OF NON-RANDOM SEGREGATION
In 1963, we began a series of experiments on DNA replication (and eventually segregation) in bacteria (Lark et al., 1963; Lark and Bird, 1965; Lark, 1966b,c). A decade had passed since the announcement of the structure of DNA (Watson and Crick, 1953) during which ingenious experiments had: (i) verified that structure (Joussot et al., 1961); (ii) demonstrated the existence of semi-conservative replication in eu- and pro-karyotes (Taylor et al., 1957; Meselson and Stahl, 1958); and (iii) suggested a mechanism for regulating the initiation of DNA synthesis in bacteria (Jacob et al., 1963).

Four experimental tools were essential to these results: pulse chase as a technique for in vivo analysis of sequential intracellular events (Roberts, 1964); autoradiography of tritiated thymidine labeled DNA (Taylor et al., 1957; Painter, 1958); density labeling of DNA(dT2N; Meselson and Stahl, 1958; or 5-Bromo-uridine: Lark et al., 1963); and the use of conditional lethal mutations to dissect intracellular bacterial processes (Epstein et al., 2012).

The cell biology of bacterial growth also had been analyzed during that decade (Schaechter et al., 1958), demonstrating, among other things, that the cellular content of RNA and DNA changed when bacteria grew at different growth rates in different media. In poor media (slower growth rates), the content of RNA and DNA were lower than during more rapid growth in richer media. Our experiments utilized a strain of Escherichia coli, 15T−, which could contain either two separate replicating chromatids per cell, or only one depending on the growth rate determined by different nutrients. When grown rapidly (in glucose), the two chromosomes would replicate at the same time, whereas at somewhat less rapid rates of growth (succinate) first one would be replicated and then the other (Lark and Lark, 1965; Lark, 1966a). As far as could be determined the two chromatids were biologically identical, since when grown very slowly (acetate) cells contained only one chromosome, but could regenerate the two chromosome content if transferred to a medium promoting faster growth.

In order to establish the chromosome content of 15T− cells grown at these different rates, we labeled cells with a pulse of tritiated thymidine and then grew them in non-radioactive medium for

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different periods (chase) and plated them at different times onto non-radioactive nutrient agar to allow the development of microcolonies. Autoradiography of either cells or the microcolonies derived from these cells established the number of radioactive DNA units labeled by the pulse (Lark and Bird, 1965). We expected that after a chase period, chromosomes would be distributed randomly – i.e., these cells each containing two chromosomes would contain two radioactive chromosomes, one radioactive and one non-radioactive chromosome, or two non-radioactive chromosomes in frequencies predicted by a binomial distribution. A surprising result was that labeled and unlabeled daughter chromosomes were not distributed randomly into daughter cells. Instead, each daughter received one of each!! This suggested to us that during DNA segregation, the cells somehow distinguished between apparently identical chromosomes on the basis of the age of their template strands (Lark, 1966c).

We were curious if such discrimination might occur during eukaryotic somatic growth, but we had not worked with any eukaryotic system. In order to test this we decided to use a cell culture system and approached a colleague, “Dick” Consigli, who was studying polyoma virus grown on tissue cultures of mouse embryo cells (Consigli et al., 1966). In collaboration with Consigli and Minocha, we labeled primary cell cultures derived from embryonic tissue with tritiated thymidine and subsequently grew them in non-radioactive medium (“chase”). This serendipitous selection of a primary cell culture yielded dramatic results described in the 1966 Science paper.

Figure 2 presents the results of labeling the cells continuously for several generations (Figure 2(I)) or of a period of radioactive labeling followed by growth in non-radioactive medium (chase Figure 2(II)). It was immediately evident that the amount of radioactivity in cells increases or decreases discontinuously in a manner to be expected if the 40 chromosome templates that had incorporated radioactivity remained together.

An unexpected bonus of this cell system was the frequent occurrence of cells with two nuclei that had yet to divide. Radioactivity of such nuclei (grains per nucleus) are also presented in the experiments in Figures 2(I) and (II). After a two to three generation chase many of the two nucleate cells were still radioactive, but only one nucleus was heavily labeled. Segregation was not random in these mouse cells.

