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Review

Is mitotic chromatid segregation random?

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Summary. The question of whether mitotic segregation of chromatids is random or programmed assumes great significance for cellular differentiation if one recognizes that sister chromatids may have epigenetic differences and carry them from one generation into the next.

The literature was examined for evidence of non-random chromosomal and chromatid segregation. Many organisms were described as undergoing non-random homologue segregation in meiosis I. The explanations for these phenomena were attributed in some instances, to peculiarities of the meiotic spindle, though in some convincing experiments, the epigenetic heterochromatin of the kinetochores was implicated. The few existing descriptions of non-random mitotic segregation were also described.

Existing literature on ultrastructural, immunohistochemical, and physiological features of the chromatid kinetochores during the mitotic process was searched for evidence of asymmetry or structural differences between sister chromatids, which is presented. Also reported are descriptions of how epigenetic changes and cell differentiation can influence centromeric function and ultimately, kinetochore function.

Fundamental to the hypothesis of gene regulation presented here, is the assumption that genetic foci on different chromosomes interact, and must be proximate to each other and stereologically compatible for interactions to occur. Also described are spatial changes in chromosomal territories associated with function and differentiation. These territories can be in varying nuclear locations depending on gene function, and may show asymmetry between daughter cells.

Despite evidence presented for the possibility of non-random chromatid segregation at mitosis, this question will remain unanswered until the matter is specifically addressed by experiment.

Key words: Mitosis, Chromatids, Kinetochore, Epigenetics, Differentiation

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Introduction

It has largely been assumed that sister chromatids segregate at mitosis in a random manner because the genetic information of the chromatids is identical and therefore, their segregation bears no biological importance. Nonetheless there is at present great interest in the epigenetic regulation of gene expression and cell differentiation. Epigenetic differences between sister chromatids previously have been reported – such as lateral asymmetry G-banding (Angell and Jacobs, 1975; Tucci and Skalko, 1977; Goradia and Davis, 1978) after BrdU uptake (Strobel et al., 1981; Goodwin et al., 1996), differences in DNAase digestion, single strand nick translation labeling and conformational changes of metaphase chromatids - these differences have all been described in detail by Patkin (2002) in his review article The question of randomness of chromatid segregation only assumes importance if the sister chromatids are functionally different, and if there is a pattern of specific chromatids which segregate together to daughter cells.

In a previous review article (Bell, 2004), a case was made for interchromosomal functional associations, with the epigenetic structures of these chromosomes regulating their interactions. A graphic depiction of a possible interaction was provided using the helical symmetry of each metaphase chromatid as a permissive factor for interaction with an adjacent chromosome. This model was constructed from the experimental findings including electron tomography and reconstructions from confocal microscopy (Boy de la Tour and Laemmli, 1988; Baumgartner et al., 1991; Heliot et al., 1997) which described mirror image symmetrical helicality of metaphase chromatids. To date, and to our knowledge, the above findings are the only epigenetic differences morphologically detected between all sister chromatids. As well, the phenomenon of lateral asymetrical banding seen after radionucleotide uptake has been described (Angell and Jacobs, 1975; Strobel et al., 1981; Goodwin et al., 1996) though some have attributed this as an artifact (Strobel et al., 1981; Goodwin et al., 1996). The above model assumes that some degree of epigenetic distinctiveness is maintained by interphase

chromosomes.

If one supposes that chromosomes can be closely apposed spatially, and that a locus on one chromosome can produce a product which activates or represses a gene on an adjacent chromosome, a conformational or stereological change in one chromosome may deter or enable this interaction. Such a conformational change may be provided by a difference in helicality of one chromosome relative to another. If both chromosomes have the same “handedness” of helicality, they might interact. If, however, both show opposing handedness of helical twisting, they may not achieve sufficient apposition to enable interaction. If chromatid segregation were truly random, the chances for differential gene expression in daughter cells would be highly probable, though the sustained expression of specific genes through many cell generations would be very unlikely. For differentiation to occur, differing sets of genes must be activated or repressed in an ordered programmed fashion. Recent exciting understandings of the epigenetic control of gene transcription and silencing by cytosine methylation and associated histone modifications have led to the reasonable assumptions that these epigenetic changes which are heritable, are involved in cellular and embryonic differentiation. The experimental demonstration of these heritable epigenetic markers does not invalidate the existence of an additional controlling mechanism.

