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Pairing and anti-pairing: a balancing act in the diploid genome

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Abstract

The presence of maternal and paternal homologs appears to be much more than just a doubling of genetic material. We know this because genomes have evolved elaborate mechanisms that permit homologous regions to sense and then respond to each other. One way in which homologs communicate is to come into contact and, in fact, Dipteran insects such as Drosophila excel at this task, aligning all pairs of maternal and paternal chromosomes, end-to-end, in essentially all somatic tissues throughout development. Here, we reexamine the widely held tenet that extensive somatic pairing of homologous sequences cannot occur in mammals and suggest, instead, that pairing may be a widespread and significant potential that has gone unnoticed in mammals because they expend considerable effort to prevent it. We then extend this discussion to interchromosomal interactions, in general, and speculate about the potential of nuclear organization and pairing to impact inheritance.

Introduction

A poorly understood aspect of genome organization is the regulation of interchromosomal interactions and their relationship to intrachromosomal interactions within a chromosome territory (CT) [1]. For instance, in the context of three-dimensional (3D) organization, how do regulatory elements preferentially interact with gene promoters in *cis*? Likewise, how are interactions in *trans* inhibited and/or promoted in subnuclear compartments of similarly regulated chromatin domains? These issues are further complicated in the context of homologous chromosomes, which are nearly identical in sequence and protein composition and yet are somehow sensed, distinguished, and typically packaged individually inside of the nucleus. Here, we provide an overview of recent studies regarding homolog positioning across a wide array of organisms, including mammals, and propose that the infrequent nature of homologous interactions is due at least in part to active inhibitory mechanisms.

Emergent evidence for interchromosomal interactions

At first glance, the short- and long-range intrachromosomal contacts that form chromatin loops and CTs would seem to discourage interchromosomal interactions. However, techniques ranging from traditional genetics to fluorescent *in situ* hybridization (FISH) and chromosome conformation capture (e.g. 3C, 4C, 5C, Hi-C, etc.) have now produced an

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abundance of evidence for interchromosomal interactions and the capacity of those interactions to contribute to gene regulation. For example, several loci have been shown to loop out of their CT to form interchromosomal contacts with active genes, thus correlating an open chromatin conformation with gene expression [2-6]. CT intermingling has also been observed in instances of gene repression. For example, pericentromeric heterochromatin from different chromosomes cluster into repressive nuclear compartments with many repressed transposable elements and facultatively repressed genes [7-11].

In short, there is a significant amount of crosstalk between different CTs, reflecting a general tendency for loci of similar genomic content and chromatin status to be proximal to each other (reviewed by [12]). In fact, the propensity of certain chromosomal regions to participate in interchromosomal interactions is believed to constrain the distance between interacting chromosomes and thus influence the nonrandom nuclear position of CTs themselves [13, 14]. Interestingly, the nature and frequency of translocated regions in cancer suggests that the regulation of interchromosomal contacts also has functional implications for the diseased states [4, 15-19].

What about homologous chromosomes?

Chromosomes adopt a distinct position in the nucleus based on gene density, expression status, and number of repetitive elements. As such, chromosomes of similar size and gene density are more likely to interact in mouse and human cells [20-22]. Thus, if chromosome organization reflects sequence and transcriptional activity, then maternal and paternal homologs might interact more frequently than would be expected at random as they are virtually identical in size, sequence, and, most likely, associated proteins and other factors. And yet, only a few species exhibit extensive homolog interactions in somatic cells, the most noteworthy of which are Dipteran insects, such as Drosophila, which align all pairs of homologs, end-to-end, in essentially all somatic tissues [reviewed by 23]. Indeed, Drosophila homolog pairing is one of the most dramatic examples of interchromosomal interactions.

Equally notable is that no species other than Dipterans are believed to support somatic homolog pairing to this extent. For example, Heride et al. showed that human homologs lie in separate CTs and are thus far apart from each other despite their sequence similarity [24]. Similar conclusions have been drawn from DNA FISH in a wide range of species and are further supported by haplotype reconstruction of mouse and human Hi-C data sets, demonstrating that chromosome haplotypes in diploid cells do not interact frequently with each other [e.g. 25, 26].

Such paucity of pairing has led to an assumption that pairing results from an active process that is specific to Dipterans and absent in other species (Figure 1A). Another explanation, however, is that pairing is a significant potential which has gone unnoticed in other species because these species expend considerable effort to prevent it (Figure 1A) [27-30]. These interpretations are not two sides of the same coin. Just as somatic pairing is evidence for inter-homolog communication, so would a nonrandom pattern of homolog separation be indicative of inter-homolog awareness.

Advantages of somatic pairing: is being together better than staying apart?

The potential for communication between Drosophila homologs was postulated >100 years ago by Nettie Stevens and then demonstrated in 1954 by Ed Lewis, who introduced the term transvection to describe forms of gene activity that are sensitive to the proximity of homologs (Figure 1B) [31, 32]. Thus, transvection is one of the originating examples of two broad and overlapping areas of research: the field of *trans* interactions and that of homology effects [33], wherein genes are influenced by the presence of homology. In particular, transvection encompasses pairing-sensitive allelic crosstalk, pairing-sensitive silencing, and many other phenomena in a wide variety of species, including mammals [reviewed by 33-38].

What advantages might somatic pairing afford? Many models have been considered, including its potential to a) enable intragenic complementation by, for example, the *trans* action of regulatory elements (Figure 1B) [33-37 and reviewed by 39-41], b) facilitate co-regulation [42], c) contribute to chromosome counting and dosage compensation [36, 43-52], as well as d) promote mitotic recombination or homolog-templated repair [53] and, thus, e) accelerate positive and negative selection of variants by effecting loss-of-heterozygosity (LOH). Indeed, extensive stretches of homologous pairing might maximize accurate alignment and, hence, viable recombinant events [54]. Considering that homolog pairing may antagonize or promote sister chromatid cohesion, homolog pairing could also f) control processes, such as sister chromatid templated repair and sister chromatid exchange, that are influenced by sister chromatid cohesion [28]. Furthermore, as homolog pairing is likely to impact chromosome topology, it may also g) affect chromosome compaction and extension [29, 30, 55] as well as accessibility, such as through linear-locking [44, 56].

