

Selective chromatid segregation mechanism for *Bruchus* wings piebald color

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1. ABSTRACT

The mechanisms of asymmetric organ development have been under intensive investigation for years, yet the proposed mechanisms remain controversial (1-3). The female *Bruchus quadrimaculatus* beetle insect develops two black-colored spots bilaterally located on each upper elytra wing by an unknown mechanism. Fifty percent of the *P* (for piebald, two colors) gene homozygous mutant insects, described in 1925, had a normal left elytrum (with two black spots) and an abnormal right elytrum (with two red spots) and the balance supported the converse lateralized pigment arrangement (4). Rather than supporting the conventional morphogen model for the wings pigmentation development, their biological origin is explained here with the somatic strand-specific epigenetic imprinting and selective sister chromatid segregation (SSIS) mechanism (5). We propose that the *P* gene product performs the selective sister chromatid segregation function to produce symmetric cell division of a specific cell during embryogenesis to result in the bilateral symmetric development of elytra black color spots and that the altered chromatid segregation pattern of the mutant causes asymmetric cell division to confer the piebald phenotype.

2. HOW TO DIVIDE SYMMETRICALLY OR ASYMMETRICALLY?

For the development of all multicellular organisms, as well as for the maintenance of their tissues homeostasis, temporally regulated symmetric and asymmetric cell divisions are required. A key developmental biology question has been to understand the mechanisms that produce developmentally equivalent or nonequivalent daughter cells at specific cell divisions, such as during embryogenesis. Most research has been devoted to discovering the biological basis of asymmetric cell divisions through studies of diverse organisms. Asymmetric divisions are generally thought to occur through regulated distribution of differentiation-specifying cellular factors by the parental cell to daughter cells and/or by differential exposure of daughter cells to cell-extrinsic factors (6). Multiple mechanisms likely have evolved to conform to requirements of the ever-evolving biology in evolutionarily diverse organisms. In comparison, mechanisms for producing symmetric cell divisions are not as extensively investigated. In the field of adult stem cell division kinetics, however, specific mechanism of symmetric cell division and symmetric self-renewal have been investigated and reported extensively. For example,

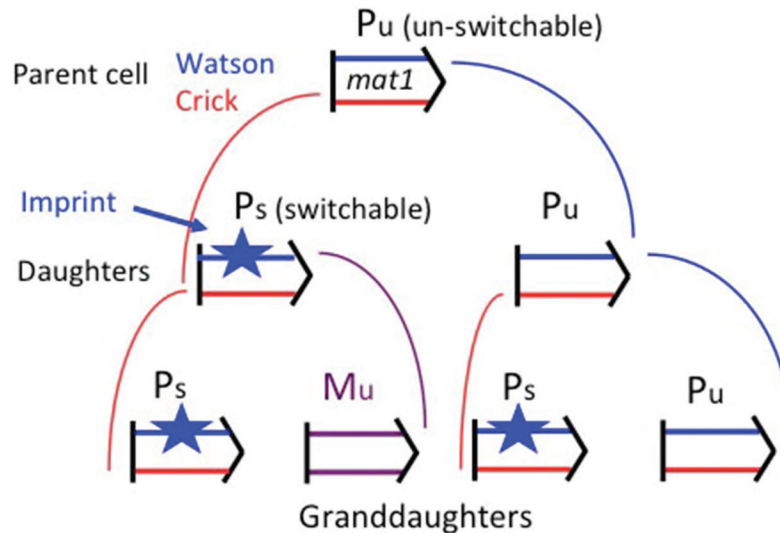


Figure 1. DNA strand-specific epigenetic imprinting/segregation (SSIS) mechanism. The mechanism explains the switching patterns found in cell pedigrees of fission yeast (Figure modified from (10, 11)). The newly synthesized Watson (W, indicated in blue line) DNA strand in the *mat1* locus is imprinted during its synthesis, while the Crick (C, represented by red line) strand is never imprinted. Most uniquely, the imprint is installed during DNA replication by a strand-, site-, and sequence-specific alteration/imprint at the *mat1* locus (10, 11, 22, 45, 46). The daughter cell inheriting the imprinted chromosome from the parental cell becomes a Ps cell. Next, during DNA replication in the Ps cell, the chromatid containing the imprinted strand suffers a transient double-stranded chromosomal break at *mat1* because the imprint blocks chromosome replication. This break induces recombination to result in switching of the *mat1* allele by the DNA transposition/substitution reaction. This mechanism forms the biological basis of two types of asymmetric cell divisions and it precisely explains the one-in-four granddaughter cells switching pattern observed in yeast cell pedigrees. The DNA sequence of the *mat1-M* allele is depicted in a different color because it differs from that of the *mat1-P* allele. The wide arrows indicate orientation of the *mat1* gene in the chromosome.

