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Discovery of the mitotic selective chromatid segregation phenomenon and its implications for vertebrate development

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The asymmetric cell division process is required for cellular differentiation and embryonic development. Recent evidence obtained in *Drosophila* and *C. elegans* suggest that this process occurs by non-equivalent distribution of proteins or mRNA (intrinsic factors) to daughter cells, or by their differential exposure to cell extrinsic factors. In contrast, haploid fission yeast sister cells developmentally differ by inheriting sister chromatids that are differentiated by epigenetic means. Specifically, the act of DNA replication at the mating-type locus in yeast switches it's alternate alleles only in one specific member of chromosome 2 sister chromatids in nearly every chromosome replication cycle. To employ this kind of mechanism for cellular differentiation, strictly based on Watson-Crick structure of DNA in diploid organism, selective segregation mechanism is required to coordinate distribution of potentially differentiated sister chromatids to daughter cells. Genetic evidence to this postulate was fortuitously provided by the analysis of mitotic recombinants of chromosome 7 in mouse cells. Remarkably, the biased segregation occurs in some cell types but not in others and the process seems to be chromosome-specific. This review summarizes the discovery of selective chromatid segregation phenomenon and it suggests that such a process of Somatic Sister chromatid Imprinting and Selective chromatid Segregation (*SSIS* model) might explain development in eukaryotes, such as that of the body axis left-right visceral organs laterality specification in mice.

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Introduction

Every multi-cellular organism consists of a variety of different cell types that give rise to different tissues and organs. The development of an organism usually starts from a single cell, which after multiple cell divisions gives rise to different tissues, with each tissue containing cells of specific types. The significance of asymmetric cell division for the development of multi-cellular organisms, including vertebrates, is widely recognized. For example, a stem cell often generates one daughter cell that is committed to differentiation, while the other daughter cell maintains stem-cell characteristics to generate cellular diversity of each organism.

Categories of major models for cellular differentiation

Two categories have been proposed for explaining the phenomenon of asymmetric cell division: 1) those that suggest that asymmetry is imposed by the cells' exposure to extra-cellular environment, and 2) those that postulate that the information for cellular differentiation is found internally in the cell because of asymmetric cell division of the progenitor cell. Prominent among the first type, the morphogen-gradient model, suggests that two initially identical daughter cells become different because they encounter different microenvironments that induce as well as repress different sets of genes of a cell [1–3]. A special case of this hypothesis concerns different stem-cell niches. According to this, precise cellular location influences the stem cell's division by integrating signals emanating from adjoining cells in the nitch to induce daughter cells to differ from one another [4–6]. While the cellular signalling phenomenon is well established, the fate of each daughter cell may also be determined by cell intrinsic factors. In the latter case, either the cytoplasmic cell-fate determinants are segregated into only one of the two daughter cells during mitosis, or alternatively, non-random chromatid segregation to daughter cells occurs to confer non-equivalency on them. It is likely that different combinations of these mechanisms operate in different tissues [7,8].

Sister chromatids are inherently non-equivalent both by DNA strand sequences and by their replication history

Recently, a model suggested that asymmetric cell division might be promoted by differentiation of sister chromatids by epigenetic means, followed by selective segregation of thus differentiated sister chromatids to daughter cells. The chromosomal DNA strands carry genetic information complementary to one another, and both strands serve as a template for the synthesis of the other strand during chromosome duplication [9]. Each chromosome replication event produces two paired

daughter chromosome copies that in the G2 phase of the cell cycle are conventionally referred to as sister chromatids. One chromatid contains a DNA replication template ('older') strand, designated as the Watson [W, Figure 1] strand, and the other is a complementary Crick (C′) strand synthesized in the previous replication cycle. Consequently, its sister chromatid consists of the older C strand and the younger W′ strand (designated W′C chromatid) [10]. Thus, because of differences in the replication history of DNA strands, the sister chromatids are formally non-equivalent even though they carry identical base sequences. It is generally believed that sister chromatids derived from a homologous pair of chromosomes are segregated randomly to daughter cells. In principle, selective chromatid segregation phenomenon might have evolved in diploid organisms to accomplish cellular differentiation [11,12,13•].