Identification of pairing and anti-pairing factors by high-throughput screening

Much has been learned regarding the mechanisms that underlie the pairing process [57, 58], with studies using FISH targeting euchromatic and heterochromatic regions beginning to identify the underlying genes. Among the first genes to be identified were Suppressor of Hairy-wing [59] and topoisomerase II [28], both of which led to reduced pairing when they were disrupted. Perhaps most intriguingly, however, was the discovery that overexpression of the Cap-H2 component of the condensin II complex in Drosophila promotes disassembly of polytene chromosomes and antagonizes transvection [29]. This finding was consistent with the proposal that pairing can be actively inhibited and suggested that condensin II is a candidate for embodying anti-pairing activity [27-30, 60].

More recently, three whole-genome screens were conducted to identify genes involved in somatic pairing in Drosophila. The first applied FISH in embyros and returned the surprising finding that essentially no zygotic transcription is necessary to establish pairing [61]. The second thus turned to Drosophila cultured cells and involved a high-throughput FISH technology, called Hi-FISH, which permits >2,000 FISH assays to be conducted and imaged

per day in 384-well plates and enables FISH-based screens for factors involved in interphase genome organization [30]. (Also Shachar et al.[62], which describes another high-throughput pipeline.) The third screen also used cell culture, in this case, assaying pairing of the X chromosome through the localization of the X-enriched MSL dosage compensation machinery [63].

In total, the Hi-FISH screen, which targeted two heterochromatic loci, revealed 105 candidate pairing genes [30]. Excitingly, many of these genes were also identified by MSL localization [63], implying that these genes regulate the pairing of whole chromosomes. Consistent with this conclusion, many of these genes were also found to influence pairing at euchromatic regions by FISH [30]. Therefore, the pairing of heterochromatic and euchromatic regions may be regulated by related mechanism(s) or, perhaps, through overlapping forces, with the potential of each contributing *in cis* to the proximity or repulsion of the other. Of course, a fuller picture of pairing awaits a parallel screen for factors specifically involved at euchromatic loci in Drosophila. The most surprising outcome, however, was that the majority (62%) of the 105 genes exhibited anti-pairing activity, strongly supporting a model in which pairing can be both promoted and inhibited. Among the candidate anti-pairing genes are those that encode for the chromatin proteins HP1a and ORC1 in addition to components of the condensin II complex, including Cap-H2. These results are consistent with the role of condensin II *in vivo* [29] and implicate chromatin compaction as a mechanism by which *trans* interactions of this type are inhibited [29, 30]. Moreover, these studies revealed novel genetic interactions between Cap-H2 and several pairing promoting genes, providing further evidence that condensin II regulates chromosome pairing and that many other proteins involved in the regulation of pairing depend on condensin II for this function [30]. In particular, the SCF^{slmb} ubiquitin ligase complex was identified as a novel inhibitor of condensin II-mediated nuclear reorganization [30, 64], lending further support to the idea that chromosome pairing can be promoted by simply removing anti-pairing activity. Also identified as anti-pairing factors were proteins involved in the G1-S transition, which is consistent with earlier observations correlating stages of the cell cycle to differing levels of homolog pairing [23, 34, 35].

Collectively, these findings argue against the view in which pairing is an active process and unpairing represents the default state. Instead, the paired state may reflect a balance of two antagonistic pathways (pairing and anti-pairing), each of which could be modulated at the gene-, chromosome-, tissue-, or species-specific level [29, 30, 65].

A new model for homolog positioning in humans

While extensive somatic pairing is not typically observed outside of Drosophila, localized and/or transient homolog interactions have been identified across a wide array of species, including mammals. Interestingly, mammalian pairing is often associated with critical cellular processes, including DNA repair and V(D)J recombination, in addition to transcriptional regulation during X-inactivation, imprinting, and cell fate establishment [45, 46, 48, 49, 66-77]. Homologous association of pericentromeric regions has also been documented for human chromosomes 1, 7, 8, 10, and 17 [78-81]. As such, the capacity of homologous pairing to alter gene activity in *trans* is no longer irrelevant in mammalian

somatic cells and may even account for some puzzling features of allelic crosstalk [75, 82, 83].

What remains unclear is whether transient pairing events in mammals are mechanistically related to the genome-wide pairing observed in Drosophila. If not, then it would seem that pairing evolved multiple times in metazoans, further highlighting the potential importance of these interactions. However, another, perhaps simpler, model is that pairing and anti-pairing pathways were both present in the common eukaryotic ancestor (Figure 2). Indeed, over 90% of Drosophila candidate pairing genes have human orthologs [30], consistent with eukaryotes having retained a potential to pair homologs. Therefore, the reason we see extensive pairing in Dipterans and not humans may be because the balance might favor pairing activity in the former and anti-pairing activity in the latter (Figure 2).

Extensive pairing in humans may be associated with disease

If somatic pairing is a widespread potential of genomes, then any disruption of anti-pairing should increase homologous contacts in humans. Remarkably, this may have already been observed by Koeman *et al.* who, as part of their investigation to reveal why renal oncocytomas overexpress genes on the q arm of Chromosome 19, discovered that this arm, in particular, is paired in its entirety, from centromere to telomere, in over 50% of transformed cells [84]. This most dramatic example, by far, of somatic pairing outside of Drosophila led the authors to suggest that a transvection-like mechanism may be responsible for the elevated gene activity of 19q and, furthermore, that pairing be considered an associated feature of tumorigenesis in general. The contrast between the diseased and normal tissue suggests that 19q pairing may result from a clonally heritable change and raises two possibilities: the change generated a novel activity – i.e., somatic pairing – or it disrupted a mechanism that had been inhibiting pairing, as would be predicted by a model in which pairing is balanced by anti-pairing. Although the capacity of mutations to generate novel activity is not unheard of, we find the latter explanation more plausible, since spontaneous changes in the genome are more often destructive than they are creative (Figure 1). Thus, all human cells may have the capacity for genome-wide somatic pairing (Figure 2), and disruptions of this balance may be indicative and perhaps even causative of some diseased states.

