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Review

The role of specialized transcription factories in chromosome pairing

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ABSTRACT

Homologous chromosomes can pair in somatic and germ line cells, and many mechanisms have been proposed to explain how they do so. One popular class of models involves base-pairing between DNA strands catalyzed by recombination proteins, but pairing still occurs in mutants lacking the relevant functional proteins. We discuss an alternative based on two observations: transcription occurs in factories that specialize in transcribing specific gene sub-sets, and chromosomes only pair when transcribed. Each chromosome in the haploid set has a unique array of transcription units strung along its length; we suggest each is organized into clouds of loops tethered to specialized factories. Only homologs share similar strings of clouds and factories. Pairing begins when a promoter on one chromosome initiates in the homologous and specialized factory organized mainly by its homologous partner. This transiently ties the two homologs together, to increase the chances that adjacent promoters initiate in their homologous factories and that the two homologs will be zipped together. Then, interactions between promoters and RNA polymerases in the factories mediate pairing.

1. Introduction

It is widely known that an intimate pairing between homologs in the germ line of many diploid organisms is essential for successful meiosis; generally only paired homologs recombine and form the bivalents required for correct segregation into haploid sets. Perhaps less well appreciated is the extent of pairing seen in somatic cells. For example, early during *Drosophila* development histone genes on different homologs are distributed randomly, but two-thirds pair at the mid-blastula transition [1]; when the adult fly emerges, most homologs in most somatic cells in most tissues are at least partially paired [2]. In organisms with more complex genomes, such high levels of pairing are rarely seen. Even so, the existence of "knock-out" mice attests to the fact that small pieces of input DNA can occasionally find their homologous targets in somatic cells with complex genomes. What mechanisms underlie such pairing in both germ line and soma?

The hydrogen bonding that mediates base-pairing between two DNA strands, and the cohesion between two sister chromatids, will be of secondary interest here as participants are born side-by-side and do not need to seek out and identify their partners. We also distinguish between the mechanism underlying the initial identification of partners, and the final verification that correct partners have been found (which involves base pairing). We exemplify this by reference to meiosis. During leptotene, each duplicated chromosomal pair searches for its (duplicated) homolog, so by the beginning of zygotene most lie roughly parallel to their partners, ~300 nm apart. We use the term "pairing" to describe the mechanism – which is of central interest here – that creates this distant association; it should be distinguished from the familiar "base-pairing" involving H-bonds. During zygotene, the synaptonemal complex draws the two closer together, so that by pachytene (duplicated) homologs lie ~100 nm apart in register. Recombination then takes place between two DNA duplexes that must lie within nanometers of each other; validation that pairing is correct depends on H-bonds between bases in individual DNA strands of the interacting partners.

Many models have been suggested to explain how pairing might occur [3–6]. Most involve H-bonding between bases, and these are undoubtedly involved during the later steps of recombination and validation; we will argue they are unlikely to play major roles during pairing in either mitotic or meiotic cells. Here, we review results supporting an alternative model for pairing based on the DNA–protein interactions involved in transcription [7].

2. A brief history: the rise and fall of a role for H-bonding

We begin by briefly reviewing some of the many different mechanisms that have been invoked. Most involve some – or all – of three features [8]: "stirring" to generate accidental contacts between homologs (through active processes that complement Brownian motion), non-random chromosomal positioning to ease the homology search (e.g., through side-by-side alignment in the "Rabl" or "bouquet" arrangements), and weak interactions (to facilitate a trial-and-error homology search, and subsequent "zipping" together of partners).

In general, recognition must also involve long-range interactions acting over many tens of nanometers, where homologs "feel" other

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chromosomes as they search for homologous sites before moving together [3,9,10]. Early models often involved pairing proteins, but once H-bonds were found to play a central role in the pairing of individual strands in the DNA duplex, it was immediately obvious they might also be involved in pairing. Thus, a single-strand or double-strand DNA break could enable a single-strand from one chromosome to extend and feel for its homolog, and – once found – base-pairing would stabilize the connection. Then, as other single-stranded extensions succeeded in their searches, the two partners would be zipped together. Such models are attractive because DNA:DNA base pairing provides a clear precedent for the required complementary interaction, and a DNA break could initiate both pairing and recombination. However, homologs pair well before the relevant breaks in DNA can be detected, and they can do so in recombination-deficient cells and/or the complete absence of breaks.

