recruitment and subsequent deposition of 5-methylcytosine marks for *de novo* methylation (Figure 1). However, the dependency of *de novo* methylation on DADs remains unexplored, for example, upon DNMT overexpression or perturbation of H3K4me3 methyltransferase activity. The mechanisms driving histone remodeling for DAD organization are also unsolved. A tantalizing candidate is the piRNA pathway due to direct relationships with the removal of H3K4me2 and establishment of *de novo* methylation and H3K9me3 patterns on TE sequences [8,9]. The authors speculate that *de novo* methylation related to DADs is likely not sequence specific due to the promiscuous nature of DNMTs, but given the requirement for piRNAs in *de novo* methylation of LINE-1 sequences, further analysis of their overall contribution to DAD organization is warranted [4,6]. Finally, since female germ cells regain methylation postnatally and only after enduring massive LINE-1-driven oocyte death [10], it will be intriguing to determine whether oocytes use the same chromatin-remodeling strategy elucidated by Yamanaka *et al.* in males [6].

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Forum

Paircounting

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X inactivation presents two longstanding puzzles: the counting and choice of X chromosomes. Here, we consider counting and choice in the context of pairing, both of the X and of the autosomes.

Allelic pairing at the X inactivation center (Xic) during X inactivation in mouse embryonic stem cells has been one of the most thought-provoking observations of mammalian genome regulation since its discovery over a decade ago [1–3]. Indeed, pairing has extraordinary potential for resolving two cornerstone puzzles of X inactivation: counting and choice. Here, we speculate on what pairing could theoretically offer.

Perhaps most saliently, pairing can simplify counting by reducing the number of independent entities in a population by half. This capacity of pairing is especially significant with respect to genetics because, when restricted to homologous chromosomes, it can function as a check on ploidy. Thus, when 'paircounted', 2 homologous chromosomes would give a count of 1, while all 46 chromosomes of a normal human diploid cell (46XX or 46XY) would give a paircount of 23 (22 pairs of autosomes and 1 pair of sex chromosomes, the latter paired at the pseudoautosomal region in males), with other outcomes signaling departures from diploidy (Figure 1A).

Another consequence of pairing is its potential to generate singularity and, by this singularity, drive choice. This is best exemplified when considering the number 2, which is the number of homologs for any autosome in a diploid cell. Specifically, paircounting reduces 2 to 1 and, in this way, creates a singularity; whereas, before pairing, there are two copies of any particular genomic region in a normal diploid cell, upon pairing, the resulting coupled regions generate a structural entity that is present exactly once. The point of emphasis here is that singularity could have implications for regulatory processes that involve allelic skewing, such as X inactivation and other forms of monoallelism, wherein the cell makes a choice between two allelic regions that subsequently assume different fates. For instance, if pairing generates a unique structure, then allelic distinction could be achieved if that structure were subsequently bound by a single copy of a factor that impacts, marks, or, after unpairing, persists on just one of the two allelic regions (Figure 1B). The single factor could, for example, designate the future active (or inactive) allele. This proposal is reminiscent of