Why might human cells favor the unpaired state?

If the default and/or ancestral state of chromosomes is to be paired with their homologs then why would humans and other species expend effort to prevent it? As suggested by renal oncocytomas, one explanation might be a need to disrupt trans-communication of alleles. Active separation of homologs may also facilitate allele-specific expression, such as monoallelism [reviewed by 24, 85], although, ironically, an initial pairing event might actually facilitate the coordination of monoallelic expression through allelic crosstalk, which is consistent with elevated homolog contacts that have been documented at imprinted loci in humans [66-69, 72, 73, 75]. Similarly, although transient pairing of the two X-chromosomes may be an important step in the sensing and counting of X-chromosomes during X-inactivation in mammals, the subsequent separation of the X's, possibly mediated by anti-

pairing mechanisms, may then be required to achieve chromosome-wide allele-specific expression [45-49, 51, 52]. In the case of biallelically expressed genes, separation of homologs may better ensure a wider distribution of products in the cytoplasm or the generation of polarity, should there be any differences between the chromosomes.

In addition to its potential effects on transcription, the unpaired state may serve to minimize the likelihood of mitotic recombination, which could reduce the frequency of LOH and, hence, penetrance of recessive deleterious mutations [24]. The unpaired state may also contribute to genome stability by removing entanglements between homologs or sister chromatids, which might otherwise increase the frequency of chromosome missegregation and, consequently, aneuploidy. In fact, an imbalance of pairing and anti-pairing activities that favors pairing may be a common underlying cause of diseases associated with gene misexpression, aneuploidy, and LOH. In this context, one might ask why Drosophila and other Dipterans support extensive somatic homolog pairing if the proximity of homologous sequences can give rise to such detrimental outcomes. Here, we would suggest that Dipterans may have evolved mechanisms for controlling or mitigating the consequences of pairing by, for example, preventing crosstalk or effecting local unpairing.

Pairing as a model for long-range interactions

Ultimately, the manner in which the paired and unpaired state of homologous sequences is regulated must fold into the greater picture of intra- and interchromosomal interactions, and it will be critical to understand how all these interactions come together to guide the genome through the cell cycle and development. For instance, are all genomic regions subjected to antagonistic forces that act to promote and inhibit their interactions with other loci? Additionally, what is the mechanistic relationship between intra- and interchromosomal interactions at the local and chromosome-wide level, and are they in competition or cooperation with each other? Intriguingly, the identification of condensin II as an anti-pairing factor is in line with the intrachromosomal functions of compaction and chromatin looping being a mechanism by which long-range interchromosomal interactions, such as pairing, are inhibited [11, 29, 30, 55]. Consistent with this model, depletion of condensin, or other architectural proteins such as CTCF and cohesin, often shows that long- and short-range chromosomal contact frequencies are inversely correlated [11, 86-90]. In this viewpoint, the mechanisms of pairing may overlap with that of intra- and interchromosomal interactions in general, with all types of long-range interactions being precluded by the formation, size, and/or density of small chromatin loops (Figure 3) [11, 29, 30, 55]. Indeed, given its robust and simplistic nature, pairing is proving to be a powerful experimental system for elucidating the intricate balance between intra- and interchromosomal contacts.

Closing remarks and a consideration of inheritance

What milestones lie ahead? Technologically, improvements in high-throughput FISH [30, 62] will likely enhance our capacity to identify genes involved in genome organization, while strategies that enable Hi-C [e.g. 25, 26] and FISH [91] to distinguish homologs will clarify the contributions of pairing and anti-pairing. Technologies for visualizing the genome, including live [reviewed by 92] and super-resolution microscopy [91 and reviewed

by 93] will also improve. These may reveal that regions of the genome that are accessible for expression or intra- and interchromosomal interactions are distinguished by signature conformations or simply by how dynamically they shift from one conformation to another. Similarly, pairing may run the gamut between a base-by-base alignment to a more laissez-faire arrangement in which homologous sequences are only loosely apposed and vary in a locus-, temporal-, and/or cell-type-specific fashion.

Conceptually, we may discover that genome organization is as important a component of heritable information as are nucleic acids and epigenetic marks. Thus, we may find it equally likely to be altered and then passed from one generation to the next in the form of the altered configuration, itself, or as simply the effect of the alteration. While inheritance of an altered configuration may be easily envisioned if changes occurred in the germline lineage, the capacity of nonautonomous factors to transmit information between cells leaves open the possibility of somatic changes in nuclear organization being transmitted to the germline and thus also the next generation (Figure 4A) [94-96]. Therefore, genome organization may account for instances of transgenerational inheritance, acquired traits, and traits acquired via maternal or fetal microchimerism [97] and transplantation. We may even discover that it contributes to the missing heritability that confounds the mapping of disease traits. As such, a full personal genome may ultimately include tissue-specific descriptions for all aspects of genome positioning.

Finally, we speculate on how the defining principle of pairing may confer a unique capacity on genomes. In particular, by aligning homologous sequences, pairing may enable single cells to assess and respond to the degree of structural heterogeneity between parental genomes and thus, indirectly, that of the population from which those genomes were drawn (Figure 4B). Indeed, such a process has been proposed for the ultraconservation of sequences and maintenance of genome integrity through evolution, suggesting that pairing can exert long-term consequences [98, 99]. In particular, assessment in cell lineages that give rise to germ cells might enable such lineages to influence genomic diversity in ensuing generations by suppressing or promoting *de novo* changes, reducing or enhancing fertility, modulating repair, triggering apoptosis, or inducing meiotic drive in response to the degree of heterogeneity detected [98, 99] (Figure 4B; M. Jakubik and C-t. W. unpublished). Might this comparison of parental genomes be a key, or even the primary, function of the end-to-end alignment of homologs in meiosis? An analogous process in non-germline cells could further afford organisms some control over the degree of structural heterogeneity in their soma [99]. Intriguingly, studies have correlated sites of sequence heterogeneity with higher local mutation rates and, in the germline, attributed the heightened rates to an instability or compromised state of meiotic pairing [100, 101]. Here, we suggest that cells may embody a process in which they exert a directed influence on future generations by assessing parental and population heterogeneity and then modulating mutation rates (Figure 4B). In brief, of the many intra- and interchromosomal interactions that contribute to nuclear organization, the pairing of homologous sequences may be outstanding with respect to its conceptual simplicity and yet magnitude of impact. By definition, it is merely the coming together of homologous sequences, and yet this minimal requirement gives it license to virtually the entire genome and perhaps even future generations. With such potential for impact, it would be no wonder if pairing had evolved hand-in-hand with anti-pairing.