If breaks are not involved, perhaps pairing between intact DNA molecules plays a role. For example, an intact duplex loop could "kiss" chromatin loops in other chromosomes, and – once a partner had been found – proteins involved in recombination (e.g., RecA-like proteins) could line them up through base pairing, stabilizing them [8]. Unfortunately, chromosomes still pair in the relevant mutants [11–13]. Therefore, we are forced to consider DNA:DNA pairing through special types of H-bonds (e.g., through Hoogsteen pairing between four, parallel, DNA strands). If this does not appeal, we can consider RNA:RNA base pairing involving sense and anti-sense transcripts still tethered through their polymerases to their respective homologs [8].

All models involving base pairing face two central problems. First, pairing occurs far too quickly. For example, most double-strand breaks in yeast are repaired by homologous recombination within 2 h ($\sim 10^4$ s), so any homology search based on base pairing would require successive independent searches each lasting $\sim 10^{-3}$ s to search through the $\sim 10^7$ bases in the genome. There remains no satisfactory mechanism as to how such rapid searches might be made [6]. Moreover, they should depend on the concentration of the target gene, but varying the concentration of a (mammalian) target ~800-fold has no effect [14]. Second, the many DNA repeats found in complex genomes should defeat such a homology search.

We now seem to be at a stage in history where many current models for recombination just ignore how homologs might find their partners, or suppose that homologs must be "pre-paired" in some way, perhaps through the action of "pairing" proteins [6]. However, no single candidate protein has emerged from the various genome projects, and we are left with a multitude of different ones that must play different roles in different contexts – for example, yeast Taz1, mouse SUN1, maize PHS1, and wheat Ph1 [15–19]. Against this background, we update our model.

3. A model for genome organization

Our model is based on one view of how genomes are organized (Fig. 1). We imagine that RNA polymerases do not track along their templates as textbooks depict; rather, fixed enzymes reel in their templates as they extrude their transcripts [20]. At the larger scale, engaged polymerases and transcription factors cluster into "factories" to loop the intervening DNA [21–23]. Clustering of active polymerases will inevitably be driven by an entropic "depletion attraction" that acts in the crowded nucleus [24]. [For other models of chromosome organization, see refs [25–27].] Support comes from various sources [23]: (i) A polymerase that tracks along a helix generates a transcript entwined about its template; however, no satisfactory untwining mechanism has yet been proposed. Immobilizing the polymerase ensures no such problem arises. (ii) After permeabilization, active polymerases resist detachment by nucleases which place them close to points where loops are tied to the sub-structure. (iii) High-resolution imaging shows nascent RNA to be concentrated in a few sites — the factories. As there are more active molecules of RNA polymerase II (in human, mouse, newt nuclei) than such factories, and as only one polymerase is typically engaged on a transcription unit, each factory must contain many different units [28]. (iv) Chromosome conformation capture (3C) and fluorescence *in situ* hybridization (FISH) show sequences lying far apart on the genetic map can nevertheless lie close together in 3D space; significantly, contacting sequences are usually transcriptionally active. Examples of such contacts include those between mouse *Hbb-b1* and its locus control region (LCR), the T_H2 LCR and its transcriptionally-poised interleukin targets, *H19* and *Igf2*, plus the H enhancer and its target OR genes [29–33]. 3C also shows active units on different chromosomes cluster together [34–37]. (v) Nascent transcripts are invariably associated