guanine ribonucleotide pools and lonosie-5'-monophosphate dehydrogenase, the rate-limiting enzyme for guanine nucleotide biosynthesis, are important factors for p53-dependent asymmetric cell renewal (7, 8). It is concluded here that both inherent DNA strand sequence differences and their replication asymmetries, followed by selective chromatid segregation, form the physical basis for generating regulated symmetric and asymmetric cell divisions essential for body laterality development.

3. EPIGENETICALLY DIFFERENTIATED WATSON VERSUS CRICK STRAND INHERITANCE OF THE PARENTAL CHROMOSOME DIFFERENTIATES SISTER CELLS IN TWO YEAST SPECIES

The mechanism of asymmetric cell division has been best understood in studies of laboratory model organisms whose cells undergo developmentally asymmetric cell divisions. A prime example of such an organism is the fission yeast *Schizosaccharomyces pombe*. The cells of this haploid yeast divide by equatorial division, and therefore it is named fission yeast. Individual cells

exist in either of the two mating types, called P (for plus) and M (for minus). The cell types are dictated by the alternate alleles of the mating-type locus (*mat1*), called *mat1-P* and *mat1-M* (Reviewed in (9)). There is a phenomenon of mating-type switching by which yeast cells change their mating/cell type by interconverting *mat1* alleles through the highly regulated recombination/substitution reaction of the *mat1* gene. Most remarkably, only a single cell switches *mat1* among four granddaughters of a cell (Figure 1). The Pu (u for un-switchable) cell always produces one Pu daughter, while the other daughter achieves switching competence, so named Ps (s for switchable), in more than 80 percent of cellular pedigrees. The Ps cell produces one switched Mu daughter cell while the other one remains Ps in over 80 percent cell divisions. Therefore, over 80 percent cell divisions follow a stem cell-like pattern of asymmetric cell division, in which two types of patterns alternate in consecutive cell divisions occurring in cellular pedigrees. The newly switched Mu cell likewise produces one Pu switched cell among four granddaughters of a cell by following the same two regulated cell-lineage defined switching patterns. Remarkably, instead of the usual

one-in-four granddaughter cells switching (Figure 1), two (cousins)-in-four granddaughter cells switch in stocks genetically engineered to contain inverted *mat1* duplication (10, 11). Thus, normally asymmetric cell division becomes symmetric in cells containing inverted *mat1* duplication. This was the first convincing result supporting the sister chromatids differentiation model presented in Figure 1. In short, fission yeast uses intrinsic DNA strands and replication asymmetry to switch mating/cell type through asymmetric cell division mechanism.

Facing insurmountable technical challenges, no studies have been initiated to determine the existence of such a DNA strand-based mechanism of asymmetric cell division during embryonic development in any multicellular organism. We have been searching for another system where the sophisticated tools of biology available for research with *S. pombe* can be applied to ascertain whether such a mechanism operates elsewhere. The *Schizosaccharomyces japonicus* (44 percent GC content) fission yeast is highly diverged from the well-studied *S. pombe* species (36 percent GC content); their protein orthologs are only 55 percent identical at the amino acid level (12). We found that the genomic locations and DNA sequences of the *mating-type loci* of *S. japonicus* differ greatly from those of the *S. pombe* species. Despite evolutionary differences, and remarkably, *S. japonicus* cells switch cell/mating type after undergoing two consecutive cycles of asymmetric cell divisions and therefore only single cell switches among four granddaughters, as first described in *S. pombe* (Figure 1). The DNA strand-specific epigenetic imprint at *mat1* initiates the recombination event, which is required for cellular differentiation (13). Therefore, the *S. pombe* and *S. japonicus* mating systems provide the first two examples in which the intrinsic strand asymmetry of the double-helical structure of DNA plus strand-specific imprint installed by the DNA replication process at a single locus constitutes the mechanism of asymmetric cell division. Thus, this unique, strand-specific imprinting/segregation epigenetic mechanism is evolutionarily conserved in these two highly diverged yeast species. Only in these yeasts has it been possible to determine the existence of the sister chromatid differentiation mechanism and both are found to employ it for their cellular differentiation. As compared to other mechanisms invoking cascade of regulatory events, this mechanism is relatively easy to comprehend because the DNA strands asymmetry provides the

physical basis for the sister cells' differentiation, although it is only demonstrated to operate in these single-cell, haploid organisms.