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References

1. Cremer T, Cremer M. Chromosome territories. Cold Spring Harbor perspectives in biology. 2010; 2(3):a003889. [PubMed: 20300217]
2. Mahy NL, Perry PE, Bickmore WA. Gene density and transcription influence the localization of chromatin outside of chromosome territories detectable by FISH. J Cell Biol. 2002; 159(5):753–63. [PubMed: 12473685]
3. Osborne CS, et al. Active genes dynamically colocalize to shared sites of ongoing transcription. Nat Genet. 2004; 36(10):1065–71. [PubMed: 15361872]
4. Branco MR, Pombo A. Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. PLoS Biol. 2006; 4(5):e138. [PubMed: 16623600]
5. Zhao Z, et al. Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. Nat Genet. 2006; 38(11):1341–7. [PubMed: 17033624]
6. Schoenfelder S, et al. Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. Nat Genet. 2010; 42(1):53–61. [PubMed: 20010836]
7. Dernburg AF, et al. Perturbation of nuclear architecture by long-distance chromosome interactions. Cell. 1996; 85(5):745–59. [PubMed: 8646782]
8. Dimitri P. Fluorescent in situ hybridization with transposable element probes to mitotic chromosomal heterochromatin of Drosophila. Methods Mol Biol. 2004; 260:29–39. [PubMed: 15020800]
9. Bantignies F, et al. Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila. Cell. 2011; 144(2):214–26. [PubMed: 21241892]
**10. Clowney EJ, et al. Nuclear aggregation of olfactory receptor genes governs their monogenic expression. Cell. 2012; 151(4):724–37. [PubMed: 23141535] The authors examined the nuclear position of olfactory receptor genes in mouse olfactory neurons and found that the silencing of thousands of genes located on different chromosomes is achieved by the spatial clustering of the genes into heterochromatic foci.
**11. Li L, et al. Widespread rearrangement of 3D chromatin organization underlies polycomb-mediated stress-induced silencing. Molecular cell. 2015; 58(2):216–31. [PubMed: 25818644] The authors analyzed Hi-C interactions in Drosophila cells following heat-shock-induced stress, which revealed a surprisingly plastic organization and highlighted an inverse relationship between long- and short-range chromosome contacts.
12. Gibcus JH, Dekker J. The hierarchy of the 3D genome. Molecular cell. 2013; 49(5):773–82. [PubMed: 23473598]
13. Misteli T. Beyond the sequence: cellular organization of genome function. Cell. 2007; 128(4):787–800. [PubMed: 17320514]
14. Fraser J, et al. An Overview of Genome Organization and How We Got There: from FISH to Hi-C. Microbiol Mol Biol Rev. 2015; 79(3):347–72. [PubMed: 26223848]
15. Roix JJ, et al. Spatial proximity of translocation-prone gene loci in human lymphomas. Nat Genet. 2003; 34(3):287–91. [PubMed: 12808455]

16. Fudenberg G, et al. High order chromatin architecture shapes the landscape of chromosomal alterations in cancer. Nat Biotechnol. 2011; 29(12):1109–13. [PubMed: 22101486]

**17. Engreitz JM, Agarwala V, Mirny LA. Three-dimensional genome architecture influences partner selection for chromosomal translocations in human disease. PloS one. 2012; 7(9):e44196. [PubMed: 23028501] The authors showed that many translocation breakpoints observed in human diseases have elevated Hi-C contact frequencies in normal cells, suggesting a broad role for 3D chromatin organization in determining the frequency of translocations between loci.

18. Evdokimova V, et al. Formation of carcinogenic chromosomal rearrangements in human thyroid cells after induction of double-strand DNA breaks by restriction endonucleases. Endocr Relat Cancer. 2012; 19(3):271–81. [PubMed: 22323563]

**19. Zhang Y, et al. Spatial organization of the mouse genome and its role in recurrent chromosomal translocations. Cell. 2012; 148(5):908–21. [PubMed: 22341456] The authors used a novel combination of Hi-C and high-throughput, genome-wide translocation sequencing (HTGTS) to argue 3D genome organization and spatial proximity among loci strongly influence patterns of chromosomal rearrangements and translocations.

20. Croft JA, et al. Differences in the localization and morphology of chromosomes in the human nucleus. J Cell Biol. 1999; 145(6):1119–31. [PubMed: 10366586]

21. Tanabe H, et al. Non-random radial arrangements of interphase chromosome territories: evolutionary considerations and functional implications. Mutation research. 2002; 504(1-2):37–45. [PubMed: 12106644]

22. Lieberman-Aiden E, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science. 2009; 326(5950):289–93. [PubMed: 19815776]

23. McKee BD. Homologous pairing and chromosome dynamics in meiosis and mitosis. Biochim Biophys Acta. 2004; 1677(1-3):165–80. [PubMed: 15020057]

**24. Heride C, et al. Distance between homologous chromosomes results from chromosome positioning constraints. J Cell Sci. 2010; 123(Pt 23):4063–75. [PubMed: 21084563] The authors used 3D FISH analysis of 10 chromosomes in human epithelial cancer cells and found that inter-homolog distances are generally larger than inter-heterolog distances, supporting a model in which a critical distance is maintained between homologous chromosomes in human cells.

**25. Selvaraj S, et al. Whole-genome haplotype reconstruction using proximity-ligation and shotgun sequencing. Nat Biotechnol. 2013; 31(12):1111–8. [PubMed: 24185094] The authors carried out haplotype reconstruction of mouse and human Hi-C data sets, which revealed that chromosome haplotypes, including those on homologous chromosomes, do not interact frequently with each other.

