

Chromosomes, positions please!

Ruth R. E. Williams and Amanda G. Fisher

Chromosome organization in the interphase nucleus is largely regarded to be non-random. However, the exact nature of this non-randomness and the mechanism for conveying positional information to daughter nuclei is a subject of intense debate, as two recent studies reveal.

Interphase chromosomes occupy discrete non-overlapping territories and evidence suggests that the position of these territories in nuclear space is organized and hence might be functionally relevant¹. Two modes of organization have been reported. First, chromosomes can be ordered in a radial manner, from the centre to the periphery of the nucleus. This type of organisation has been correlated with gene density and the size of chromosomes^{2–5}. Second, although not mutually exclusive, it has been reported that chromosomes can have non-random neighbours^{6,7}, a finding that might account for preferential translocations and interactions between specific chromosomes.

Non-random territory organisation also implies the need for a mechanism to successfully convey chromosome positional information to daughter nuclei (cellular memory). Two recent papers suggest not only different types of chromosome organization in the nucleus, but also different mechanisms for ‘memorising’ chromosome position. A study by Walter *et al.* in *Journal of Cell Biology*⁸ reports that positional information is lost at metaphase and that radial, but not neighbourhood, organization is re-established in early G1 phase. Conversely, a study by Gerlich *et al.* in *Cell*⁹ reports that individual chromosomes maintain information about their previous nuclear positions through mitosis and, as a consequence, chromosome neighbourhoods are preserved in daughter cells. How did two such polemic views arise and, more importantly, how might they be resolved?

Walter *et al.* were interested in whether chromosome neighbourhoods are maintained from one cell cycle to the next. To analyse chromatin distribution from mother to daughter nuclei, they synchronised HeLa cells expressing a histone H2B–green fluorescent protein (GFP) fusion protein in G2 and then bleached all but a thin subsection of the nuclear volume. Cells were then observed from G2, through M phase, to G1 using time-lapse confocal microscopy. Of 48 daughter cells examined, 20 showed a single cluster of unbleached chromatin, reflecting the original arrangement in the mother nucleus. In contrast, 28 out of 48 showed either some clustering, with patches of fluorescence in remote parts of the nucleus, or a widespread distribution of fluorescent patches throughout the nucleus.

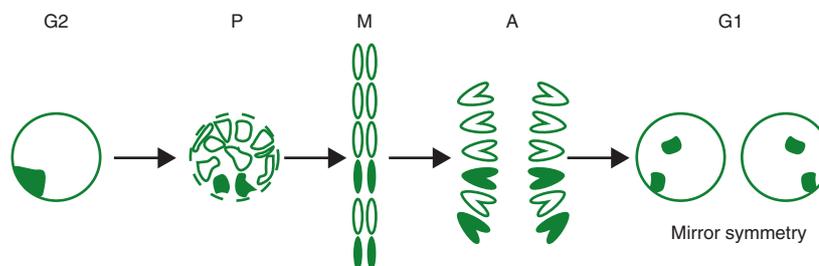


Figure 1 Chromatin movements at cell division. A schematic representation of mitosis, showing how unbleached fluorescent chromatin (shaded area) may be redistributed as a result of chromosomes converging from prophase (P) to metaphase (M). There is no movement of chromosomes perpendicular to the spindle axis, resulting in a mirror-symmetrical pattern in daughter cells. Walter showed that the fluorescent signal may or may not remain as a single patch through mitosis. P, early prophase; G2, Gap phase 2; A, anaphase; G1, Gap phase 1.

In general, the patterns showed mirror symmetry in the two daughter cells — this would be expected to result from a lack of chromosomal movement perpendicular to the mitotic spindle axis (Fig. 1).

Walter *et al.* then used fluorescent *in situ* hybridization (FISH) to examine particular chromosome territories in HeLa cells undergoing multiple divisions. They observed the positions of chromosomes 7 and 10 and found that after one cell division, chromosome arrangements in the two daughter nuclei showed mirror symmetry. However, by the four-cell stage, although there was apparent symmetry between two cells of a pair (one division), this was not the same symmetry as that of the neighbouring pair. These results, together with the patterns of unbleached chromatin in the live cell analysis, indicate that chromosome neighbourhoods are not maintained through mitosis.