4. DISCOVERY OF THE SELECTIVE SISTER CHROMATID SEGREGATION PHENOMENON

The fission yeast studies have revealed a unique mechanism of strand-specific epigenetic marking that can bestow developmental asymmetry upon the two daughter cells that receive subsequently replicated DNA. We recognized that the mechanism of asymmetric cell division that gives rise to the phenomenon of *mat1* switching could also explain the vertebrate developmental differentiation that gives rise to body laterality (5) and asymmetric brain hemispheres development in humans (14). However, in order for that epigenetic mechanism to work in diploids the marked DNA strand from the two homologous chromosomes will have to be segregated selectively. We proposed the SSIS model to postulate that certain regions of the genome in higher eukaryotes use the strand marking by epigenetic moiety to be followed by coordinated strand/chromatid segregation as a mechanism to establish developmental symmetry or asymmetry. Thus the strand inheritance mechanism proposed for the development of diploid organisms requires selective segregation of epigenetically differentiated sister chromatids of both homologs of a chromosome to specific daughter cells. The SSIS model might be applied to multiple chromosomes should multiple developmental genes be simultaneously regulated epigenetically for cellular differentiation during mitosis.

Most interestingly, the designated W, W :: C, C segregation (W, Watson and C, Crick template DNA strand-containing chromatid) of mouse chromosome 7 occurs in endoderm and embryonic stem cells, while the W, C :: W, C pattern occurs in neuroectoderm cells, and a random pattern is observed in pancreatic, mesoderm and cardiomyocyte cells (15) (Figure 2). Both nonrandom patterns changed to random mode when the left-right dynein (LRD) function was inactivated in cells. Furthermore, the *LRD* gene is expressed in three cell types undergoing only selective segregation while it is inherently not expressed (perhaps epigenetically) in three other cell types undergoing random segregation (16). The *LRD* gene controls the distribution of visceral organs left-right laterality in mice (17). In the *LRD* mutant,

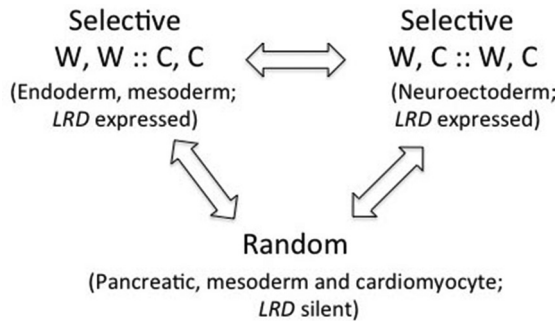


Figure 2. Cell-type regulated inter-conversion of modes of mouse chromosome 7 sister chromatid segregation. The left-right dynein-encoding gene (*LRD*) governs the selective chromatid segregation process (15, 16).

50 percent of mice develop with normal situs and the balance develops situs inversus of the internal organs. Taken together, these findings suggest that the *LRD*-encoded protein controls selective segregation of chromosome 7 chromatids in a cell type-specific fashion (Figure 2) and perhaps this is the mechanism of visceral organs body laterality development (16). A recent study of *Drosophila* employed the chromosome orientation fluorescence *in situ* hybridization approach to find that autosomal sister chromatids segregate only with the W, W :: C, C mode (18, 19). Also, a high frequency of nonrandom template strand segregation during differentiation of embryonic mouse stem cells was recently reported (20). Together, these studies demonstrate that biased and random sister chromatid segregation mechanisms function in a chromosome(s)- and cell type-specific manner in both vertebrate and invertebrate organisms. It is predicted that mutations in the segregation machinery would alter a specific mode in cells of a specific cell type to uncover the default mode from which the specific mode had originally evolved (Figure 2).

