Does random X-inactivation in mammals reflect a random choice between two X chromosomes?

Benjamin R. Williams* and Chao-ting Wu*:†, 1

*Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115
†Station for Natural Studies, Brookline, Massachusetts 02446
Address for correspondence: Department of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115.
Email: twu@rascal.med.harvard.edu

Running head: Two models for X-inactivation

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Corresponding author:
Chao-ting Wu
Department of Genetics
Harvard Medical School
77 Avenue Louis Pasteur, Boston, Massachusetts 02115
617-432-4431 Office
617-432-7663 FAX
Email: twu@rascal.med.harvard.edu
ABSTRACT

Inactivation of the maternal or paternal X chromosome in a mammalian embryonic XX cell is believed to involve random choice between the two Xs. We propose two alternative models. One suggests that choice is not random, while the other is consistent with random choice, but not one between two Xs.
Two studies, one by Amar Klar (2002) regarding the chromosomal basis of schizophrenia and of mating-type switching in *S. pombe* and another by Merok *et al.* (2002) from the laboratory of James Sherley concerning chromosome segregation in asymmetrically dividing murine cells, have led us to wonder whether certain developmental decisions that have been assumed to be random are, in fact, not random. They raise this issue by highlighting the predictive significance of Mendelian ratios, the potential of asymmetric DNA marks, and the merit of comparing sister cells resulting from cell division. In conjunction with a proposal by Robin Holliday, in which developmental switches in gene expression are simplified by the silencing of one of two copies of a genetic element through genomic imprinting (Holliday 1990), these studies suggest two new models for how only one X chromosome in a mammalian XX cell is permitted to be active in the process called X-inactivation.

While applicable to several aspects of X-inactivation, the two models are most readily illustrated by addressing that form in which choice of an X chromosome for inactivation (or activation) results in a 1:1 ratio of active maternal Xs to active paternal Xs in an XX cell population. Current prevailing models of X-inactivation interpret this ratio to indicate that the selection of either the maternal or paternal X chromosome for inactivation occurs by a process involving random choice between the two Xs (reviewed in Brockdorff 2002; Plath *et al.* 2002; Lee 2003). In contrast, the first model presented here argues that choice may in fact be predetermined and, therefore, not random. It is described below in its simplest form and then juxtaposed with a second model in which the outcome of X-inactivation is truly random yet not due to a choice between two X-chromosomes. Even if ultimately proven not applicable to X-inactivation, the bases of these models may pertain elsewhere, especially in situations involving monoallelic expression and other manifestations of allelic skewing.
The first model proposes that choice occurs in the cell generation preceding X-inactivation through an asymmetric mark, either parentally inherited or induced, on just one DNA strand of one X chromosome, the other X being immune to marking and/or the consequences of marking by, for example, parental imprinting (Fig. 1). The single-stranded, and therefore asymmetric, nature of the mark dictates that after cell division only one daughter cell will carry a marked X. If the marked X in the daughter cell is designated by the mark to be the future active X and its homologue responds by becoming the future inactive X, and if in the other daughter cell the unmarked X is designated by the lack of a mark to become the future inactive X and its homologue responds by becoming the future active X, then sister cells will show opposite patterns of inactivation and, in this way, maintain a 1:1 ratio of active maternal to paternal Xs in the cell population. As such, the model predicts the phenotypic relationship between sister cells (or their clonal derivatives) to be nonrandom and in striking contrast to that anticipated by models involving random choice between two X chromosomes. A good test of the model, therefore, would be the assessment of the pattern of X-inactivation among sister cells (or their clonal derivatives) generated immediately after X chromosomes commit to an active or inactive fate.

This first model directly parallels the proposal by Klar (1987; 2001; Dalgaard and Klar 1999) in which an asymmetric mark on one strand of DNA explains the observation that only one of the two daughters of an S. pombe cell will produce a daughter cell that switches mating type. The mark may be a bound proteinaceous factor, modification of DNA, or break in the DNA strand. It may also be the consequence of an event during replication in which either the old or the newly synthesized strand acquires a distinctive feature, such as, in the case of a newly synthesized strand, RNA moieties or single-stranded breaks introduced via Okazaki fragments. That a newly synthesized strand can be distinguished from its older template has been demonstrated by Merok et al. (2002),
wherein cultured murine cells that have been induced to adopt stem cell-like growth kinetics segregate chromosomes carrying the original and oldest templates to one daughter cell and those carrying more recently synthesized strands to the other daughter. The mark may even be directly or indirectly related to two transcripts, *Xist* and *Tsix* (reviewed in Brockdorff 2002; Plath *et al.* 2002; Lee 2003), which are transcribed from opposite DNA strands of the X inactivation center and, by their antisense nature, reflect the asymmetry inherent in DNA.

The suggestion that one X chromosome is immune, possibly by parental imprinting, is key and follows the proposal by Holliday (1990) that reduction of the number of genetic players from 2 to 1 by genomic imprinting greatly simplifies developmental choices in a cell. Here, this concept is extended to include a step subsequent to choice in which the immune X is brought back into play and assumes a state that is opposite to that of the determined X. In this way, the model is able to accommodate either the maternal or paternal X being active in a cell. That parental imprinting of the X chromosome can persist through early development and influence X-inactivation has been established by the preferential inactivation of the paternal X chromosome in extraembryonic tissues and, in marsupials, in the embryonic tissues as well (reviewed in Migeon 1998; Plath *et al.* 2002; Lee 2003; most recently Huynh and Lee 2003; Okamoto *et al.* 2003; also see Bean, Schaner, and Kelly 2004).