If chromosomes lose their positional information at mitosis, then how is order re-established in daughter cells? A mechanism was suggested by observations of chromosome movement over long periods of the cell cycle. Using the same cell line, the team labelled DNA during S phase (by fluorescent nucleotide incorporation) and subsequently allowed the cells to divide multiple times until chromosome segregation resulted in one or two labelled ‘territories’ per nucleus.

The distances between labelled domains (in cells with more than one) and from labelled domains to the nuclear centre were measured at regular intervals and the variation in distance (movement) was recorded. These experiments revealed notably greater chromosome movement during G1 than in S or G2 phase. The results of the FISH experiments and the live-cell analysis suggested that neighbourhood organisation is not maintained. Thus, the increased movement in G1 was interpreted as movement of territories to re-establish their non-random radial positions.

A report by Gerlich *et al.*⁹ provides startlingly contradictory results. They used rat kidney cells expressing histone H2B–GFP to study chromatin movement during interphase. They bleached half the nuclear volume in early G1 cells and observed the bleached/unbleached boundary for approximately 5 h. Over this period, and in contrast to Walter *et al.*⁸, they detected no movement, nor did they detect movement in S phase or G2. Thus, they predicted that for positional chromosome information to be conveyed from mother to daughter cells, a mechanism whereby chromosomes re-establish their preferred positions in G1 is unlikely. Instead, they propose that chromosome positioning might be maintained through mitosis.

To better understand the behaviour of chromosomes at mitosis, the group devised

a computer simulation based on four key criteria. First, chromosomes occupy discrete nuclear volumes (territories); second, chromosomes condense and decondense isometrically; third, there is no movement perpendicular to the spindle axis as chromosomes congress to the metaphase plate; finally, individual chromosomes move at random times to each other. The simulation predicted that if mother nuclei were bleached parallel to the spindle axis then daughter nuclei would reveal no detectable position exchange, but if mother nuclei were bleached perpendicular to the spindle then daughter nuclei would have a random arrangement of bleached and non-bleached areas (Fig. 2a). To their surprise, however, when half-bleached nuclei were observed from prophase through M phase to G1, none showed a random transmission of bleached/unbleached regions, regardless of whether bleaching had been performed parallel or perpendicular to the spindle axis. This is inconsistent with a model that predicts random positioning of chromosomes at mitosis (Fig. 2b).

To understand how three-dimensional information about interphase chromosomes is transmitted through mitosis, they examined the specific movement of fluorescently labelled centromeres from G2 to metaphase. This revealed that chromosomes at the metaphase plate represent a simple flat projection of their prophase positioning and retain no information about their distance along the spindle in the preceding prophase, providing strong indications that a mechanism for restoring such distance information occurs during metaphase to telophase.

By observing individual chromosomes in more detail at the onset of anaphase, the group found that chromosomes destined for a position near centrosomes separated from their sister chromatid earlier than chromosomes destined to be nearer the site of cytokinesis (Fig. 2c). Because sister chromatid separation initiates at centromeres, they asked whether by perturbing centromere function they could in turn perturb cellular memory of position. The answer is yes. Hoechst treatment, which prevents heterochromatinisation of centromeres, also prevented chromosome positional information being conveyed through mitosis. Together, these results provided a mechanism to explain their observation that chromatin is not randomly distributed to daughter nuclei; that is, chromosome neighbourhoods are inherited.

The striking difference in conclusions from these two papers highlights the need for more detailed investigation. But where do we begin? Why do Walter *et al.* see movement of chromatin in G1, but Gerlich *et al.* do not? Gerlich *et al.* argue that the movement detected in G1 by Walter *et al.* is a