5. THE SSIS MODEL PROPOSED TO ACCOUNT FOR SYMMETRIC ELYTRA SPOT PIGMENTATION OF *BRUCHUS*

In each cell, the two chromosomal DNA strands carry DNA sequences that are complementary to one another and they have opposite chemical polarity (21). Moreover, each strand serves as a template for the synthesis of the complementary strand during chromosome duplication through a semi-conservative replication mechanism. The chromosome replication process

produces paired daughter chromosome copies, which, in the G2 phase of the cell cycle are called sister chromatids. Notably, one chromatid always contains the original template Watson (W) strand and the newly synthesized Crick (C) strand, and the sister chromatid contains the original template C strand and the newly synthesized W strand (Figure 3). Thus, the replication history of a chromatid is always different from that of its sister. Moreover, the leading-versus lagging-strand replication of opposite strands employs different enzymatic activities. In principle, such inherently asymmetric replication could differentially affect expression versus silencing of developmentally important genes in DNA strand/chromatid-specific fashion. It has been a long-held belief that sister chromatids, because they consist of identical DNA sequence copies, are randomly distributed to each new daughter cell during mitosis. In contrast, the SSIS mechanism proposes a unique cellular biology concept of selective chromatid segregation during cell division to exploit chromatid-specific epigenetic differentiation as a mechanism for cellular differentiation.

The SSIS model was initially proposed as a mechanism to explain the developmental origin of the left-right laterality of vertebrates' visceral organs (5) and the hemispheric laterality of the human brain (14). To explain the development of symmetric wing spots of *Bruchus*, a variation of this model is proposed here (Figure 3). It suggests that symmetric cell division might be based on the designated W, C :: W, C strands/chromatids segregation pattern of the chromosome on which the black pigment-specifying gene resides and that the gene is epigenetically regulated as diagrammed. The model suggests that at specific stages in development, developmentally important genes will be differentially regulated via somatically installed heterochromatin assembly during chromosome replication in the chromatid-specific fashion. Indeed, in *S. pombe*, the DNA polymerase-alpha replication factor is required for imprinting (Figure 1) at *mat1* (22) and for gene silencing through heterochromatin assembly (23). Also, both transcriptionally active and silenced epigenetic states of gene expression are very stable and can be inherited as classical Mendelian/chromosomal epigenetic markers in both mitosis and in meiosis of fission yeast (24-26). In this yeast, individual mitotic haploid cells were subjected to Mendelian meiotic genetic analysis to discover cell lineage-regulated DNA strand-specific epigenetic entities existing in somatic cells (27) and these entities form the mechanism of asymmetric cell