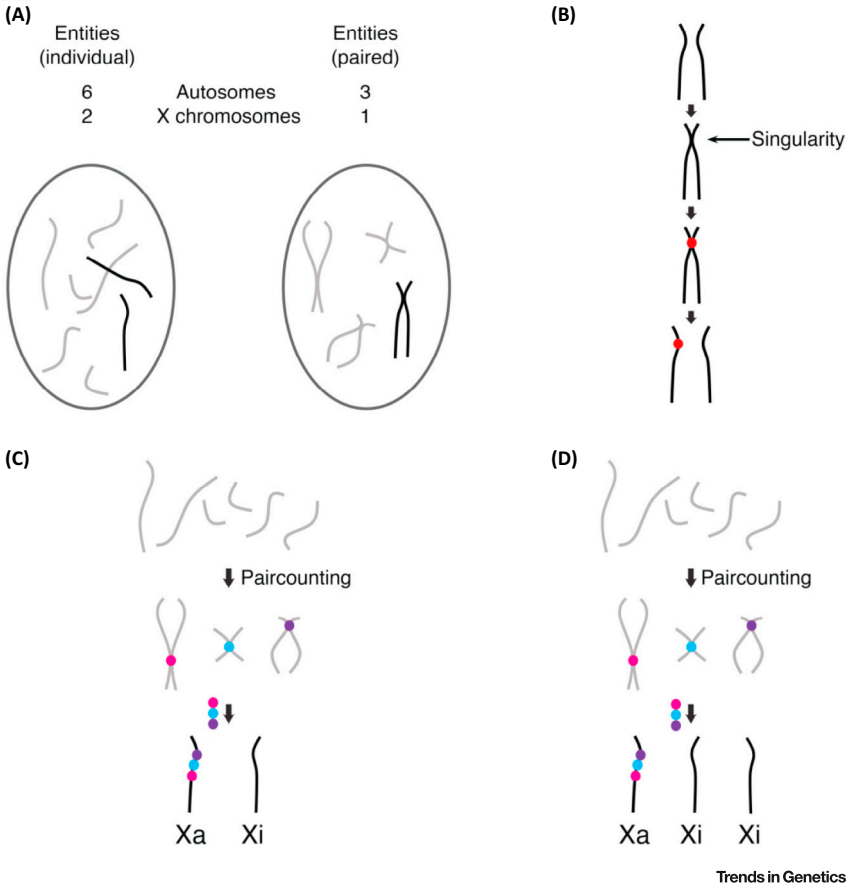

Figure 1. Paircounting.

(A) Homolog pairing reduces the number of entities to be counted by half. Autosomes are shown in gray and X chromosomes in black. (B) Once singularity has been generated by pairing, binding of that singularity by one copy of a factor (red circle) that then impacts, marks, or persists on just one of the two allelic regions will necessarily result in allelic distinction. Given that there is only a single binding site for the factor, the proposed mechanism could achieve allelic distinction regardless of how many copies of the factor are in the nucleus. As for the nature of the factor and its subsequent mark, many forms are possible, including protein, nucleic acid, epigenetic, structural, and/or combinations thereof. (C,D) In these hypothetical cells carrying three pairs of autosomes and either two (C) or three (D) X chromosomes, X inactivation begins with paircounting of the autosomes and the generation of singularities; here, we show one paircounted region per chromosome, although there could be more, followed by the binding of each paired region by a factor (colored circles), aggregation of the factors into a singularity, and then selection by the singularity of one X to remain active (Xa), with all other Xs becoming inactive (Xi). Singularity could also be achieved by aggregation of (or sequential interaction between) the paired autosomal regions, themselves. Observations of telomere aggregation would be consistent with the paircounted autosomal regions being telomeric or subtelomeric, one study explicitly tying (sub)telomeric pairing to X inactivation [6].

previous models describing how pairing can lead two Xs to be asymmetrically expressed [1,2] by, for example, transvection [4] or the partitioning of factors [5,6]. In our case, it is the binding of a single copy of a factor to the singularity, after that singularity has been generated by pairing, that drives choice. Alternatively, pairing could induce conformational changes, such that the two allelic regions become structurally and, thus, functionally different after separating.

It was surprising, therefore, when further consideration of our model led us to question whether the singularity proposed to underlie choice would necessarily be that arising from the pairing of genomic regions undergoing choice. In this way, our model recalls a recent study questioning whether Xic pairing is essential for X inactivation [3]. Indeed, we had previously suggested that singularity could be generated if one of the Xs were taken out of play by, for example, imprinting [7]. Alternatively, if a cell were able to produce an X-targeting factor in just one copy, then pairing of the Xs would no longer be essential for X inactivation because, since a single factor can target only one X, it cannot avoid effecting choice as soon as it binds an X. This implication of a factor present in only one copy was highlighted decades ago as a potential explanation for random X inactivation [8]. Thus, the challenge of our modeling shifted to the question of how a cell might generate just one copy of any factor. The answer may again lie with pairing because, as described earlier, pairing can produce a singularity when it couples two allelic regions into one structure. That is, the resulting paired structure may, itself, be the factor. Alternatively, the paired structure could bind and activate a single *trans*-acting factor; so that only one factor is galvanized in action, activation would need to be followed by unpairing or some other process by which the paired structure is taken out of play. Mary Lyon [8] suggested nearly half a century ago that 'a pair of autosomes jointly synthesizes a single molecule'. Here, we have merely suggested how two autosomes could accomplish such a task.

Box 1. In the Ever Expanding Realm of Pairing...