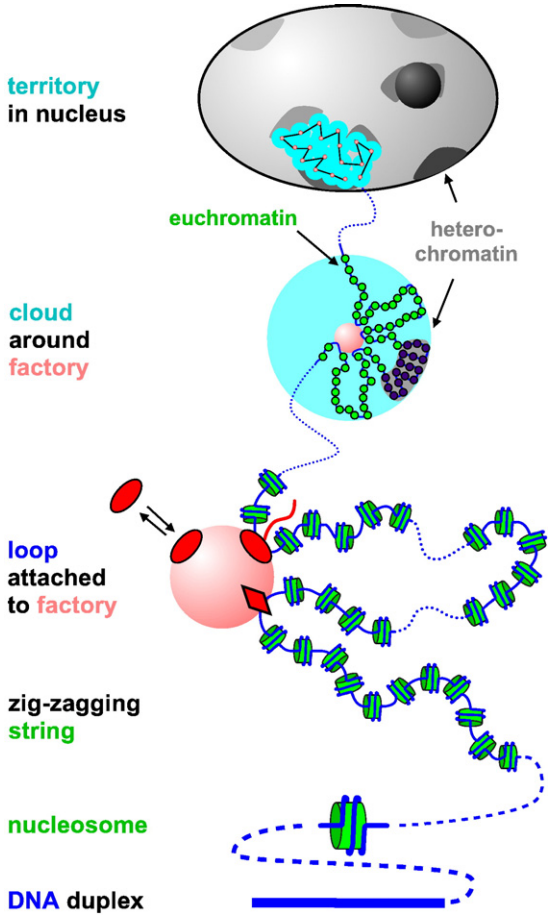

Fig. 1. A model for genome organization. In eukaryotes, DNA is coiled around the histone octamer, and runs of nucleosomes form a zig-zagging string. At the intermediate level in the hierarchy, this string is organized into loops (in HeLa, these have an average contour length of ~86 kbp; range 5–200 kbp) by attachment to transcription factors (diamond) and engaged RNA polymerases (ovals). [There are other ties, in addition to these major ones.] 10–20 such loops (only a few are shown) form a cloud around the factory, to give a structure equivalent to that of the bacterial nucleoid. [Active transcription units that are nearest neighbours are shown bound to one factory here, but the structure is more complex; units distant on the genetic map (perhaps on different chromosomes) will sometimes bind to the factory.] Active polymerases do not track along their templates; they are bound to a transcription factory and act both as motors that reel in their templates and as one of the critical structural ties that maintain the loops. Loops inevitably appear and disappear as polymerases initiate and terminate, and the factors bind and dissociate. Nucleosomes in long loops are static and acquire a (heterochromatic) histone code that spreads down the fibre; they also aggregate on to the lamina, nucleoli, and chromocentres. Each transcription factory contains one type of RNA polymerase (i.e., I, II, or III) to the exclusion of others, and some factories are richer in certain transcription factors than others (and so are involved in the transcription of specific sets of genes). Individual components in the factor exchange continually with others in the soluble pool. Successive clouds strung along the chromosome form a territory (the general path of DNA between clouds is shown). [There are 50–100 clouds in a human cell.] Modified from [59]; this material is used by permission of John Wiley and Sons, Inc.