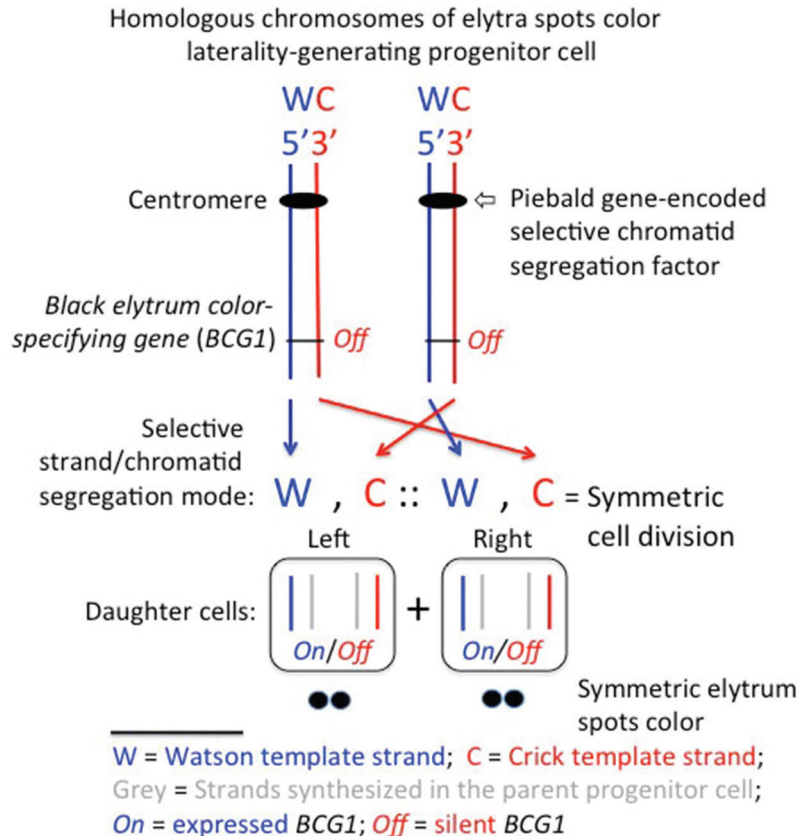


Figure 3. The SSIS mechanism explains symmetric elytra spot pigmentation development in *Bruchus*. The SSIS model (modified from (5, 14)) suggests three postulates: (A) the progenitor cell carries the hypothetical *Black Color-specifying Gene 1 (BCG1)*, but it is epigenetically silenced (*Off*) on both homologs of a chromosome; (B) *BCG1* is activated (*On*) during replication in the template W (blue line) strand-containing chromatid at a specific cell division, but the template C (red line) strand-containing sister chromatid maintains the *Off* state like that of the parental cell; and (C) the progenitor cell will divide in such a way that specific chromatids are selectively segregated to the resulting left- versus right-side situated daughter cells with respect to predetermined embryonic anterior-posterior and dorso-ventral axes of the embryo. The *P* gene-encoded factor dictates the denominated W, C :: W, C (by referring specifically to the template strands) chromatid segregation mode by functioning at centromeres of the specific chromosome to deliberately produce symmetric cell division. Next, symmetrical regulation of a key developmental gene(s) in daughter cells could start a cascade of downstream-regulated events to promote symmetric development of laterality such that two black-pigmented spots (indicated by dots) develop on each wing. The W and C strands are defined through their DNA sequence, 5'- and 3'- chemical polarity and replication history. To help appreciate segregation mode, W template strands are represented as blue colored straight lines, C template strands in red; the grey lines represent strands synthesized in the present replication cycle. In addition to the well-appreciated function of DNA for carrying genetic information of the organism (21), the SSIS model provides a physical basis for cellular differentiation as a courtesy of the double-helical structure of DNA (5).

division (reviewed in (9)). Because such an approach cannot be applied for research with somatic cells of higher organisms for technical reasons, studies with the yeast model organism have led the way to the discovery of this hitherto unappreciated principle of cellular biology. In principle, both asymmetric and symmetric cell division mechanisms may have evolved to exploit the epigenetic mechanism of gene regulation for accomplishing eukaryotic development. Based on the recent findings that regulated patterns of selective chromatid segregation

occur in mice and *Drosophila* (Figure 2), we employ the SSIS mechanism to explain the wing phenotype. Specifically, we propose that the bilateral symmetry results from a W, C :: W, C segregation pattern occurring in a single cell, producing symmetrical cell division, when the left-right distribution of the color spots is initially executed during embryogenesis in the wild type *Bruchus* (Figure 3). We hypothesize that the *P* gene factor executes specific chromatid distribution to dictate the W, C :: W, C segregation pattern to result in symmetric cell division.

6. THE SSIS MECHANISM EXPLAINS THE *BRUCHUS* PIEBALD ELYTRA MUTANT PHENOTYPE

The female *Bruchus quadrimaculatus* beetle has two black spots bilaterally located on each elytrum (Figure 3). Interestingly, due to a spontaneous recessive mutation described in a 1925 publication, 50 percent of the mutant homozygote females developed a normal left elytrum (with two black spots) and an abnormal right elytrum (with two red spots), and the remaining 50 percent supported the converse type (4). The biological mechanism of the symmetry in wild type insects and asymmetry of the mutant has not been defined. The gene for elytrum black pigment determination was named P (for piebald locus) because its homozygous mutant develops the piebald phenotype; the identity of the gene has not been determined. Notably, all of the p^-/p^- mutant insects developed bilateral piebald asymmetry, and, equally interestingly, the asymmetry was randomly distributed to the left or the right body side; that is, numbers of insects with red spots on the right were equal to those with red spots on the left.

In principle, the P factor might dictate the W, C :: W, C pattern starting from either one of the two default modes existing in the progenitor p^-/p^- mutant cell; random or another selective W, W :: C, C mode (Figure 2). To explain the random left-right distribution of pigmentation, we propose that the default mode of W, W :: C, C co-segregation operates in p^-/p^- embryos due to the lack of the P-encoded factor to result in only asymmetric cell division of the laterality-generating progenitor cell (Figure 4). Note that this hypothesized segregation pattern is the one that inherently operates in *Drosophila* autosomes in male germline cells, in which co-segregation of non-sister chromatids occurs in all mitoses; instead of left-right laterality, however, the specific chromatid pairs are randomly segregated to the stem or the differentiated cell of the embryo (18). This way asymmetric development results in *Bruchus*, and therefore, 50 percent mutant insects have black spots only on the right elytrum and 50 percent have them only on the left. Instead, should the default mode be of the random type, then 50% insects will develop left black/right black insects pigmentation like that of the wild-type insects: 25% left black/right red insects: 25% left red/right black insects. Such an outcome was indeed observed in *LRD* mouse mutants concerning visceral organs laterality development (1, 3). Because 100 percent insects developed piebald pigmentation (4), accordingly we propose here that

the default mode selective W, W :: C, C segregation occurs in the piebald mutant. We conclude that the SSIS mechanism explains the mutant phenotypes very well and that this work suggests a mechanism of how inherited bilateral symmetry normally develops in this insect, and failing that, randomly distributed left-right asymmetry develops in the mutant