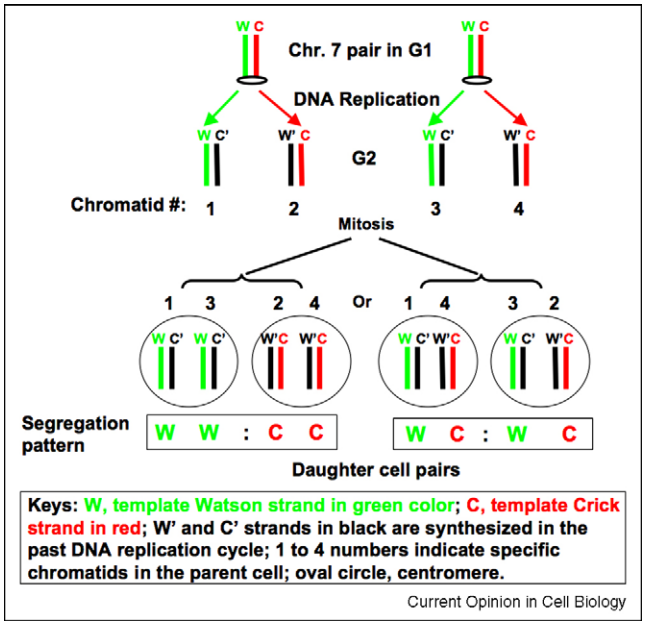
In mammals, experiments that monitor genome-wide distribution of labeled nucleotides to sister cells suggest that non-random segregation of DNA strands might occur in cells undergoing asymmetric cell division [14–18,19•,20]. Such is the tenet of the Cairns [21] Immortal Strand Hypothesis for segregating older template-DNA strands to asymmetrically dividing self-renewing B stem cells. This hypothesis was proposed as a mechanism to protect stem cells from inheriting DNA replication errors so as to avoid cancer development in future. However, it has not been possible to definitively ascertain whether all chromosomes undergo asymmetric segregation as biased segregation of only a set of chromosomes can explain the findings. In addition, in most studies it has not been possible to unequivocally identify the stem and non-stem cells in culture or *in vivo*.

Therefore, the biased segregation cannot be considered as a generally applicable model for stem-cell division.

Discovery of the chromosome-specific chromatid segregation phenomenon

For chromatid asymmetry to serve as one of the mechanisms for cellular differentiation in multi-cellular diploid organisms, a process for selective chromatid segregation is required. Such a *S*omatic *S*trand-specific *I*mprinting and selective chromatid *S*egregation (*SSIS*) model would generate non-equivalent daughter cells in mitosis [11]. This model postulated that one daughter cell inherits both W′C chromosomes, one copy from each homolog; the other cell thereby inherits both WC′ copies from the progenitor cell (Figure 1). By specifying only the older chromosome strands of chromatids/chromosomes for simplicity and brevity, the term of WW:CC segregation was coined, where W reflects WC′ and C reflects CW′ strand-containing chromatid/chromosome [10]. The key insight that led to the proposal of this model was derived from an unusual result obtained by inducing mitotic recombination between mouse chromosome (Chr.) 7 homologs [10].