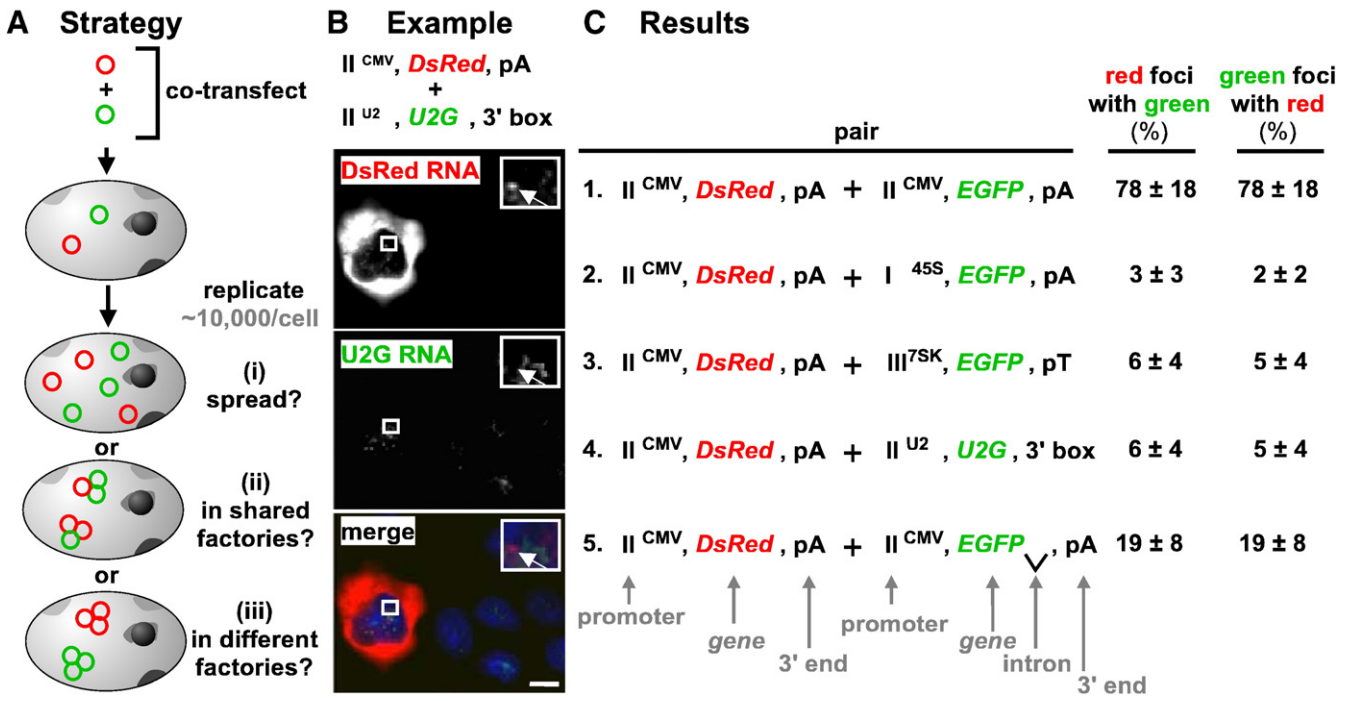

Fig. 2. Factories specialize in transcribing particular genes. (A) Strategy. Are minichromosomal templates (and nascent transcripts; not shown) spread throughout nuclei, or concentrated in the same or different factories? (B) Cells are transfected with plasmids encoding *DsRed* (the reference) or *U2G* snRNA (the test gene) driven by different promoters (pair 4 in C); RNA FISH shows nascent RNA is concentrated in nuclear foci containing either *DsRed* or *U2G* RNA — but not both (insets, arrows). DNA was counterstained with DAPI. Bar: 10 μm. (C) Percentage of red nuclear foci containing green signal (and *vice versa*) after co-transfecting a reference (encoding *DsRed*) and test plasmid. Pair 1: Nascent RNA made from identical promoters is found in the same foci (percentage is high). Pairs 2–4: Nascent RNA made from the reference CMV promoter is in different foci from that from the others (percentages all low). Pair 5: Most intron-containing nascent RNA is in different foci (percentage low). Results show possibility A iii applies. Adapted from [48].

with huge (i.e., 87-nm) structures of ~10 MDa — the factories [38]. This model would apply to all genomes, and key details are specified (e.g., the nature of ties maintaining loops, loop length, number of active polymerases/factory). Initially controversial, it is increasingly being considered as a likely possibility (e.g., refs [39–41]).

4. Factories specialize in transcribing specific gene sub-sets

The clustering of components in a factory ensures high local concentrations, enabling efficient interactions. For example, HeLa nuclei contain a ~1 μM pool of RNA polymerase II, but the concentration in a factory is ~1 mM. As a result, few transcripts – if any – would then be made outside factories. Moreover, we also know that factories specialize in the transcription of specific gene sub-sets. Thus, it is well known that polymerase I is concentrated in nucleoli where it transcribes repeated rDNA cistrons [42,43], but what of the nucleoplasmic polymerases? We can envisage two types of organization. In one, a cluster of polymerase II molecules forms a factory that transcribes only class II units; an analogous cluster of polymerase III molecules would work only on class III units. In the other, one factory might contain both types of enzyme to generate both types of transcript. Various studies indicate the former applies, and the most convincing exploits the steric hindrance that occurs between immunolabelling probes [44]. An anti-polymerase II antibody blocks access to RNA being made by polymerase II, but not to polymerase III protein or its transcripts; conversely, anti-polymerase III blocks access to RNA being made by polymerase III, but not to polymerase II protein or its transcripts.