We soon discovered that established tissue culture lines (HeLa or CHO) had lost this property (Lark et al., 1966). Had we begun with established cell lines, we would probably have concluded that the non-random segregation we had documented in bacteria was not a property of somatic mammalian cell division, and we would have abandoned the investigation. Instead, we speculated that the polyploid nature of these established cell lines had obscured the non-random segregation of diploid chromosome sets. Although the distinction between cell lines that had acquired immortality and primary cell lines with programmed longevity was known (Hayflick and Moorhead, 1961; Hayflick, 1965), we had not considered this distinction as a possible explanation for the difference between a primary mouse line and the HeLa or CHO lines.

Our desire to avoid possible changes in ploidy during prolonged tissue culture led us to search for alternative preparations in which the non-random segregation could be studied in vivo. Autoradiographic analysis of mitosis in plant root tips had been used with great success by Taylor et al. (1957), who elegantly used autoradiography to establish that a chromosome was one DNA molecule that replicated semi-conservatively. We decided to use this system and settled on a pulse-chase protocol in which root tips were first grown for a period in radioactive thymidine (pulse) and then grown in non-radioactive medium for a much longer time (chase).
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FIGURE 2 | Non-random segregation of radioactive chromosomes in primary cultures of mouse embryo cells (data taken from Figures 1 and 2 of Lark et al., 1966). Distribution of silver grains in autoradiographs of mouse embryo cells:

(I) Grown as a primary tissue culture for 1, 2, or 3 generations in H3-thymidine (0.025 mc/ml) (A) An inoculum of 2.5 × 10⁶ cells per Petri dish (150 mm) was grown for 24 h. (B) 1.25 × 10⁶ cells were grown for 48 h. (C) 0.63 × 10⁶ cells were grown for 72 h.

(II) Grown as a primary tissue culture for one generation in H3-thymidine (0.025 mc/ml) and for two subsequent generations in non-radioactive medium (chase).

(A) 0.3 × 10⁶ cells per Petri dish (35 mm) were grown for 24 h, in medium containing H3-thymidine; (B) 0.15 × 10⁶ cells were grown for 24 h as in (A) and then the medium was replaced with non-radioactive medium and cells grown for an additional 24 h; (C) 0.08 × 10⁶ cells were grown for 24 h as in (A) and then for 48 h in non-radioactive medium. For details see Lark et al. (1966). The solid curves represent the result expected under a null hypothesis of random segregation of equally labeled chromosomes.

We analyzed root tips of the diploid bean, Vicia faba that has 12 easily visualized chromosomes, and in which sister chromatid exchange had been studied in detail by Pedana (1963). By examining anaphase preparations we could compare the amount of radioactivity in the two sets of chromosomes separating after the pulse-chase had been completed. The data (Lark, 1967), corrected for sister chromatid exchange, clearly supported non-random segregation of chromosomes in which “parent template” radioactive DNA was separated from non-radioactive “grandparent template” DNA (Figure 3).

To test the idea that polyploidy might obscure non-random segregation we also examined root tips of wheat, Tritium aestivum (2n = 42) a hexaploid composed of three sets of similar but not identical homeologous chromosomes. The results of our pulse-chase experiments were not clear-cut. The difference between chromosome sets in anaphase preparations was less apparent than in V. faba (Lark, 1967), but left open the possibility that there might be a sub population of cells in which segregation was non-random. In contrast, chromosome segregation was clearly non-random in Tritium buetum (4n = 28, AAAA) a tetraploid relative of modern wheat. Cytological studies of mitosis in wheat (Feldman et al., 1966) had just been published suggesting that during inter-phase, chromosomes did not completely de-condense and that homeologous sets of chromosomes were not randomly distributed throughout the nucleus thus opening the possibility that segregation of each of the three diploid sets that composed the hexaploid might be regulated autonomously. We therefore decided to further analyze segregation in Tritium using genetically different, but related, plants.