In any system which dictates differentiation by inherited epigenetic changes, and by random chromatid segregation at mitosis, the epigenetic markers of both chromatids must be identical to facilitate the production of identical cells of specific tissues. If the chromatids are not identical both genetically and epigenetically, (and there is evidence for this), then mitotic segregation of chromatids must be non-random and programmed in order to effect progressive differentiation of the cells.

In the ensuing article, chromosome and chromatid segregation is examined in both meiotic and mitotic situations, and existing evidence presented which might support the notion of non-random chromatid segregation at mitosis.

Evidence for non-random segregation in meiosis

The most compelling evidence for non-random chromosomal segregation exists in examples of meiotic division in diverse phyla (Pardo-Manuel de Villena and Sapienza, 2001). In yeast, non-random homologue segregation has been recorded (Davis and Smith, 2003). Meiotic drive, the favoring of one homologue over the other, has been described in plants (Comai, 2000), in sciarid flies (Fuge, 1997) where paternal chromosomes with undefined kinetochores are eliminated in MI by non kinetochore microtubules, in flea beetles (Kupfer and Wise, 2000; Green-Marroquin et al., 2001) where there is nonrandom selection of sex chromosomes in spermatocytes, in mice where the segregation of sex chromosomes in MI has been attributed to spindle mediated factors (LeMaire-Adkins and Hunt, 2000) and to the influence of autosomal factors (Hodges et al., 2001). In microsurgical experiments with grasshopper spermatocytes, MI chromosomes still exhibit the same segregation patterns after transplantation to MII spindles, thereby implicating the kinetochores as the cause of the selection (Paliulis and Nicklas, 2000). It has been suggested that mutations in centromeric tandem repeats might lead to "weak"centromeres causing a segregation imbalance and a "meiotic drive" for selection of "strong" kinetochores (Copenhaver, 2004). The centromeric DNA tandem repeats translate to si RNA which induce the heterochromatin of the kinetochore; defects in translation are seen to cause chromosomal missegregation (Huitorel and Kirschner, 1988; Sears et al., 1995; Hall et al., 2003).

Non-random segregation of sister chromatids in mitosis

Besides theoretical and speculative articles (Jablonka and Jablonka, 1982; Klar, 2002; Patkin, 2002; Bell, 2004), experimental evidence for this phenomenon can be found in two situations. One concept involves the preferential nonrandom segregation of recombinant homologues to opposite poles in Drosophilia. This segregation is described as x segregation and is thought to occur in part because of sister chromatid cohesion (Pimpinelli and Ripoll, 1986; Beumer et al., 1998). In yeasts, however, both x and z segregations occur with equal frequency (Chua and Jinks-Robertson, 1991). The other situation has been described in in the growing tips of fungal mycelia (Rosenberger and Kessel, 1968) and in mammalian stem cells, where the original DNA template is preferentially conserved and the newly created homologues are segregated (Merok et al., 2002) as described in the "Immortal Strand Hypothesis" which really describes a manner of z segregation.

Labelling experiments on stem cell reproduction have supported the so-called “Immortal Strand Hypothesis” by indicating that newly replicated stem cells show labeling in only one of the daughter cells after division. The rationalized purpose for this phenomenon was to maintain the original genetic material in an intact form, not subjected to the possibility of modification or mutation by replication errors. This finding is, however, perfectly compatible with the hypothesis of chromosomal interactions regulated by the chromosomes’ helicality. If we assume that in stem cells, most or all developmental “switches” are “open, then stem cells should maintain this ”open” configuration of gene switches, and newly synthesized chromatids with opposite helicality will be entirely segregated to one daughter cell. This daughter cell can still function as a “stem cell” because all of the replicated chromatids will still bear the same relative helicality to each other. The implication of the above finding is that there is a non-random segregation of chromatids to the daughter cells produced by mitotic division of stem cells.

Overview of chromosome segregation

The structure of the mitotic spindle is composed of two centriolar poles and associated microtubules which morphologically displays an apparent "mirror image symmetry". The microtubules attach to kinetochores of bioriented sister chromatids, which also exhibit "mirror image symmetry" Functional analysis of the spindle reveals differences in origin, structure and function between the two spindle halves and components that belie the initial impression of symmetry and an inferred equivalence.