Regarding the elytra spot color phenotype of the p^-/p^- insects, it can be concluded that the P factor acts to distribute elytrum spot color determinants to left-right body sides, rather than to determine color development. This conclusion also leads us to suggest that the potential for red color development normally exists but it is not executed in wild type insects, presumably because the black color-specifying mechanism prohibits operation of the default mode, red color-specifying mechanism. Since a single mechanism brings about the development of black/red-colored lateralized spots in the p^-/p^- mutant, we surmise that the color developed on one side is dependent on that developed on the other side. Accordingly, our model proposes that the elytrum spot color gene normally exists in bivalent states, perhaps transcriptionally *On* or *Off* epigenetic states in a chromatid-specific fashion, and only at a certain single-cell division during embryogenesis a specific chromatid distribution mode is specified (Figure 3). Consistent with the “cryptic red color state” idea, experimental injury to the developing pupa in wild type insects leads to the development of red spots on the elytrum on the injured side. Such an injury results in a piebald phenotype, which, unlike the p^-/p^- mutation, is not inherited by subsequent generations. Interestingly, such an experimentally-induced color variation was originally thought to be caused by an injury-induced mutation of the black color gene (4). We propose instead that the *BCG1* gene is in the *On* state temporally, only during a specific developmental window, and that it is epigenetically turned *Off* at other stages of development. Thus, we consider red color development as the default mode when the *BCG1* gene is epigenetically turned off, and also that the *BCG1* function might inhibit red color development. Experimental injury, therefore, only uncovers the default *Off* state of the gene, causing only red spots to develop. The males lack spots altogether, thus, elytra spot development is a sex-linked trait. The mechanism of why males do not develop spots is not known.

Visceral organs left/right laterality is fixed with respect to the dorso-ventral and anterior-posterior developmental axes in higher

Homologous chromosomes of elytra spots color laterality-generating progenitor cell of the p/p^- piebald mutant