Figure 1

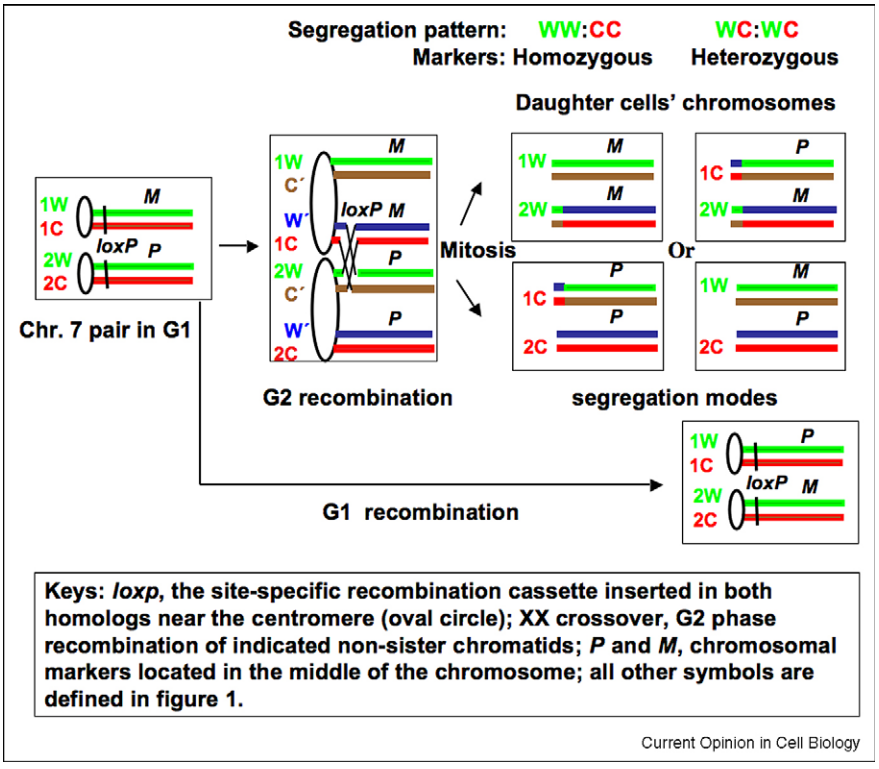


Two theoretical possibilities of selective chromatid distribution of Chr. 7 in mouse cell mitoses.
For clarity and brevity, chromosomal DNA strands found normally in the double helix configuration are presented as straight lines. The W and C strands are defined by their specific 5′–3′ DNA sequence orientation. In the WW:CC designated pattern, both template [older] W [arbitrarily coloured green] strand-containing chromatids are segregated to one daughter cell and both older C [red] strand-containing chromatids are segregated to the other daughter cells to cause asymmetric cell division. Equivalent daughter cells are produced in the WC:WC segregation mode because both inherit WC′ plus W′C chromosomes.

Both the chromatid origin and the distal marker *P* (for paternal allele) or *M* (for maternal allele) in the recombinant lines were detected with Southern analysis, by performing methylation-sensitive restriction enzyme digestion of the *Snrpn* gene located at the middle of Chr. 7 (Figure 2); the *M* epigenetic allele is methylated in the maternal homolog and unmethylated in the paternal P allele by conventional parent-of-origin imprinting [22•,23•]. The result of experimentally induced recombination with the site-specific Cre/loxp system in mouse embryonic stem cells [ES] [23•] was subsequently interpreted to suggest the existence of the selective chromatid segregation phenomenon [10].

The remarkable result obtained was that in all 432 Chr. 7 recombinants analyzed, each produced an *M*/*M* and *P*/*P* pair of homozygous progeny (Figure 2). Normally, G2 recombination events between non-sister chromatids are resolved to generate a mixture of homozygous and heterozygous progeny for markers located distal to the crossover point. The result of preferentially homozygous products was also observed in *Drosophila* by employing the distinct FLP/FRT recombination system [24,25]. For

Figure 2



The recombination model employed to discover the biased chromatid segregation phenomenon. Mitotic crossover at the *loxp* sites is experimentally induced by transiently expressing Cre recombinase in cells (modified from [22•,23•] refs.). The crossover event generates one chromatid with a functional hypoxanthine phosphoribosyl transferase minigene and those colonies inheriting the marker are selected by growing in an appropriate selective medium. The *P* and *M* allelic constitution is determined with Southern analysis. To obtain the result of all recombinants becoming homozygous for *P* and *M* alleles, as in ES and endoderm cells [Table 1], recombination must occur not in the G1 but in the G2 phase, only between specific non-sister chromatids [e.g. WC′ with W′C], and it must be followed by selective distribution of sister centromeres, as indicated in the drawing. Therefore, all *M*/*M* and *P*/*P* homozygous recombinants are interpreted to reflect the WW:CC segregation, and all *P*/*M* recombinants reflect the WC:WC segregation process. All notations are defined in Figure 1.

explaining unusual results with both the recombination systems it was suggested that somehow the recombination systems themselves cause recombinant chromatids to segregate always from each other [23•,24,25]. Specifically, a meiotic-reduction-division 1-like process was postulated in which sister chromatids remain attached to each other in regions distal to the crossover point and therefore segregate together to one pole of the mitotic spindle causing markers homozygosis in both daughter calls. Such a 'sister chromatid cohesion model' is unlikely, as it requires chromatid segregation to occur through chromosome regions other than the centromeres. Also, this constraint is unlikely to be imposed by two different site-specific recombination systems, that, too, in two different organisms where recombination was induced by systems not indigenous to cells of either species. Moreover, such constraints imposed by the recombination process in mouse cells should have resulted in a similarly biased segregation in other cell types where, instead, an unbiased segregation pattern was observed (see below).