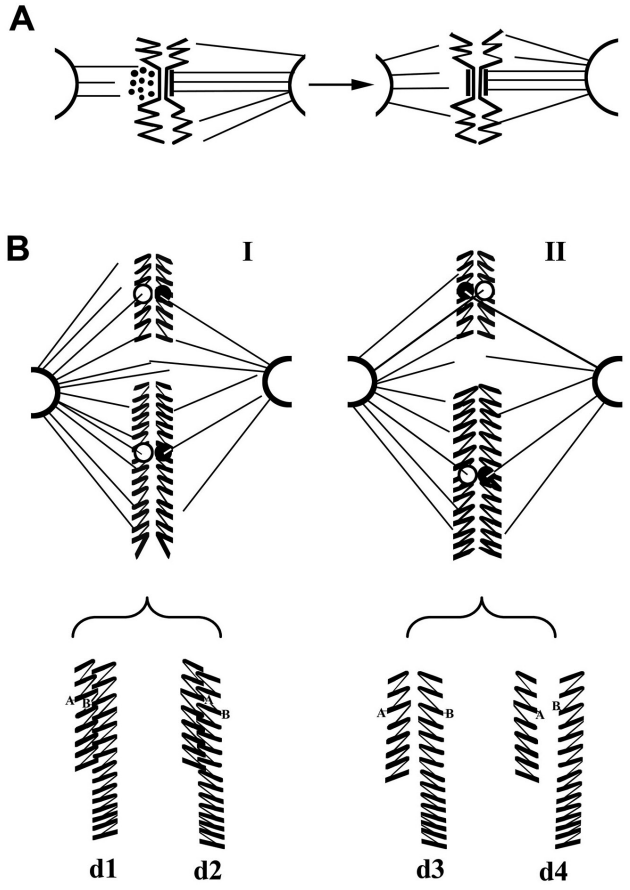

Fig. 1A. Schematic representation of asynchronous microtubule kinetochore attachments in a chromatid pair. At the left, one kinetochore shows a mature trilaminate structure with microtubular attachment. The corresponding sister kinetochore shows an amorphous globular structure with no microtubular attachment. At the right hand, the sister kinetochore has matured and is ready for attachment. **B.** The upper row shows two possibilities of chromatid segregation involving two hypothetically interacting chromosomes as shown in columns "I" and "II". In column "I", both chromatids with the same "handedness" of spiral are drawn to the same spindle pole. In column "II", chromatids with opposite "handedness of spiral are drawn to the same pole. The lower row shows the configuration of these hypothetical chromosomes in daughter cells. Both daughter cells, "d1" and "d2" arise from the segregation as shown in column "I". Both cells should have the same phenotype as both sets of chromosomes can be interposed, and hypothetical genes "A" and "B" are apposed and can interact. The daughter cells "d3" and "d4" arise from the mitotic segregation as shown in column "II". In these cells the chromosomes have opposite "handedness" of spiral and genes "A" and "B" cannot be apposed. These two cells should also show identical phenotype where no interaction between "A" and "B" occurs.

Centrioles

There are reported functional and morphological differences between the centrioles in each cell. In fertilized ova of starfish (Wu and Palazzo, 1999; Uetake et al., 2002) and sheep (Crozet et al., 2000), the centrosome appears to be of paternal origin. Within eukaryotic cells, after each division, the "mother centrosome" replicates. Functional differences have been reported between "mother" and daughter centrioles (Piel et al., 2000; O'Toole et al., 2003). The "mother" centriole shows greater maturity than the daughter with differing sets of proteins associated with the mother- the mother centrosome is associated with ninein, Odf2, cynexin (Morgensen et al., 2000; Nakagawa et al, 2001).

Functional differences in kinetochores during congression

Ultrastructural investigations of cultured PtK1 cells undergoing mitosis, have revealed that the number of microtubules attached to the trailing kinetochore (closest to the metaphase plate) during congression is twice the number of microtubules attached to the leading, anti-poleward kinetochore (McEwen et al., 1997). This disparity in microtubular attachment has not been linked to the direction of chromosomal migration or to the displacement force of the migration. The initial attachment of microtubules to kinetochores has been described as asynchronous (Khodjakov and Rieder, 1996), suggesting an increased affinity and "preferential" attachment of a particular aster to the accessible kinetochore. Ultrastructural differences have been demonstrated between an attached kinetochore and an unattached sister kinetochore which has been reported as larger in area and lacking a discernable three layered organization with an excess of amorphous material surrounding it (Rieder and Alexander, 1990; Cassimeris et al., 1994). The mechanical forces of the attached microtubules are shown to distort the architecture of both the attached and as yet unattached sister kinetochore. In vertebrates, striking differences in concentration of centromeric proteins such as BubR1, CENPE, dynein and Mad2 are seen - the unattached kinetochore showing much higher levels of the above (Hoffman et al., 2001). A different report (Taylor et al., 2001) shows equal reactivities for BubR1, but differential reactivity for Bub1 with the weaker staining reactivity on the kinetochore closer to the spindle pole. Gorbsky and Ricketts (1993) demonstrated differential immunohistochemical staining for a phosphoepitope labeling kinetochores of congressing chromosomes in cultured Ptk1 cells- the kinetochore of the leading

chromosomes stained stronger than that of the trailing one. A schematic depiction of asynchronous kinetochore maturation is shown in Figure 1A.