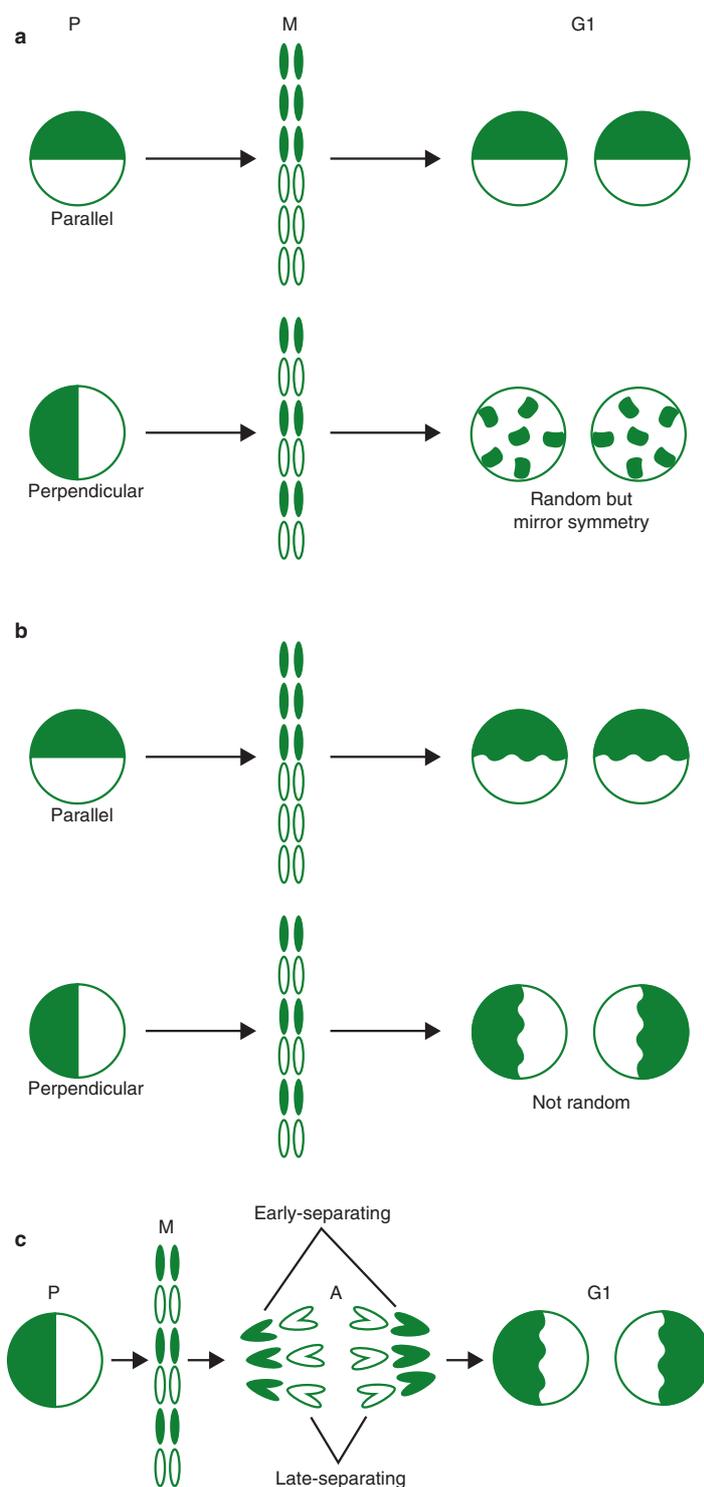


Figure 2 Models for chromatin redistribution at mitosis. a, b, A schematic representation of Gerlich's computer predictions (a) and actual observations (b) of fluorescent signal distribution through mitosis in nuclei bleached parallel or perpendicular to the spindle axis, as indicated. P, early prophase; M, metaphase; G1, Gap phase 1. Parallel bleaching pattern predictions were observed experimentally (compare parallel bleaching in a and b), indicating no movement of chromosomes perpendicular to the spindle axis. However perpendicular bleaching pattern predictions were not observed experimentally (compare perpendicular bleaching in a and b). c, A schematic representation demonstrating how different separation timing of sister chromatids can convey the perpendicular bleaching pattern from mother to daughter nuclei.