Table 1

LRD protein implicated in selective segregation.

		Wild type	LRD knockdown	
Cell type	lrd gene	Segregation pattern	LRD status	Segregation pattern
ES and Endoderm	ON	WW:CC	OFF	Random
Neuroectoderm	ON	WW:CC	OFF	Random
Pancreatic, mesoderm and cardiomyocytes	OFF	Random	ND	ND

Expression of the *lrd* dynein gene is inherently regulated by cell type; ON denotes expressed and OFF indicates silent. ND, not done.

An alternative model, proposed to explain the results in ES cells, is that selective distribution of sister chromatids to progeny cells occurs irrespective of mitotic recombination and that it involves centromere-based segregation [10,22•] [Figure 2]. We advance that this kind of distribution evidences the WW:CC segregation pattern of ES cells. By altering growth conditions, the ES cells containing the site-specific recombination system were changed to several other cell types. Only WW:CC segregation was observed in endodermal cells (Table 1). By contrast, pancreatic, mesodermal, and cardiomyocyte cultures exhibited near random segregation patterns [22•]. Thus, the analysis of recombinants constitutes a procedure to discern the segregation mode of a specific chromosome in mitoses. To obtain the homozygous result, it is necessary that a specific pair of non-sister chromatids participate in recombination in the G2 phase, followed by selective segregation of sister centromeres [10]. Furthermore, recombination in the G1 phase must not have occurred, because such events would produce *P/M* heterozygous recombinants (Figure 2). Perhaps the biased chromatid segregation process itself restricts which pair of chromatids is permitted to recombine in the G2 phase, and second, somehow it also prohibits Chr. 7 recombination in G1 [22•]. Overall, these results demonstrate: 1) that recombination is cell cycle phase restricted, 2) support the existence of patterned Chr. 7 segregation in ES cells, 3) suggest that this pattern is not invariant, as it changes with differentiation state [22•,23•], and 4) this process appears to be chromosome-specific because a similar analysis of Chr. 11 produced a mixture of *P/M* and *M/M* plus *P/P* types of the usually expected recombinant products [23•].

Another unanticipated result was obtained with neuroectoderm cells [22•], which showed a distinct non-random pattern. In this case, all 160 recombinants analyzed maintained the *P/M* constitution, a result consistent with the biased WC:WC segregation pattern (Figures 1 and 2) and/or recombination preferentially occurred in G1 phase (Figure 2). If neuroectoderm cells indeed recombine in G2 (discussed below), it remains to be determined whether the ES/endoderm and the neuroectoderm results differ because of cell-type regulated distinction between chromatids that are permitted to recombine or due to differences in the mode of chromatid segregation. It is difficult to imagine that a chromatid choice mechanism has evolved for a site-specific recombination system not indigenous to mouse cells. Therefore, we envision that probably a chromosome-specific non-random segregation process inherently operates in some cell lineages, and that such a process indirectly influences the choice of chromatids that are permitted to recombine. Furthermore, this process might also disallow recombination in the G1 phase by constraining chromosome interaction, perhaps through chromosome compartmentalization in the nucleus [26].