The above described sequential attachments of sister kinetichores, would decrease the chances of merotelic attachments as well enable a "programmed" segregation in the context of preferential microtubule binding to a particular centriole.

Epigenetic influences on the centromere

The kinetochores are usually formed at the centromere, morphologically recognized as a primary constriction and formed of stretches of tandem repeat DNA associated with histones that impart a heterochromatic superstructure which is responsible for epigenetic influences (Cimini et al., 2003). It is believed that the genomic coding of the centromere is not as important as its heterochromatinic structure in establishing the centromeric site (Sullivan et al., 2001). Neocentromeres can be induced at alternate chromosomal sites as evidenced by pollen "killer" genes which induce neocentromeres which influence preferential gene segregation to favour propagation of the "killer" genes (Comai, 2000). The centromeric heterochromatic structure can be modified by primary DNA mutations which are associated with developmental abnormalities involving chromosomal missegregation (Moore, 2004), in experimental situations involving yeast artificial chromosomes (Sears et al., 1995) and in chromosomal abnormalities in human cancer (Wang et al., 2004).

As well the centromeric heterochromatic can be modified by cytosine methylation and histone acytelation (Vig and Willcourt, 1998; Taddei et al, 2001; Maison et al., 2002) and may be manifested by difficulties in chromosomal separation and missegregation (Vig and Willcourt, 1998). The epigenetic structure of the centromere and subsequent chromosomal division, both meiotic and mitotic in yeast has been shown to be modified by iRNA (Hall et al., 2003; Fukagawa, 2004).

There is evidence for differentiation associated modification of centromeric heterochromatin. During differentiation the protein TIF1â (a histone phosphorylation agent and a component of the histone deacetylase N-CoR1complex) associates with centromeric heterochromatin (Cammas et al., 2002) as does the transcription factor Ikaros in lymphocyte differentiation (Cobb et al., 2000). In different tissues, the centromeres of specific chromosomes cluster in differentiation specific patterns (Alcobia et al., 2000) A specialized histone H2AZ, which is absent in early embryos forms with differentiation and is localized to the pericentromeric heterochromatin (Rangasamy et al., 2003).

Comparative studies have implicated centromeric mutations as one of the causes for "meiotic drive" (Moore, 2004; Talbert et al., 2004)- a nonrandom preferential chromosome segregation at meiosis. The above discussion provides support to the thesis that the epigenetic modifications associated with differentiation can alter centromeric structure and function, and influence kinetochore function and chromosomal segregation.

Sister Chromatid Separation

For separation of sister chromatids to occur, decatenation of intertwined coils must occur and topoisomerase II is believed to be implicated in decatenation as well as in the separation of chromatids at mitosis (Giminez-Abian et al., 1995; Sumner, 1996) The Cohesin complex of proteins is believed to be central in chromatid cohesion as well as separation (Hirano, 2000; Nasmyth et al., 2000). Sister chromatid cohesion and the formation of homologue bivalents have been implicated as factors in the nonrandom mitotic segregation of recombinate homologues in Drosophilia (Pimpinelli and Ripoll, 1986; Beumer et al., 1998). The prevalence of x segregation in mitosis would also insure that parental strand homologue chromatids would be distributed between the daughter cells. The results of *z* segregation would produce conditions as described later in the "Immortal Strand Hypothesis".

Modifications to heterochromatin structure by histone hyperacetylation has been reported to prevent sister chromatid separation and promote syntelic attachments (Cimini et al., 2003). Heterochromatin formation is also bound to chromatid cohesion and is cell cycle phased by activation of an S phase kinase Hsk-Dfp1 (Bailis and Forsberg, 2004).

In addition to the effects of heterochromatin modifications, segregation of homologues in meiosis I is dependent upon the presence of a "monopolin" protein complex in Saccharomyces cerevisiae (Page and Hawley, 2003; Rabitsch et al., 2003). A homologue of a "monopolin" component, Psc1 in Schizosaccharomyces pombe is essential for accurate mitotic chromatid segregation (Rabitsch et al., 2003).