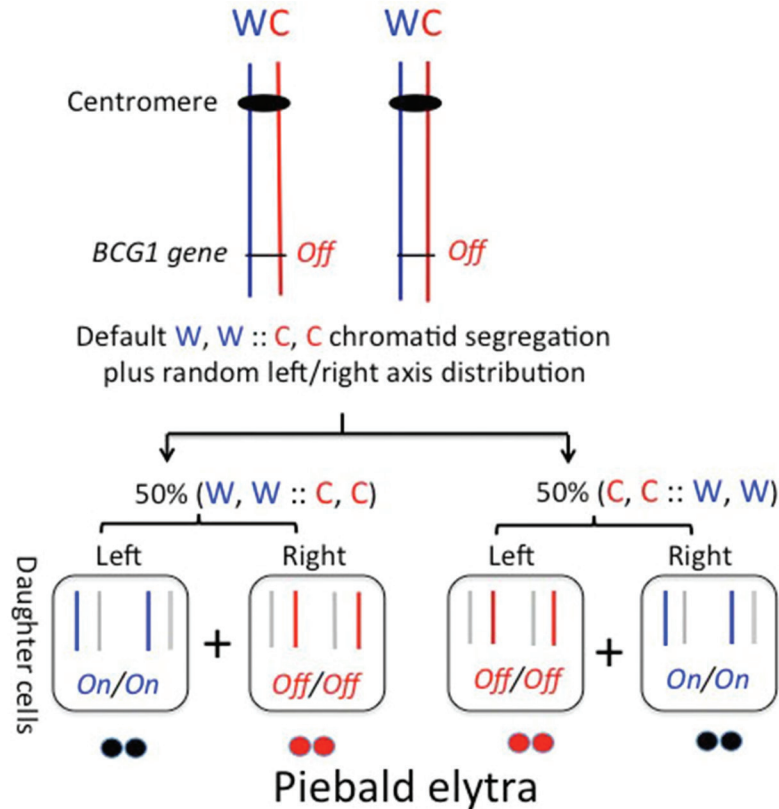


Figure 4. The SSIS mechanism explains the piebald mutant phenotypes of *Bruchus*. When the hypothetical *BCG1* gene is epigenetically regulated during chromosome replication as diagrammed, followed by the default mode of W, W :: C, C chromatid segregation occurring in the absence of the P factor, asymmetric cell division results in all embryos. When the *BCG1* gene is expressed in the elytrum, black spots result, and in the Off state, the default mode red spots develop. The round dots drawn at the bottom indicate the color of the elytrum spots on indicated sides of the beetle. All other symbols used here are defined in the Figure 3 legend.

animals (1, 2, 28). In contrast, the wing asymmetry is randomly distributed to the left- versus right-side in the *Bruchus* piebald mutant. Because the black/red wings combination likely results from a single event occurring in the mutant, their origin of asymmetry should reflect a single parsimonious mechanism. Interestingly, the author of the piebald mutant stated, “Probably some delicately adjusting mechanism shifts this asymmetry right or left, according to chance (4).” We extend the author’s foresight by suggesting that random distribution of selected chromatid pairs to the left-right axis at a critical single-cell division during development constitutes the mechanism of asymmetry development in the mutant. Stated another way, color developmental symmetry/asymmetry of wings is dictated by the mode of distribution of two particulate entities

to daughter cells at a specific cell division in embryogenesis, entities perhaps comprising the On state of the *BCG1* gene (Figures 3 and 4). This rationale, we feel, is the strongest one supporting the SSIS mechanism for wing development although no direct evidence exists supporting all the postulates of the model applied here to explain wings pigment development. This model could be used to guide future research on this insect’s body laterality development.

7. THE SSIS MECHANISM APPLIED FOR LATERALITY DEVELOPMENT OF EUKARYOTES

In addition to *Bruchus* wings development discussed above, the SSIS mechanism has been

advanced to explain variations of body laterality development due to respective gene mutations and for a case concerning chromosomal translocations in diverse organisms. The 50 percent penetrance of mouse embryonic lethality due to symmetric visceral organs development in the *lrd* mouse mutants (1, 3), the 50 percent congenital mirror hand movements disorder penetrance due to *rad51/RAD51* constitution in humans (29), and the 50 percent psychoses disorders penetrance in families containing chromosome 11 translocations (reviewed in (30)) are other such examples invoking SSIS mechanism. We propose that the *LRD* gene in mouse, the *rad51/RAD51* constitution in humans, and the piebald gene of *Bruchus* function to perform selective chromatid segregation of the relevant chromosome at critical mitoses during embryogenesis. Although mechanistic details remain unknown for all these systems and require future research, developmental symmetry/asymmetry is proposed in each case to be the result of selective segregation of precisely two particulate cellular entities to daughter cells at a critical cell division during embryogenesis. In each case, these entities are probably coincident with the *On* state of the developmental gene located on non-sister chromatids of a homologous pair of chromosomes. SSIS has likely evolved as one of the mechanisms for accomplishing cellular differentiation and development in diverse organisms. Another unrelated hypothesis advanced for a different biological purpose, the immortal strand hypothesis, has suggested genome-wide biased segregation of DNA strands to avoid distribution of potentially cancer-causing DNA replication errors to stem cells (reviewed in (31)). In contrast, SSIS comprises a chromosome-specific epigenetic mechanism strictly employed for cellular differentiation and development.

8. COMPARING SSIS AND MORPHOGEN GRADIENT DEVELOPMENTAL MODELS

As stated above, the SSIS model was initially proposed as a potential mechanism for laterality development of visceral organs in mice (5). Immediately, this proposal drew very strong criticisms from developmental biologists; they argued that yeast is a haploid organism and discoveries in yeast cannot be readily applied to a diploid organism, that selective strand segregation has not been demonstrated in vertebrates, and moreover, that development is well explained by the morphogen-gradient model (32, 33). In our response, we had disagreed with these criticisms and reasoned

that because developmental mechanisms are not yet defined, and therefore, further work is required to define them (34). Likewise, our conclusions for selective chromatid segregation in mouse cells (15) were argued to be invalid because the site-specific chromosome recombination system we had used could have produced the same results and also that no precedence existed for selective chromatid recombination proposed to occur to derive the SSIS model (35). We had also discounted these arguments (36). Moreover, the authors of the *Drosophila* study did not highlight the W, W :: C, C autosomes' segregation results (18), and we subsequently pointed out the significance of their result (19). Concerning the issue of laterality development of visceral organs, directional fluid flow driven by hundreds of primary cilia that develop in the embryonic node structure (i.e. the morphogen-gradient model) and asymmetric expression of ion channels in sister cells are other models advanced; however, the basis for breaking early left-right body symmetry during embryogenesis in vertebrates remains unknown and highly controversial (2). Recently it was reported that development was not impeded in mouse mutants having as few as two cilia even when they are situated at any location in the entire node (37). We surmise that this observation is not consistent with the morphogen model.