Left–right dynein protein [LRD] is implicated in both the selective chromatid segregation process and the mouse visceral organs left–right axis determination

The left–right dynein (*lrd*) is an axonemal dynein heavy chain-encoding gene of mice. In addition to symmetric expression in the embryonic node, transient asymmetric expression of *lrd* occurs in the head-fold region of 0–5-somite stage mouse embryos. At later stages, *lrd* is expressed symmetrically in the floorplate of the neural tube, a midline-signalling centre, and in a region of the embryo shown to be involved in visceral organs' left–right [LR] development [27–30]. In addition, expression is seen in nonciliated cells [31], in several ciliated cell types in newborn mice, the epithelial lining of the nasal cavity, and in the ependymal lining of the third ventricle of the brain [32]. A spontaneous missense mutation in *lrd* causes randomization of LR laterality such that one-half of mice develop with mirror-imaged visceral organs, as compared with wild-type mice [33]. The LRD protein's function in visceral laterality specification in mice was confirmed by generating a targeted deletion of the ATP binding domain of the *lrd* gene. Like the missense mutation, the deletion mutant similarly produced LR axis randomization [34]. Similarly, homozygous mutants of different subunits of dynein exhibit visceral organs' randomization in humans [35].

The SSIS model was initially proposed to explain the LR randomization phenotype of the *iv* [*situs inversus*] mutant mice. By the model, the *iv* gene mediates WW:CC segregation [Figure 1] to produce asymmetric cell division in the embryo when initially the visceral laterality is specified [11]. According to the molecular data discussed above from studies of *Drosophila*, mice and of humans, it is possible that LRD is involved in LR asymmetry through participation in the selective chromatid segregation mechanism. A direct test of this proposal in the selective Chr. 7 segregation process revealed a perfect correlation between *lrd* mRNA presence/absence and the Chr. 7 segregation mode in all the six cell lineages that were examined [Table 1]. Specifically, *lrd* is expressed in cultures that follow selective segregation pattern, and it is 'silent' in those that follow an unbiased pattern [36•]. Second, after *lrd* inhibition by RNAi, each of the ES, endoderm, and neuroectoderm cell lines disrupted their selective segregation mode [Table 1]. Third, neuroectoderm cells only after LRD depletion produced *M/M* heterozygous recombinants; these must have been generated by recombination in G2 [Figure 2]. Thus, neuroectoderm cells must normally follow the WC:WC segregation mode to produce only *P/M* heterozygous recombinants where recombined chromatids are always delivered to the same daughter cell. As both the WW:CC and WC:WC selective patterns are found in different lineages, the recombination process itself could not have dictated the specific segregation

mode. Rather, selective segregation process is postulated to operate in specific cell lineages [22•,36•]. These results support the proposal that LRD probably functions directly in the selective Chr. 7 segregation mechanism and support the existence of the selective strand segregation feature of the SSIS model proposed for LR axis determination in vertebrates [11].

The mechanism of how the LR symmetry in the mouse embryo is initially broken remains one of the key unanswered question in developmental biology [11,37–40]. Defining the molecular function of the *lrd* gene in axis development is required to explain the fascinating LR axis randomization phenotype of the mutant mice. The most popular model for the LRD protein function postulates its role in the motility of monocilia developed on nodal cells of the mouse embryo, called the 'nodal flow' hypothesis [41]. Notably, the mutant mice develop immotile cilia. According to the nodal flow hypothesis, the cilia-generated leftward embryonic fluid flow establishes an asymmetric gradient of a 'morphogen' across the embryo, constituting a mechanism for LR patterning [42]. However, it remains a controversial model when applied to LR axis determination. The work in mouse cells supports the possibility that the dynein motor protein plays a cytoplasmic role in LR patterning that is distinct from its function in ciliary motility [38].

The SSIS model was proposed as an alternative to the popular morphogen-gradient model for explaining development of LR asymmetry through asymmetric cell division. This chromosome-based model is based on cell lineage. It is designed to exploit the inherent base sequence difference of W and C strands [43], their replication history, and the epigenetic entities that might be installed at the time of chromosome replication during development [10–12,13•,44,45]. The result of random chromatid segregation in LRD knockdown cells is consistent with the random LR visceral phenotype of the */rd-/lrd*-mutant mice [46•]. Also, the findings of only one-half of heterozygous Chr. 11 translocation carriers develop schizophrenia and bipolar brain psychiatric diseases have been argued to support the SSIS model for human brain LR laterality development by postulating random chromatid segregation occurring owing to rearrangements [10]. The same logic might explain the Kartagener LR axis randomization syndrome, characterized by immotile cilia due to cytoplasmic dynein defects in humans [35].