result of global nuclear shape changes after telophase; that is, expansion and flattening of the cell to the substrate (personal communication). However, Walter *et al.* report that occasionally, the distance between chromosome territories became smaller during early G1. This indicates that increased general movement in G1 is not simply a result of rapid nuclear volume increase after telophase, but rather movement of territories to their final radial location. In concordance with this observation, it was shown previously that localisation of chromosome 18 to the nuclear periphery in fibroblasts is established in a 2–4-h window at the start of G1 (ref. 3). Additionally, it has been shown that movement of a transcriptionally activated GFP-tagged locus from the nuclear periphery to interior occurs immediately after M phase¹⁰. Gerlich *et al.* studied interphase movement by bleaching half the nucleus and looking for mixing of the bleached/non-bleached boundary. In contrast, Walter *et al.* measured movement between the centre points of two or more labelled chromosome domains. Potentially, this approach could be more sensitive to changes in movement than visual observation. However, Gerlich *et al.* also studied movement of centromeres and noted that their relative positions did not change in G1, and thus neither did relative neighbourhoods.

Gerlich *et al.*'s centromere-driven mechanism for non-random neighbourhood inheritance immediately begs the question: what happens to translocated chromosomes? It has been shown that gene-dense chromosome 19 and gene-poor chromosome 18 are radially arranged in the nucleus, located centrally and peripherally, respectively². In cells from an individual

with a balanced reciprocal translocation of chromosomes 18 and 19, positions of the translocated portions reflect that of the donor chromosome; that is, chromosome 19 material was more centrally located than the chromosome 18 onto which it was joined and *vice versa*². This indicates that the centromeres did not influence positioning of the translocated material and consequently, Walter *et al.* conclude that although a centromere-directed mechanism for non-random chromosome positioning cannot be excluded, it cannot alone account for establishing non-random nuclear architecture (personal communication).

Could the different conclusions of the two groups be a result of interpretation? Gerlich *et al.* point out that they were looking for any deviation from the computer model as an indication of non-randomness, whereas the hypothesis of Walter *et al.* was that for neighbourhoods to be maintained, there should be no mixing (personal communication). Gerlich *et al.* note that accuracy of transmission of chromosome position from mother to daughter cells is not 100% and some intermixing occurs within one cell division. Their results indicate that although inheritance is not random, it is also not perfect, and predict that order may be diluted over several cell cycles. This flexibility would allow a mechanism for cell-type-specific organisation to be achieved during development.

So what is the biological significance of chromosome order at interphase? Investigating the specific arrangements of chromosomes in different cell types, during development and identifying diseases/cell states where arrangements are perturbed will be an important step towards deciphering its

meaning. Non-random clustering of chromosomes 12, 14 and 15 occurs in both normal mouse splenocytes and in a lymphoma cell line⁷. Furthermore, homologues of chromosomes 7, 8 and 16 have also been reported to localize to preferred nuclear locations in quiescent fibroblasts⁶. During myogenesis, it has been reported that centromeres move from a random distribution in the nucleoplasm to a non-random association with the nuclear periphery¹¹, whereas in human lymphoblasts it was shown that chromosome territory position was unaffected by transcriptional activity². Thus, although debate continues over the exact nature of chromosome organisation and its mode of inheritance, studies such as these might prove to be a divining rod for functional relevance. □

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Memorable transcription

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Experiments in the yeast *Saccharomyces cerevisiae* have shown that the enzyme Set1 preferentially targets the 5' coding regions of transcriptionally active genes, where it catalyses di- and tri-methylation of histone H3 Lys 4. This methylation mark is retained after transcription has subsided, suggesting that it provides a memory of recent transcription.

Complex eukaryotes contain several hundred different cell types, each with a distinctive set of properties defined by a unique pattern of gene expression. Every cell (with minor exceptions) carries the same complement of genes, the defining patterns of gene expression are put in place and stabilized by epigenetic mechanisms during cellular differentiation.

Stabilization of gene expression patterns often persists through many cell generations and has been termed cellular memory. Now, experiments in the budding yeast *S. cerevisiae* reported by Ng *et al.*¹ and Krogan *et al.*² in *Molecular Cell*, raise the possibility of a new type of memory — perhaps appropriate for single-celled organisms — short-term memory. As is often the

case, the results of these experiments on the yeast may provide a new perspective on the mechanisms of gene regulation in higher eukaryotes.

In recent years it has become clear that the nucleosome core particle — the basic unit of chromatin structure in all eukaryotes — is a central component of gene regulation mechanisms and a vital carrier of