But do factories specialize even further? Various types of evidence indicate they do. [For reviews, see [45,46].] For example, 3C shows that transcribed regulators (i.e., the globin and T_H2 LCRs, *H19*, the H enhancer) and their targets are often together (above) — presumably in the appropriate factories. Similarly, *MYC* and *IGH* (on chromosomes 15 and 12) are the most frequent translocation partners in plasmacytoma and Burkitt lymphoma, and when B cells are activated the two are recruited to the same factory — presumably again in one specializing in transcribing immediate early genes [47].

New, and decisive, evidence comes from a systematic analysis using minichromosomes [48]. The approach (Fig. 2A) was to co-transfect plasmids encoding different transcription units and an origin of replication into monkey cells. Plasmid DNA is assembled into nucleosomes, and the resulting minichromosomes are replicated and transcribed by the host's machinery. By 24 h there are ~200 minichromosomes in each of ~20 factories per transfected cell. In the example shown in Fig. 2B, one plasmid has the CMV promoter driving *DsRed*, the other the *U2* promoter driving a marked *U2* snRNA gene (i.e., *U2G*); both units are transcribed by polymerase II. RNA FISH shows there to be considerable amounts of *DsRed* RNA – but not *U2G* RNA – in the cytoplasm of the transfected cell on the left. This is expected; "standard" messages are exported to accumulate in the cytoplasm, while *U2G* transcripts are detected using a probe complementary to rapidly-degraded sequences. Nuclear signal is again seen in ~20 foci against a diffuse background. Foci (insets) mark nascent RNA at synthetic sites, while the diffuse pool represents completed transcripts on the way to the cytoplasm. Individual foci contain either (nascent) *DsRed* or *U2G* RNA — but not both (Fig. 2B, insets). Quantitative analysis confirms that red foci rarely contain green signal, and *vice versa* (Fig. 2C, pair 4). Clearly, CMV and U2 units are targeted to different factories (consistent with possibility iii in Fig. 2A). Analogous experiments show polymerases I/III promoters target minichromosomes to different factories from the CMV promoter (Fig. 2C, pairs 2,3,5), while identical promoters target minichromosomes to the same factories (pair 1). Introns can also direct minichromosomes to special factories – presumably ones involved in splicing (pairs 1 and 5). 3C confirms that minichromosomes with similar promoters lie closer together, and that *U2G* units share factories with host *U2* units.

The above experiments also provide good evidence that active minichromosomes with similar promoters pair (Fig. 3A).

5. Chromosomes that pair are transcriptionally active

It is a remarkable fact that chromosomes only pair when transcriptionally active [7,9]. Compare, for example, mitotic and meiotic chromosomes. During mitosis, chromosomes condense, lose transcriptional activity, but do not pair (although vestiges of any preexisting pairing may be retained); in prophase I of meiosis, chromosomes condense but retain transcriptional activity — and pair. This correlation between activity and pairing is carried over into interphase in some somatic cells: in *Drosophila* embryos, homologs only pair when transcription begins at the mid-blastula transition [1], and – later – giant polytene chromosomes are both active and paired. Significantly, the first meiotic pairing sequence to be mapped precisely turned out to be the key transcriptional element – a promoter – with the copy number of that promoter determining the degree of pairing [9]. Another well-characterized mitotic pairing site is the transcriptional regulator of the *Drosophila* brown locus [49]. And as we have seen, similar minichromosomes pair when transcribed in the same specialized factories (Fig. 2).