Concluding remarks

On the basis of the discovery of new cell biological phenomenon of cell-type regulated selective chromatid segregation that we review here, an interesting new avenue for research has opened up to explain cellular differentiation through asymmetric chromatid distribution [13•,47•]. Studies of the Cre/loxp and FLP/FRT site-specific recombination systems quoted above were designed only to develop tools for chromosomal manipulation. We propose that this approach also identifies the mode of sister chromatid segregation in normal mitosis. Liu *et al.* [23•] conducted their study only with Chr. 7 and 11. Fortuitously, Chr. 7 exhibited two types of selective segregation patterns, which are LRD-dependent, and which change with the cell type [22•,36•]. These results raise the tantalizing possibility that Chr. 7 might specify LR axis determination in mice. If so, one genetic test of this suggestion is that mice genetically engineered to contain cetromeric inversion in both Chr. 7 homologs should develop *situs inversus* in all animals. Furthermore, some of the cell intrinsic factors implicated in promoting asymmetric cell division might function by dictating cellular polarity [48], that we suggest should be required for selective chromatid distribution. For example, the cell-fate determining Numb protein is located in the 'mother' centrosome [49], a structure found at the pole of the mitotic spindle. Also, the basal body of monocilia originates from one of the two centrosomes of the spindle. It has been speculated that the sister centromeres might be differentiated during replication by epigenetic means to identify them for biased segregation [22•,50•,51]. In sum, we hypothesize that SSIS mechanism may be crucial for evolution of form, for cellular differentiation and development, a different sets of chromosomes might be involved in different cell lineages, and for maintaining the integrity of parent-of-origin-specific imprints in somatic lineages by discouraging mitotic recombination through chromosome compartmentalization in the nucleus [26].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Ephrussi A, Johnston D: **Seeing is believing: the bicoid morphogen gradient matures**. *Cell* 2004, **116**:143-152.
2. Tabin C, Wolpert L: **Rethinking the proximodistal axis of the vertebrate limb in the molecular era**. *Genes Dev* 2007, **21**:1433-1442.
3. Tickle C: **Morphogen gradients in vertebrate limb development**. *Semin Cell Dev Biol* 1999, **10**:345-351.
4. Yamashita YM, Fuller MT: **Asymmetric centrosome behavior and the mechanisms of stem cell division**. *J Cell Biol* 2008, **180**:261-266.
5. Lin H: **The stem-cell niche theory: lessons from flies**. *Nat Rev Genet* 2002, **3**:931-940.
6. Lin H: **To be and not to be**. *Nature* 2003, **425**:353-355.
7. Knoblich JA: **Mechanisms of asymmetric stem cell division**. *Cell* 2008, **132**:583-597.

8. Gönczy P: **Mechanisms of asymmetric cell division: flies and worms pave the way**. *Nat Rev Mol Cell Biol* 2008, **9**:355-366.

9. Meselson M, Stahl FW: **The replication of DNA**. *Proc Natl Acad Sci USA* 1958, **44**:671-682.

10. Klar AJS: **A genetic mechanism implicates chromosome 11 in schizophrenia and bipolar diseases**. *Genetics* 2004, **167**:1833-1840.

11. Klar AJS: **A model for specification of the left-right axis in vertebrates**. *Trends Genet* 1994, **10**:392-396.

12. Klar AJS: **The developmental fate of fission yeast cells is determined by the pattern of inheritance of parental and grandparental DNA strands**. *EMBO J* 1990, **9**: 1407-1415.

13. • Klar AJS: **Lessons learned from studies of fission yeast mating-type switching and silencing**. *Ann Rev Genet* 2007, **41**:213-236.
This review describes the evidence of asymmetric cell division in fission yeast concerning *mat1* locus, where a strand-specific imprint leads to the production of non-equivalent sister chromatids. It also gives more insight about the biological importance of segregation phenomenon that might be operating in mammalian cells.

14. Fuchs E: **Skin stem cells: rising to the surface**. *J Cell Biol* 2008, **180**:273-284.

15. Karpowicz P, Morshead C, Kam A, Jervis E, Ramunas J, Cheng V, Van der Kooy D: **Support of the immortal strand hypothesis: neural stem cell partition DNA asymmetrically in vitro**. *J Cell Biol* 2005, **170**:721-732.