Mice undergo two forms of X inactivation (reviewed in [15]). One targets the paternal X at the four–eight-cell stage and is maintained in extraembryonic tissues. The other occurs in the embryo proper, but only after the paternal X has been reactivated at the ~60–64-cell stage. This second form, which targets either the maternal or the paternal X, has been deeply studied using mouse embryonic stem cells, where pairing has been observed between allelic X inactivation centers [1,2].

Pairing of homologous chromosomal regions outside the realm of meiosis has been reported in a variety of species and comes in many guises, from the genome-wide telomere-to-telomere pairing seen in *Drosophila* to the transient, localized pairing observed in mouse embryonic stem cells and associated with mammalian imprinting, allelic skewing, V(D)J recombination, stem cell differentiation, DNA repair, and cancer. In fact, the implication of homolog pairing in such a diversity of mammalian phenomena has vanquished the long-held perception that it is a peculiarity of *Drosophila* (reviewed in [13,14]). It may even be that homolog pairing is a universal feature of all somatic cells, wherein the degree of pairing reflects the extent to which it is counterbalanced by mechanisms acting to keep homologs apart. Thus, *Drosophila* would be an organism in which pairing 'wins', while humans and other mammals would be organisms in which antipairing generally prevails. The transient and reproducible nature of mammalian pairing argue further that it is meticulously controlled, a hallmark of phenomena with potent consequences (reviewed in [13,14]).

What might the consequences of pairing be? Not surprisingly, much effort has focused on the impact of pairing on gene expression, with one of the more well-understood mechanisms involving the action of enhancers in *trans* on a promoter located on a separate chromosome. Not only does transcription lend itself well to quantitative analysis, but genetic studies have also provided ample evidence that pairing can lead to striking changes in phenotype that are correlated with changes in gene expression. Much less studied has been the possibility of pairing generating or altering functions that do not result directly (or ever) in a change in transcription at the loci undergoing pairing. Such functions would include homologous recombination during DNA repair or mitotic crossing over. Here, we suggest that pairing may contribute to counting and choice by reducing the number of entities to be counted and generating states of singularity, respectively.

As described thus far, our model suggests that, with respect to choice and X inactivation, the genomic region undergoing pairing could be either X linked or autosomal. If X linked, pairing of the X would be an essential step for choice of the future active X, while involvement of autosomal pairing would release the X from any need to pair. Importantly, the latter would also render choice mechanistically responsive to the autosomal complement of a cell, in other words, counting. In fact, it is well established that X inactivation reflects the number of Xs as well as the number of autosomes in a cell, wherein the number of active Xs in a euploid cell generally approximates half the number of sets of autosomes. Consistent with this, studies have suggested that X inactivation results from an interplay of X-linked and autosomal factors, including blocking [8] and competence factors [9], as well as dosage-sensitive repressors of X inactivation [10].

Here, we speculate that autosomal paircounting may underlie the responsiveness of X inactivation to the autosomal complement. In fact, pairing has previously been considered as a mechanism for assessing genome ploidy [11,12] as well as achieving dosage compensation (reviewed in [13]). Here, we suggest that it is a singularity generated by autosomal paircounting that translates the count of autosomes into the number of Xs that remain active, with a paircount of 1 for any autosome in a diploid cell corresponding to one active X. Importantly, while a single paircounted autosomal region could serve as a proxy for the autosomal complement of an entire genome, it is also possible that paircounting targets at least one or even multiple regions on all autosomes (Figure 1C). This latter scenario would require genome-wide coordination of paircounting through, for example, the aggregation of (or sequential interactions between) paired regions (and/or the factors they generate) to achieve singularity. Figure 1D illustrates how paircounting in a cell that is trisomic for the X but otherwise diploid can ensure that only a single X remains active. Note that, while the simplest form of our model does not require Xs to pair, it also does not preclude a key role for X chromosome pairing during X inactivation. Indeed, we imagine that a genome-wide assessment of ploidy, perhaps even for purposes beyond X inactivation, would necessarily involve the X.

For over a century, homolog pairing has straddled the line between being an anomaly of *Drosophila* to being a widespread form of genome organization (reviewed in [13,14]). Pairing enthusiasts are placing bets on the latter, anticipating a greater prevalence of somatic homolog pairing than previously expected across species, cell types, and cell states (Box 1).

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