An outstanding achievement of 20th century evolutionary cytogenetics was the research by Hitoshi Kihara (Japan), Earnest Sears (USA), and Nikolai Vavilov (Russia; reviewed by Crow, 1994) that led to an understanding of the origins of hexaploid wheat. Their analysis established that wheat was a hexaploid composed of three diploid sets of similar, but not identical, homoeologous chromosomes (A, B, and D; 2n = 14/set). Their research had produced a number of genetically different Tritium lines. We obtained seeds from Sears and in the process learned about the history of wheat and the use of polysomic/nullisomic lines to associate phenotypes with specific chromosomes or portions of chromosomes. In polysomic/nullisomic lines of wheat, particular chromosomes, or arms of chromosomes, of one homoeologous set are replaced by extra copies of the same chromosome from another, different, homoeologue. For example nulli5B-tetra5D (2-5A:0-5B:4-5D) or nulli5D-tetra5B (2-5A:4-5B:0-5D) lines lack homoeologous chromosome 5B or 5D, respectively. The results (Figure 4) of comparing radioactive segregation in anaphases of cells from pulse-chased root tips from these different lines led to the conclusion that a locus, or loci, on chromosome 5 regulated non-random segregation (Lark, 1969a): 5B promoted non-random segregation; 5D promoted random segregation.
Additional experiments varying the chromosome 5 dosage of different homeologs (A, B, or D) in tetraploid lines of *Triticum Lark (1969a)* led to the conclusions that: (i) non-random segregation was a normal process in wheat, (ii) a focus on chromosome 5D suppressed non-random segregation, (iii) the magnitude of this effect in the presence of 3B was dose dependent, and (iv) that although 5A alone (e.g., tetra 5A in this effect in the presence of 5B was dose dependent, and (iv) that

The department in Utah was, and still is, united by a common deep interest in evolutionary phenomena and my exposure to this from different faculty, ranging from population ecologists to this from different faculty, ranging from population ecologists...
to molecular biologists, returned my thoughts if not my hands
to non-random segregation. John Cairns, a friend and colleague,
who shared a deep interest in DNA replication, visited for a few
weeks in 1972 and 1973, during which we discussed the non-
random segregation experiments. Our results interested him and
eventually led to his hypothesis on the role of non-random segre-
gation in maintaining template fidelity, consequently reducing the
frequency of cancer in populations of rapidly reproducing intesti-
nal epithelial cells (Cairns, 1975). Cairns’ ideas thus were the first
formal exposition of the concept that in higher eukaryotes non-
random segregation would serve to differentiate between cells of
different generations whose function and fate would thenceforth
be different.

At that time very little genetic analysis of interactive networks
had been carried out. Genetics was still focused on single gene
effects. Phenotypes were mostly qualitative and the techniques
for analyzing quantitative genetic systems were just beginning to
be developed (e.g., Falconer and Mackay, 1996). Today, we are
very aware of genome wide interactions and their importance in
regulating a multitude of quantitative phenotypes. Thus, the selec-
tive advantage of non-random mitotic chromosome segregation
in preserving networks of inter-chromosome information now
seems evident. Cairns’ ideas focused on the exclusion of deleter-
iouss mutations thus maintaining the integrity of stem cells with
“healthy” genomes. However, if we invoke the idea of directed epi-
genetic variation involving multiple chromosomes, non-random
segregation can be viewed as a mechanism for preserving cell
lineages with useful adaptations. Such lineages could then be
maintained throughout development and morphogenesis (Reik,
2007; Fedoriw et al., 2012). The ability to maintain such networks
also would provide flexibility in coping with environmental vari-
ation constituting a powerful selective advantage for plants as well
as for animals that lack intrinsic homeostatic control (e.g., see Feil
and Berger, 2007). These, as well as other consequences, promise
an exciting future for the investigation of phenotypes benefiting
from non-random chromosome segregation during mitosis.

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