**26. Rao SS, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell. 2014; 159(7):1665–80. [PubMed: 25497547] The authors used in situ Hi-C to construct interaction maps of each homolog separately and, in a homolog-nonspecific manner, achieved a resolution down to 1kb.

27. Joyce EF, McKim KS. When specialized sites are important for synapsis and the distribution of crossovers. Bioessays. 2007; 29(3):217–26. [PubMed: 17295219]

28. Williams BR, et al. Disruption of topoisomerase II perturbs pairing in drosophila cell culture. Genetics. 2007; 177(1):31–46. [PubMed: 17890361]

29. Hartl TA, Smith HF, Bosco G. Chromosome alignment and transvection are antagonized by condensin II. Science. 2008; 322(5906):1384–7. [PubMed: 19039137]

30. Joyce EF, et al. Identification of genes that promote or antagonize somatic homolog pairing using a high-throughput FISH-based screen. PLoS Genet. 2012; 8(5):e1002667. [PubMed: 22589731]

31. Stevens N. A study of the germ cells of certain Diptera, with reference to the heterochromosomes and phenomena of synapsis. J. Exp. Zool. 1908; 5:359–374.

32. Lewis EB. The theory and application of a new method of detecting chromosomal rearrangements in Drosophila melanogaster. Am. Nat. 1954; (88):225–239.

33. Wu CT, Morris JR. Transvection and other homology effects. Curr Opin Genet Dev. 1999; 9(2): 237–46. [PubMed: 10322135]

34. Duncan IW. Transvection effects in Drosophila. Annu Rev Genet. 2002; 36:521–56. [PubMed: 12429702]

35. Kennison JA, Southworth JW. Transvection in Drosophila. Adv Genet. 2002; 46:399–420. [PubMed: 11931232]

36. Apte MS, Meller VH. Homologue pairing in flies and mammals: gene regulation when two are involved. Genet Res Int. 2012; 2012:430587. [PubMed: 22567388]

37. Kassis JA. Transvection in 2012: site-specific transgenes reveal a plethora of trans-regulatory effects. Genetics. 2012; 191(4):1037–9. [PubMed: 22879406]

38. Kassis JA, Brown JL. Polycomb group response elements in Drosophila and vertebrates. Adv Genet. 2013; 81:83–118. [PubMed: 23419717]

39. Geyer PK, Green MM, Corces VG. Tissue-specific transcriptional enhancers may act in trans on the gene located in the homologous chromosome: the molecular basis of transvection in Drosophila. EMBO J. 1990; 9(7):2247–56. [PubMed: 2162766]

40. Bateman JR, Johnson JE, Locke MN. Comparing enhancer action in cis and in trans. Genetics. 2012; 191(4):1143–55. [PubMed: 22649083]

41. Mellert DJ, Truman JW. Transvection is common throughout the Drosophila genome. Genetics. 2012; 191(4):1129–41. [PubMed: 22649078]

42. Ashburner M. Happy Birthday - Puffs! Chromosome Today. 1977; 6:213–22.

43. Erickson JW, Cline TW. Molecular nature of the Drosophila sex determination signal and its link to neurogenesis. Science. 1991; 251(4997):1071–4. [PubMed: 1900130]

44. Wu CT, Howe M. A genetic analysis of the Suppressor 2 of zeste complex of Drosophila melanogaster. Genetics. 1995; 140(1):139–81. [PubMed: 7635282]

45. Marahrens Y. X-inactivation by chromosomal pairing events. Genes Dev. 1999; 13(20):2624–32. [PubMed: 10541548]

46. Bacher CP, et al. Transient colocalization of X-inactivation centres accompanies the initiation of X inactivation. Nat Cell Biol. 2006; 8(3):293–9. [PubMed: 16434960]

47. Diaz-Perez SV, et al. A deletion at the mouse Xist gene exposes trans-effects that alter the heterochromatin of the inactive X chromosome and the replication time and DNA stability of both X chromosomes. Genetics. 2006; 174(3):1115–33. [PubMed: 16980402]

48. Xu N, Tsai CL, Lee JT. Transient homologous chromosome pairing marks the onset of X inactivation. Science. 2006; 311(5764):1149–52. [PubMed: 16424298]

49. Augui S, et al. Sensing X chromosome pairs before X inactivation via a novel X-pairing region of the Xic. Science. 2007; 318(5856):1632–6. [PubMed: 18063799]

50. Lott SE, et al. Noncanonical compensation of zygotic X transcription in early Drosophila melanogaster development revealed through single-embryo RNA-seq. PLoS Biol. 2011; 9(2):e1000590. [PubMed: 21346796]

**51. Masui O, et al. Live-cell chromosome dynamics and outcome of X chromosome pairing events during ES cell differentiation. Cell. 2011; 145(3):447–58. [PubMed: 21529716] This study used live-cell analysis to reveal the spatiotemporal dynamics of the X chromosomes during early differentiation, which indicated a direct role for pairing in facilitating monoallelic expression of Xist during random X inactivation.

**52. Sun S, et al. Xist imprinting is promoted by the hemizygous (unpaired) state in the male germ line. Proc Natl Acad Sci U S A. 2015 Using a transgenic system to study Xist imprinting, this paper found that the unpaired state plays a critical role in epigenetic transmission between generations.

53. Rong YS, Golic KG. The homologous chromosome is an effective template for the repair of mitotic DNA double-strand breaks in Drosophila. Genetics. 2003; 165(4):1831–42. [PubMed: 14704169]

54. Wilkins AS, Holliday R. The evolution of meiosis from mitosis. Genetics. 2009; 181(1):3–12. [PubMed: 19139151]

**55. Bauer CR, Hartl TA, Bosco G. Condensin II promotes the formation of chromosome territories by inducing axial compaction of polyploid interphase chromosomes. PLoS Genet. 2012; 8(8):e1002873. [PubMed: 22956908] This paper demonstrated that condensin II plays a critical role in the formation of chromosome territories in Drosophila, suggesting a mechanism in which compaction of chromosomes precludes interchromosomal associations, such as homolog pairing and clustering of pericentromeric heterochromatin.