Variations of the model are possible. The asymmetric mark may be strand- or sequence-specific or nonspecific and/or designate inactivation instead of activation, and imprinted immunity may be either maternally or paternally established. Interestingly, as the 1:1 ratio of maternal to paternal active X chromosomes depends on sister cells assuming opposite fates in the same cell generation, the model predicts that deviations from this pattern will result in skewed ratios. For example, significant distortions of the
ratio would result if temporal uncoupling of sister cells caused one sister to delay designation of the future active (or inactive) X by just a single cell generation. In this light, it is intriguing that reproducible skewed inactivation has been observed with some X chromosome variants (reviewed in Migeon 1998; Plath et al. 2002). These variants may be altering the timing of designation, although skewed inactivation would also be expected with X chromosomes that permit symmetric marking or alter the strength of, or response to, marking or immunity.

Assuming that marking, designation, and the final steps of activation and inactivation are distinct events, the model can also approximate patterns of X-inactivation seen with X-aneuploids or whole genome hyperploids. Here, we suggest a feedback mechanism which subjects uncommitted X chromosomes to a new round of marking or immunity followed by cell division when the counting mechanism, which assesses the X:autosome ratio (reviewed in Plath et al. 2002), detects patterns of marking that are in conflict with the X:autosome ratio. In this scenario, X chromosomes cycle through the various states of marking, immunity, and perhaps even designation, over successive cell generations until the correct number of X chromosomes has been marked for activation. Implicit in this explanation is the ability of marking, immunity, and possibly designation to occur over multiple generations and even be reversed.

As explained above, the first model predicts a fully nonrandom basis for X-inactivation. Less extreme models, such as those in which the mark is influential rather than deterministic, are also possible and introduce a random flavor. In contrast, the second model predicts a fully random process. As in the first model, it proposes one X to be immune early on such that random X-inactivation, again, does not result from a choice between two X chromosomes. Rather, in this second model, choice is effected through two pools of factors, one activating, the other inactivating, and neither being limited in
quantity, competing for a single nonimmune X, after which the immune X takes its cue from its homologue (Fig. 2). Once more, the role of immunity is key; by reducing the number of X chromosomes in play to just one, it permits competition between factors to effect a truly random choice. Here, the basis of skewing can be explained by different concentrations and/or efficacies of the factors or X chromosome variants with altered affinities for the factors or differing susceptibilities to immunity. Patterns of X-inactivation in X aneuploids and whole genome hyperploids, however, are more difficult to explain by this second model. Finally, we note that the two models are not mutually exclusive. Choice may result from the influence of nonrandom mechanisms on an otherwise random process or vice versa, and may involve one strategy during one phase of development and another at another phase.

In sum, we propose two models for X-inactivation, the bases of which may be applicable to other forms of allelic skewing. In contrast to other models for X-inactivation, neither rests on a step involving choice between two X chromosomes. The first model proposes the determining event to be an asymmetric mark on one X, the other X being immune to marking. The second model also assumes an immune X and, by doing so, achieves a random outcome for X-inactivation by proposing a competition between factors for the remaining X.

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LITERATURE CITED


Klar, A. J., 2001 Differentiated parental DNA chain causes stem cell pattern of cell-type switching in *Schizosaccharomyces pombe*, pp. 17-35 in *Stem Cell Biology*, edited by


Figure legends:

Figure 1. Nonrandom choice as determined by an asymmetric mark (first model). An asymmetric mark on one DNA strand, Strand 1, of the maternal X chromosome, designates the X chromosome that inherits that mark in the next generation to be the future active X. The outcome is a 1:1 ratio of cells with an active maternal X to cells with an active paternal X. In this version of the model, immunity to marking initially characterizes the paternal X chromosome. (A) Strand 1 is inherited along with Strand 3 of the paternal X chromosome and results in a cell that carries an active maternal X because the mark on Strand 1 designates the X on which it resides to become the future active X. The paternal X of this cell takes its cue from the maternal X and becomes the future inactive X. The sister cell shows the opposite pattern of inactivation because it inherits the unmarked Strand 2 of the maternal X. (B) Here, Strand 1 is inherited along with Strand 4 of the paternal X, but the outcome is the same as that resulting from cosegregation of Strands 1 and 3 in that sister cells have opposite patterns of inactivation. Black circle, asymmetric mark; grey box, immunity, which need not be localized as shown; solid line, old strand; dotted line, newly synthesized strand; m, maternal; p, paternal; a, active state; s and cross mark, silenced state.

Figure 2. Random choice by competition for a nonimmune X (second model). In this view, two classes of factors, one activating and the other inactivating, compete for the maternal X and then, after binding, designate the maternal X to be the future active or inactive X, respectively. They do not compete for the paternal X, which is immune. Again, the resulting ratio of cells with an active maternal X to cells with an active paternal X is 1:1. In contrast to the first model, choice and the consequential activation and inactivation of X chromosomes may all occur in as few as one cell generation. (A) The maternal X in this cell is bound by an activating factor and becomes the future active}
X. Taking its cue from the maternal X, the paternal X becomes the future inactive X (B)

In this cell, the maternal X is bound by an inactivating factor and becomes the future inactive X, causing the paternal X to become the future active X. Crosshatched circle, activating factor; thick cross mark, inactivating factor; all other symbols as in Figure 1.
Nonrandom choice as determined by an asymmetric mark

Replication and cell division

A or B

1:1 1:1

Figure 1
Random choice by competition for a nonimmune X

1 : 1

Figure 2