16. Merok JR, Lansita JA, Tunstead JR, Sherley JL: **Co-segregation of chromosomes containing immortal DNA strands in cells that cycle with asymmetric stem cell kinetics**. *Cancer Res* 2002, **62**:6791-6795.

17. Potten CS: **Keratinocyte stem cells, label-retaining cells and possible genome protection mechanisms**. *J Invest Dermatol Symp Proc* 2004, **9**:183-195.

18. Potten CS, Owen G, Booth D: **Intestinal stem cells protect their genome by selective segregation of template DNA strands**. *J Cell Sci* 2002, **115**:2381-2388.

19. • Shinin V, Gayrud-Morel B, Gomes D, Tajbakhsh S: **Asymmetric cell division and co-segregation of template DNA strands in adult muscle satellite cells**. *Nat Cell Biol* 2006, **8**:677-687.
The authors used pulse-chase labeling with BrdU to mark the putative stem-cell niche and identified a subpopulation of label-retaining satellite cells during growth after injury. It is suggested that some of these cells display selective template-DNA strand segregation during mitosis in the muscle fiber *in vivo*, as well as in cultures independent of their niche.

20. Smith GH: **Label-retaining epithelial cells in mouse mammary gland divide asymmetrically and retain their template DNA strands**. *Development* 2005, **132**:721-732.

21. Cairns J: **Mutation selection and the natural history of cancer**. *Nature* 1975, **255**:197-200.

22. • Armakolas A, Klar AJS: **Cell type regulates selective segregation of mouse chromosome 7 DNA strands in mitosis**. *Science* 2006, **311**:1146-1149.
By analyzing recombination events of chromosome 7 homologs of mouse cells, the study showed that embryonic stem cells and neuroectoderm cells exhibit non-random chromatid distribution, whereas the mode of distribution is random in mesoderm, cardiomyocyte, and pancreatic cells.

23. • Liu P, Jenkins AN, Copeland NG: **Efficient Cre-loxP-induced mitotic recombination in mouse embryonic stem cells**. *Nat Genet* 2002, **30**:66-72.
Here the authors demonstrate that mitotic recombination can be reproducibly induced in mouse embryonic stem (ES) cells, by Cre/loxP technology, after transient Cre expression. Notably, much of the recombination occurs in G2 and is followed by unusually by 'X segregation', thus recombinant chromatids segregate away from each other during mitosis.

24. Beumer KJ, Pimpinelli S, Golic KG: **Induced chromosomal exchange directs the segregation of recombinant chromatids in mitosis of Drosophila**. *Genetics* 1998, **150**:173-188.

25. Pimpinelli S, Ripoll P: **Nonrandom segregation of centromeres following mitotic recombination in Drosophila melanogaster**. *Proc Natl Acad Sci USA* 1986, **83**:3900-3903.

26. Tsai CL, Rowntree RK, Cohen DE, Lee JT: **Higher order chromatin structure at the X-inactivation center via looping DNA**. *Dev Biol* 2008, **319**:416-425.

27. Danos MC, Yost HJ: **Linkage of cardiac left-right asymmetry and dorsal-anterior development in Xenopus**. *Development* 1995, **121**:1467-1474.

28. Danos MC, Yost HJ: **Role of notochord in specification of cardiac left-right orientation in zebrafish and Xenopus**. *Dev Biol* 1996, **177**:96-103.

29. Goldstein AM, Ticho BS, Fishman MC: **Patterning the heart's left-right axis: from zebrafish to man**. *Dev Genet* 1998, **22**:278-287.

30. Lohr JL, Danos MC, Yost HJ: **Left-right asymmetry of a nodal-related gene is regulated by dorso-anterior midline structures during Xenopus development**. *Development* 1997, **124**: 1465-1472.

31. Bellomo D, Lander A, Harragan I, Brown NA: **Cell proliferation in mammalian gastrulation: the ventral node and notochord are relatively quiescent**. *Dev Dyn* 1996, **205**:471-485.

32. Vaisberg EA, Grissom PM, McIntosh JR: **Mammalian cells express three distinct dynein heavy chains that are localized to different cytoplasmic organelles**. *J Cell Biol* 1996, **133**:831-841.