56. Wu CT. Transvection, nuclear structure, and chromatin proteins. J Cell Biol. 1993; 120(3):587–90. [PubMed: 8425891]
57. Fung JC, et al. Homologous chromosome pairing in Drosophila melanogaster proceeds through multiple independent initiations. J Cell Biol. 1998; 141(1):5–20. [PubMed: 9531544]
58. Vazquez J, Belmont AS, Sedat JW. The dynamics of homologous chromosome pairing during male Drosophila meiosis. Curr Biol. 2002; 12(17):1473–83. [PubMed: 12225662]
59. Fritsch C, Ploeger G, Arndt-Jovin DJ. Drosophila under the lens: imaging from chromosomes to whole embryos. Chromosome Res. 2006; 14(4):451–64. [PubMed: 16821139]
60. Joyce EF, et al. Germline Progenitors Escape the Widespread Phenomenon of Homolog Pairing during Drosophila Development. PLoS Genet. 2013; 9(12):e1004013. [PubMed: 24385920]
61. Bateman JR, Wu CT. A genomewide survey argues that every zygotic gene product is dispensable for the initiation of somatic homolog pairing in Drosophila. Genetics. 2008; 180(3):1329–42. [PubMed: 18791221]
62. Shachar S, et al. Identification of Gene Positioning Factors Using High-Throughput Imaging Mapping. Cell. 2015; 162(4):911–23. [PubMed: 26276637]
63. Bateman JR, et al. A genome-wide screen identifies genes that affect somatic homolog pairing in Drosophila. G3 (Bethesda). 2012; 2(7):731–40. [PubMed: 22870396]
64. Buster DW, et al. SCFSlimb ubiquitin ligase suppresses condensin II-mediated nuclear reorganization by degrading Cap-H2. J Cell Biol. 2013; 201(1):49–63. [PubMed: 23530065]
65. Bosco G. Chromosome pairing: a hidden treasure no more. PLoS Genet. 2012; 8(5):e1002737. [PubMed: 22654678]
66. LaSalle JM, Lalande M. Homologous association of oppositely imprinted chromosomal domains. Science. 1996; 272(5262):725–8. [PubMed: 8614834]
67. Riesselmann L, Haaf T. Preferential S-phase pairing of the imprinted region on distal mouse chromosome 7. Cytogenet Cell Genet. 1999; 86(1):39–42. [PubMed: 10516430]
68. Thatcher KN, et al. Homologous pairing of 15q11-13 imprinted domains in brain is developmentally regulated but deficient in Rett and autism samples. Hum Mol Genet. 2005; 14(6): 785–97. [PubMed: 15689352]
69. Teller K, et al. Maintenance of imprinting and nuclear architecture in cycling cells. Proc Natl Acad Sci U S A. 2007; 104(38):14970–5. [PubMed: 17848516]
70. Apostolou E, Thanos D. Virus Infection Induces NF-kappaB-dependent interchromosomal associations mediating monoallelic IFN-beta gene expression. Cell. 2008; 134(1):85–96. [PubMed: 18614013]
71. Brandt VL, Hewitt SL, Skok JA. It takes two: Communication between homologous alleles preserves genomic stability during V(D)J recombination. Nucleus. 2010; 1(1):23–9. [PubMed: 21327101]
72. Leung KN, et al. Neuronal chromatin dynamics of imprinting in development and disease. Journal of cellular biochemistry. 2011; 112(2):365–73. [PubMed: 21268055]
73. Meguro-Horike M, et al. Neuron-specific impairment of inter-chromosomal pairing and transcription in a novel model of human 15q-duplication syndrome. Hum Mol Genet. 2011; 20(19):3798–810. [PubMed: 21725066]
**74. Gandhi M, et al. Homologous chromosomes make contact at the sites of double-strand breaks in genes in somatic G0/G1-phase human cells. Proc Natl Acad Sci U S A. 2012; 109(24):9454–9. [PubMed: 22645362] This study used FISH to demonstrate that contact between allelic regions of homologous chromosomes frequently occurs in somatic human cells and can be induced by DNA damage, arguing that pairing may represent a general phenomenon in human cells.
**75. Krueger C, et al. Pairing of homologous regions in the mouse genome is associated with transcription but not imprinting status. PloS one. 2012; 7(7):e38983. [PubMed: 22802932] This paper describes the pairing properties of various imprinted and non-imprinted regions in mouse tissues and ES cells and found, by allele-specific 4C-Seq and DNA FISH, that pairing is not associated with imprinted status or DNA repair, but is influenced by chromosomal location and transcription.
**76. Hogan MS, et al. Transient pairing of homologous Oct4 alleles accompanies the onset of embryonic stem cell differentiation. Cell stem cell. 2015; 16(3):275–88. [PubMed: 25748933]

The authors used DNA FISH to examine the earliest steps between pluripotency and lineage commitment in ESCs and found a critical role for transient pairing of Oct4 alleles in exiting the pluripotent state.