According to the SSIS model, regulated symmetric or asymmetric cell divisions in development might establish a transcriptional cascade by activating a specific gene or a set of genes in a cell, resulting in body laterality development later on by its offspring. For example, the *Isy-6* locus of *Caenorhabditis elegans* is "primed" through chromatin configuration in the precursor of the left, but not the right, ASEL neuron in the mitosis of a specific cell at the four-cell-stage embryo, a cell dividing six divisions before the lateralized neurons are born (38); does SSIS work here to lateralize the precursor cells? Similarly, the left-body-sided MI motor neuron and the right-sided eD3 epithelial cell in the *C. elegans* pharynx derive from the asymmetric division of a single blastomere cell dividing several cell divisions earlier in embryogenesis (39); indeed, this study invoked a model identical to the SSIS model to explain the origin of developmental asymmetry of sister cells. These *C. elegans* studies demonstrate how cells can become committed during earlier developmental stages, perhaps by epigenetic means (40), to specify an invariant cell fate later in development. Experimentally verifying

this programmed “single cell’s offspring” proposal has not been possible in mammals, although we have recently argued that the entire mouse brain hemisphere develops from the offspring of a single embryonic cell (29, 30).

Consistent with the single cell-offspring feature of the SSIS model, the brain hemispheric laterality of the frog embryo is determined at the two-blastomere stage of the embryo (41). To be noted, an undisturbed frog embryo produces a single, properly lateralized animal, however, when the two blastomeres are experimentally separated, each develops into an independent properly lateralized frog. Thus, embryonic cells can acquire new fates in response to experimental manipulations. In mammals also it is now clear that distinct cell lineages arise as early as the four-cell stage of the embryo, but these decisions are not fixed and are subject to change in response to experimentation, as observed in studies of mouse embryogenesis (42). These new findings are more in accord with the SSIS mechanism that posits cell lineage-specific developmental decisions executed early in embryogenesis, rather than with the proposal of different cell fates imposed by the morphogen concentration gradient on cells existing at different locations in the embryo (28).

Another result that is often argued to support the morphogen model is the discovery of factors that change the fate of a cell by cell–cell contact. For example, the P2 cell produces the Delta/Notch-mediated signal to establish the fate of the adjoining ABp cell in *C. elegans* by inducing epigenetic alterations (38). Similarly, growth factors secreted by specific cells are known to regulate fate of adjoining cells in the *Drosophila* testes microenvironment, called a niche (43). Likewise, the Wnt signaling factor is thought to act as a diffusible morphogen for development. Remarkably, *Drosophila* development is unaffected, albeit it is slowed down a bit, when the Wnt protein is tethered to the membrane of cell producing it to prohibit its diffusion away from the source (44). Thus, such close-range-acting cellular interaction observations do not necessarily support the morphogen gradients proposed to act through diffusion by exerting long-range controls. Many biological phenomena for development are controlled through reversible and heritable epigenetic processes. In sum, the SSIS mechanism is evolved to coordinate distribution of chromosomally borne epigenetic entities in mitosis for proper cellular differentiation and subsequent development. As presented here, the findings of

Bruchus wings spot development support the SSIS model although details of it remain to be defined by future studies.

9. ACKNOWLEDGEMENTS

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Abbreviations: SSIS, somatic strand-specific imprinting and selective sister chromatid segregation mechanism; *BCG1*, black elytrum color-specifying gene, W, Watson DNA strand; C, Crick strand; LRD, left-right dynein, *P*, piebald gene that causes symmetric wings color spots in *Bruchus*

Key Words: Piebald, *Bruchus*, Beetle, Mutant, Piebald Elytra Color, Asymmetry, Selective Chromatid Segregation Mechanism, Asymmetric Cell Division, Mechanism, Left–Right Body, Symmetry, Development, Review

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