33. Layton WM: **Heart malformations in mice homozygous for a gene causing situs inversus**. *Birth Defects Orig Artic Ser* 1978, **14**:277-293.

34. Supp DM, Brueckner M, Kuehn MR, Witte DP, Lowe LA, McGrath J, Corrales JM, Potter SS: **Targeted deletion of the ATP binding domain of left-right dynein confirms its role in specifying development of left-right asymmetries**. *Development* 1999, **126**:5495-5504.

35. Afzelius BA: **Cilia-related diseases**. *J Pathol* 2004, **204**:470-477.

36. • Armakolas A, Klar AJS: **Left-right dynein motor implicated in selective chromatid segregation in mouse cells**. *Science* 2007, **315**:100-101.
In this paper the involvement of the mouse left–right dynein (LRD) in the mechanism of differential segregation is demonstrated. Additionally, since gene mutations in the LRD gene result in randomization of visceral organs' laterality, they hypothesized that the organ laterality may be mediated by selective chromatid segregation phenomenon.

37. McGrath J, Brueckner M: **Cilia are at the heart of vertebrate left-right asymmetry**. *Curr Opin Genet Dev* 2003, **13**:385-392.

38. Qiu D, Cheng SM, Wozniak L, McSweeney M, Perrone E, Levin M: **Localization and loss-of-function implicates ciliary proteins in early, cytoplasmic roles in left-right asymmetry**. *Dev Dyn* 2005, **234**:176-189.

39. Tabin C: **Do we know anything about how left-right asymmetry is first established in the vertebrate embryo?** *J Mol Histol* 2005, **36**:317-323.

40. Tabin CJ, Vogan KJ: **A two-cilia model for vertebrate left-right axis specification**. *Genes Dev* 2003, **17**:1-6.

41. Nonaka S, Shiratori H, Saijoh Y, Hamada H: **Determination of left-right patterning of the mouse embryo by artificial nodal flow**. *Nature* 2002, **418**:96-99.

42. Brown NA, Wolpert L: **The development of handedness in left/right asymmetry**. *Development* 1990, **109**:1-9.

43. Watson JD, Crick FH: **Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid**. *Nature* 1953, **171**: 737-738.

44. Klar AJS: **Differentiated parental DNA strands confer developmental asymmetry on daughter cells in fission yeast**. *Nature* 1987, **326**:466-470.

45. Shibahara K, Stillman B: **Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin**. *Cell* 1999, **96**:575-585.

46. Klar AJS: **Support for the selective chromatid segregation**
• **hypothesis advanced for the mechanism of left-right body axis development in mice**. *Breast Dis* 2008, **26**:47-56.
This paper presents detailed predictions of the SSIS model concerning *lrd* mutant's developmental phenotypes.

47. Karpowicz P, Pellikka M, Chea E, Godt D, Tepass U, Van der
• Kooy D: **The germline stem cells of *Drosophila melanogaster* partition DNA non-randomly**. *Eur J Cell Biol* 2009, **88**:397-408.
The authors suggest that non-random partitioning of some chromosome(s), but not of the entire genome, occurs in germline stem cells in the *Drosophila* ovary where they divide asymmetrically to generate a new germline stem cell and a differentiating cystoblast. This recent study supports the SSIS model for cellular differentiation through asymmetric stem-cell division.

48. Aw S, Levin M: **Is left-right asymmetry a form of planar cell polarity?** *Development* 2009, **3**:355-366.

49. Knoblich JA, Jan LY, Jan YN: **Asymmetric segregation of Numb and Prospero during cell division**. *Nature* 1995, **377**:624-627.

50. Lew DJ, Burke DJ, Dutta A: **The immortal strand hypothesis:**
• **how could it work?** *Cell* 2008, **133**:21-23.
In this mini-review, it is theorized that sister centormeres might be biochemically marked, such as by lagging-strand and leading-strand replication, to help biased segregation of all chromosomes according to the immortal strand hypothesis or only a set of chromosomes according to the SSIS hypothesis.

51. Tajbakhsh S, Gonzalez C: **Biased segregation of DNA and centromeres: moving together and drifting apart?** *Nat Rev Mol Cell Biol* 2009, **10**:804-810.