77. Stratigi K, et al. Spatial proximity of homologous alleles and long noncoding RNAs regulate a switch in allelic gene expression. Proc Natl Acad Sci U S A. 2015; 112(13):E1577–86. [PubMed: 25770217]
78. Arnoldus EP, et al. Somatic pairing of chromosome 1 centromeres in interphase nuclei of human cerebellum. Hum Genet. 1989; 83(3):231–4. [PubMed: 2793166]
79. Arnoldus EP, et al. Interphase cytogenetics reveals somatic pairing of chromosome 17 centromeres in normal human brain tissue, but no trisomy 7 or sex-chromosome loss. Cytogenet Cell Genet. 1991; 56(3-4):214–6. [PubMed: 2055120]
80. Dalrymple SJ, et al. Correlation of cytogenetic and fluorescence in situ hybridization (FISH) studies in normal and gliotic brain. J Neuropathol Exp Neurol. 1994; 53(5):448–56. [PubMed: 8083688]
81. Atkin NB, Jackson Z. Evidence for somatic pairing of chromosome 7 and 10 homologs in a follicular lymphoma. Cancer Genet Cytogenet. 1996; 89(2):129–31. [PubMed: 8697418]
82. Spilianakis CG, et al. Interchromosomal associations between alternatively expressed loci. Nature. 2005; 435(7042):637–45. [PubMed: 15880101]
83. Sandhu KS, et al. Nonallelic transvection of multiple imprinted loci is organized by the H19 imprinting control region during germline development. Genes Dev. 2009; 23(22):2598–603. [PubMed: 19933149]
**84. Koeman JM, et al. Somatic pairing of chromosome 19 in renal oncocytoma is associated with deregulated EGLN2-mediated [corrected] oxygen-sensing response. PLoS Genet. 2008; 4(9):e1000176. [PubMed: 18773095] Using whole-chromosome paints, the authors found elevated levels of gene expression and a dramatic frequency of homolog pairing along the entirety of the long arm of chromosome 19 in cells taken from renal tumors of patients.
85. Reinius B, Sandberg R. Random monoallelic expression of autosomal genes: stochastic transcription and allele-level regulation. Nat Rev Genet. 2015; 16(11):653–64. [PubMed: 26442639]
86. Nora EP, et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature. 2012; 485(7398):381–5. [PubMed: 22495304]
87. Kim LK, et al. Oct-1 regulates IL-17 expression by directing interchromosomal associations in conjunction with CTCF in T cells. Molecular cell. 2014; 54(1):56–66. [PubMed: 24613343]
88. Tark-Dame M, et al. Depletion of the chromatin looping proteins CTCF and cohesin causes chromatin compaction: insight into chromatin folding by polymer modelling. PLoS Comput Biol. 2014; 10(10):e1003877. [PubMed: 25299688]
89. Zuin J, et al. Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. Proc Natl Acad Sci U S A. 2014; 111(3):996–1001. [PubMed: 24335803]
**90. Barutcu AR, et al. Chromatin interaction analysis reveals changes in small chromosome and telomere clustering between epithelial and breast cancer cells. Genome biology. 2015; 16(1):214. [PubMed: 26415882] This paper demonstrated that small, gene-rich human chromosomes have decreased interchromosomal associations in breast cancer cells as compared to normal tissue.
91. Beliveau BJ, et al. Single-molecule super-resolution imaging of chromosomes and in situ haplotype visualization using Oligopaint FISH probes. Nat Commun. 2015; 6:7147. [PubMed: 25962338]
92. Fujita T, Fujii H. Applications of Engineered DNA-Binding Molecules Such as TAL Proteins and the CRISPR/Cas System in Biology Research. Int J Mol Sci. 2015; 16(10):23143–64. [PubMed: 26404236]
93. Sydor AM, et al. Super-Resolution Microscopy: From Single Molecules to Supramolecular Assemblies. Trends in cell biology. 2015; 25(12):730–48. [PubMed: 26546293]
**94. Saha AK, et al. Intercellular trafficking of the nuclear oncoprotein DEK. Proc Natl Acad Sci U S A. 2013; 110(17):6847–52. [PubMed: 23569252] These authors showed that a conserved nonhistone protein, DEK, can be transmitted through the extracellular space from one cell to another, after which it enters the nucleus of the receiving cell and then effects changes in chromatin structure.

95. Sarkies P, Miska EA. Small RNAs break out: the molecular cell biology of mobile small RNAs. Nature reviews. Molecular cell biology. 2014; 15(8):525–35. [PubMed: 25053358]

96. Eaton SA, et al. Roll over Weismann: extracellular vesicles in the transgenerational transmission of environmental effects. Epigenomics. 2015

97. Jeanty C, Derderian SC, Mackenzie TC. Maternal-fetal cellular trafficking: clinical implications and consequences. Curr Opin Pediatr. 2014; 26(3):377–82. [PubMed: 24759226]

98. Derti A, et al. Mammalian ultraconserved elements are strongly depleted among segmental duplications and copy number variants. Nat Genet. 2006; 38(10):1216–20. [PubMed: 16998490]

99. McCole RB, et al. Abnormal dosage of ultraconserved elements is highly disfavored in healthy cells but not cancer cells. PLoS Genet. 2014; 10(10):e1004646. [PubMed: 25340765]

100. Amos W, Kosanovic D, Eriksson A. Inter-allelic interactions play a major role in microsatellite evolution. Proc Biol Sci. 2015; 282(1818)

101. Yang S, et al. Parent-progeny sequencing indicates higher mutation rates in heterozygotes. Nature. 2015; 523(7561):463–7. [PubMed: 26176923]

102. Morris JR, et al. Two modes of transvection: enhancer action in trans and bypass of a chromatin insulator in cis. Proc Natl Acad Sci U S A. 1998; 95(18):10740–5. [PubMed: 9724774]

103. Jack JW, Judd BH. Allelic pairing and gene regulation: A model for the zeste-white interaction in Drosophila melanogaster. Proc Natl Acad Sci U S A. 1979; 76(3):1368–72. [PubMed: 16592632]

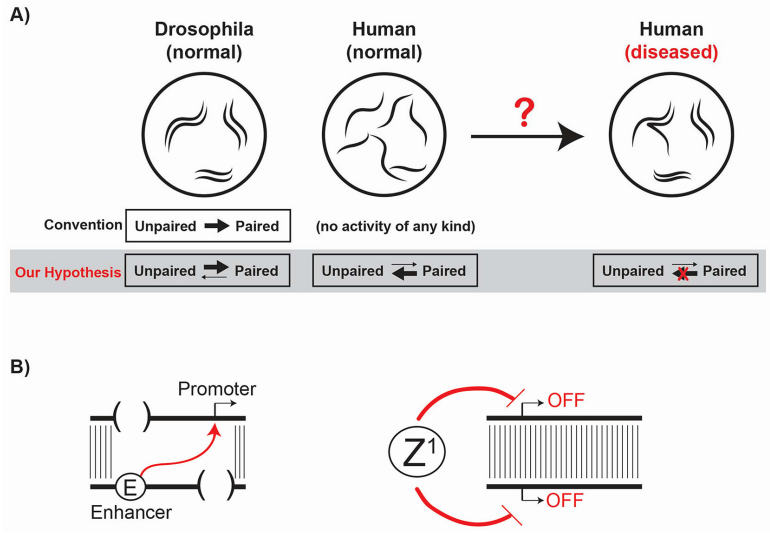

Figure 1.