Interphase nuclear organization

If one assumes that the epigenetic differences which exist between sister chromatids as well as between homologues are of functional significance not only for the regulation of ongoing transcription but also for differentiation, then one should assume that these differences must be selected at meiotic and mitotic divisions. The above discussions reviewed features of the cell division apparatus which might support a programmed selection of chromosomes and chromatids. A schematic depiction of chromatid segregation and hypothetical gene interactions is shown in Figure 1B.

A brief review of known asymmetries in chromosome function and anatomy between daughter cells and differentiating cells compliments the above discussion. It is accepted that individual chromosomes

occupy distinct nuclear territories whose locations appear to reflect transcriptional activity (Tanabe et al., 2002a; Cremer et al., 2003; Weierich et al., 2003; Stadler et al., 2004) as well as replication timing (Bickmore and Carothers, 1995; Simon et al., 1999; Tanabe et al., 2002a) These relative locations have been maintained through evolutionary changes among species (Tanabe et al., 2002a,b). Regulation of gene activity is effected by epigenetic control where initial cytosine methylation is linked to gene silencing and ensuing histone deacetylation which in turn induces heterochromatin formation with the sequestration of genes in inaccessible heterochromatinic masses. These epigenetic changes have been shown to be reproducible on newly synthesized DNA strands (Schubeler et al., 2000; Reik et al., 2001) and are heritable from mother to daughter cells (Jablonka and Lamb, 1989, 2002; Sutherland et al., 2000; Tycko, 2000; Bird, 2002; Waterland and Jirtle, 2004).

It is also recognized that epigenetic gene silencing is reversible and may not be the only factors regulating gene expression in differentiation. In embryogenesis, there are asymetrical demethylation patterns on sister chromatids (Patkin, 1997; Rougier et al., 1998). In the mouse embryo, the paternal X-chromosome is deacetylated and inactivated (Wang et al., 2001). Recent transplantation experiments of mature nuclei into embryonic cells have shown reversal of epigenetic markers (Tada et al., 1997; Kikyo and Wolfe, 2000; Wade and Kikyo, 2002; Gurdon et al, 2003; Beaujean et al., 2004; Simonsson and Gurdon, 2004; Tian, 2004), implicating cytoplasmic or paracrine factors (Horvitz and Herskovitz, 1992). Patkin (2002) has attributed chromatid asymmetries to both epigenetic changes and sister chromatid exchanges. An in vitro study of the effects of demethylating agents on cultures of Chinese hamster ovarian cells found an increased incidence of sister chromatid exchanges (Albanesi et al., 1999).

In quantitative studies of chromosomal territories, similar but different and asymmetrical geographies exist between mother and daughter cells and between sister cells (Walter et al., 2002; Thomson et al., 2004). Differences in positioning of chromosomal territories have been ascribed to gene transciption, as well as diffentiation (Koss, 1998; Weierich et al., 2003; Solovei et al., 2004; Stadler et al., 2004). Quantitative studies of chromosomal territories of homologous pairs show that they have a roughly symmetrical disportment with varying distances between the various homologues (Cremer et al., 2001). One report of human bronchial cells suggests a constant location for homologues of chromosomes 1,7, and X (Koss, 1998).

Conclusions

This review has presented evidence that chromosomes in the interphase nucleus maintain relative spatial positions dependent upon transcriptional activity, replication and differentiation. The regulation of gene activity and differentiation is believed to be dependent upon epigenetic changes involving cytosine methylation and histone acetylation. Evidence was also briefly presented for the influence of siRNA on heterochromatin function.

A premise which is made here is that chromosomes interact, and that this interaction is regulated by the epigenetic configuration of adjacent chromosomes. Epigenetic differences between genetically identical chromatids have been described. In order for these differences to be sustained through cell division, chromatid segregation must be selective and programmed. Convincing evidence exists for non random segregation of chromatids in meiosis related chiefly to epigenetic features of centromeric heterochromatin. Several studies also showed that non random chromatid segregation exists in mitosis.

With the state of present technology, the question of randomness of chromatid segregation in mitosis can be experimentally tested. One would need to pulse label synchronized cultured cells during DNA replication, identify a number of chromosomes by "painting" techniques, and examine whether the labeled chromatids are passed to daughter cells with any sort of a pattern. The results of this sort of experiment will be of great importance in helping to answer the basic question asked here.

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