A) In the conventional model, Drosophila pairs homologs because it supports a pairing activity that evolved specifically in the Dipteran lineage. In this viewpoint, both pairing and anti-pairing activity would be absent in human cells. An alternative explanation posits that all organisms support both pairing and anti-pairing activities, the relative strengths of which differ between Drosophila and humans. Importantly, this model predicts that disruption of anti-pairing in humans will induce ectopic pairing and potentially predispose individuals to disease, a notion that is consistent with the pairing of chromosome 19q in renal oncocytomas by Koeman et al. [84]. B) Two models of Drosophila transvection are shown. On the left, the enhancer of a promoter-less gene acts in *trans* on the promoter of a paired, enhancer-less homolog [39, 102]. Deficiencies are denoted as (). On the right, the gain-of-function *zeste*^{1} mutation (denoted at Z^{1}) is required to repress paired white genes. Vertical lines represent homolog pairing interactions [103].

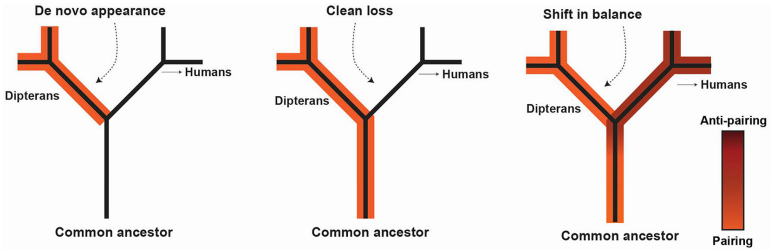

Figure 2.

Three evolutionary models to explain the singular ability of Dipterans to support genome-wide somatic pairing. The leftmost figure suggests that Dipterans evolved *de novo* a genome-wide mechanism for somatic pairing, while the middle figure suggests that a capacity for pairing had been pre-existing in the common ancestor of Dipterans and other organisms but was lost in all but the Dipteran lineage. The rightmost figure depicts an explanation wherein the paired state reflects a balance of antagonistic activities, one that promotes pairing and another that prevents pairing (anti-pairing), both of which were present in the common ancestor. A shift in balance toward pairing and anti-pairing activity would be favored in the Dipteran and human lineages, respectively.

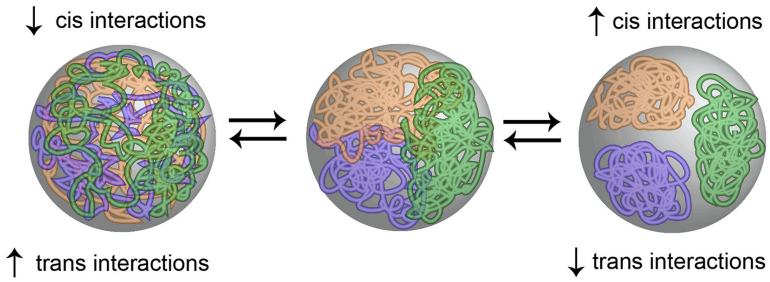

Figure 3.

Model for how intrachromosomal (*cis*) interactions (*e.g.* compaction, looping, CT formation) might influence the potential for interchromosomal interactions (*trans*) (*e.g.* pairing, recombination, translocations). We note that this antagonistic relationship between intra- and interchromosomal interactions might also be observed at the gene- or chromosome-specific level.

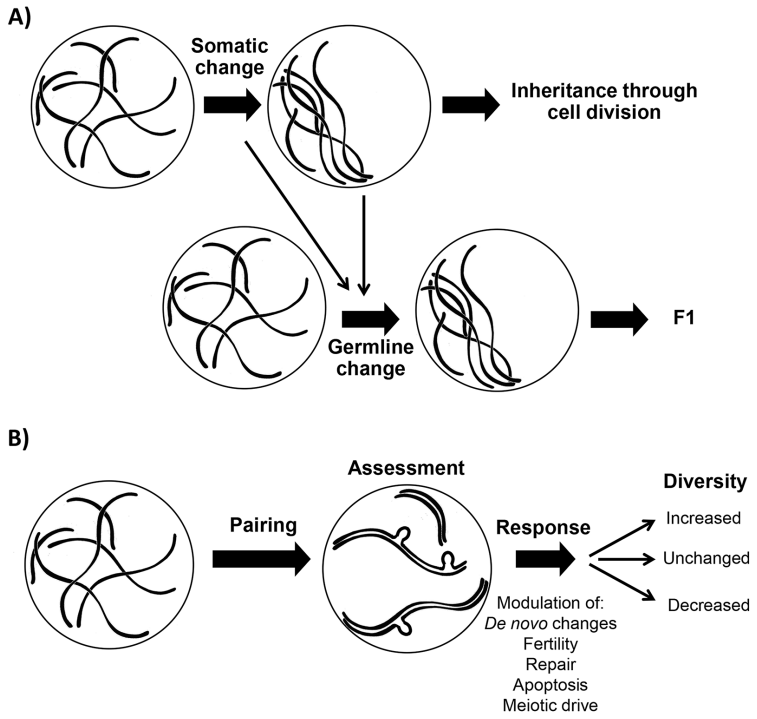

Figure 4.

A) As is the case with other genetic material, genome organization might be subject to alteration and then inheritance in its altered state, such as through cell division (top row). Furthermore, alterations transferred to the germline from the soma or occurring *de novo* in the germline would have the potential to be inherited by subsequent generations. Alterations might arise via error, mutation, stress, stochastic processes, and/or even developmentally directed cues. B) Pairing may enable cells to assess and respond to the degree of heterogeneity between parental genomes. Depending on whether it occurs in the germline or soma, this process would have the potential to alter genomic diversity in the next generation or in a population of somatic cells.