Mammalian chromosomes in somatic cells can also pair. For example, homologous α- and β-globin genes frequently associate, again only when active [50,51]. The mouse X-inactivation centre (*Xic*) provides another example; it encodes several non-genic units (e.g., *Tsix*, *Xite*, *Xist*) that control the random inactivation of one of the two X chromosomes in a female [52]. On the active X (X_a), transcription of *Xite* activates the linked *Tsix* allele, repressing anti-sense *Xist*. On the inactive X (X_i), *Xist* is transcribed, to generate transcripts that spread bidirectionally along the chromosome to promote inactivation. Female mouse ES cells recapitulate the process that establishes this state. Initially both Xs express *Tsix* (highly) and *Xist* (poorly). But at the stage when one chromosome is chosen for inactivation, the poorly-expressed *Xist* becomes more active and the two Xs pair transiently [37,53]. Pairing depends on active promoters, as deleting *Xite* and *Tsix* perturbs pairing, while adding back just the *Tsix* promoter restores it [53,54]. Moreover, the *Tsix* or *Xite* promoter inserted into an autosome pairs with a normal X [37]. Significantly, transcriptional inhibitors block X:X pairing, indicating that the act of transcription promotes pairing [55]. [We can also imagine that binding of *Xist* and *Tsix* on different chromosomes to one factory activates some switch that allows transcription in a mutually exclusive manner — and so eventual inactivation of one or other chromosome.]

This correlation with active transcription is consistent with base pairing between nascent sense and anti-sense transcripts being a driver of homolog pairing. Simultaneous transcription of opposite strands on the two homologs could generate nascent RNAs still tethered through their polymerases, and base pairing between the two transcripts would then stabilize template pairing [8]. But this correlation is also consistent with another alternative.

6. A model for chromosome pairing

We suggest two kinds of interaction drive pairing: a less-specific one between the many nucleosomes, and a more-specific one between fewer promoters and specialized factories [7]. Each chromatin fibre in the haploid set has a unique array of transcription units strung along it, and – depending on which units are active – it will self-assemble into a unique string of specialized factories surrounded by their clouds of loops (e.g., 1^m and 2^m in Fig. 3B). Only homologs will share similar arrays (i.e, 2^m and 2^p in Fig. 3B). Some clouds will be richer in long heterochromatic loops, others in shorter active loops. As nucleosomes in inactive regions tend to aggregate into heterochromatin, any contacts generated by Brownian motion will be stabilized transiently if participants carry similar heterochromatic histone codes. As illustrated in Fig. 3B, productive binding of promoters to appropriate factories will generate more lasting attachments, and binding to a homologous factory will transiently tie the two arrays together for as long as transcription of the relevant units continues. This gives time for adjacent loops to aggregate/attach, and – in turn –

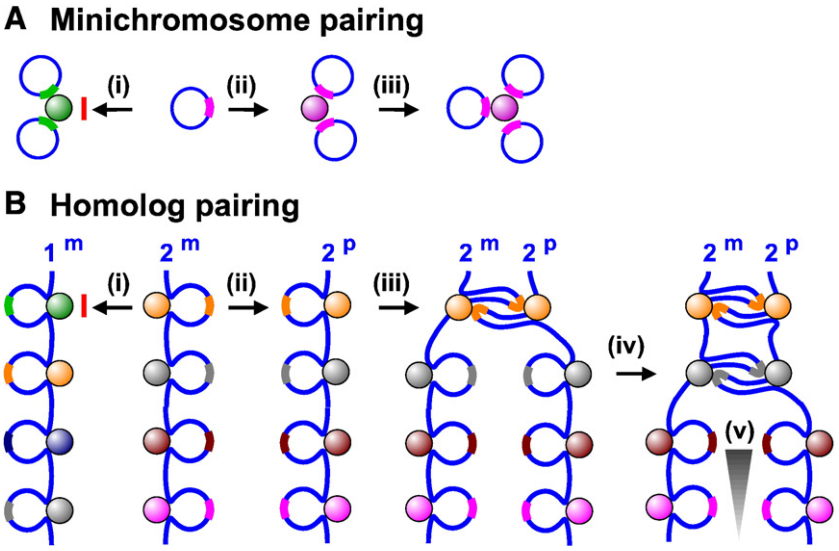

Fig. 3. Transcription-driven chromosome pairing. (A) Minichromosome pairing. (i) The minichromosome cannot bind to the green factory, as it has an inappropriate (purple) promoter. (ii). However it can bind to and initiate in a factory with the appropriate machinery (i.e., with a similar colour); as a result, it pairs with two similar minichromosomes. Adapted from [48]. (B) Homolog pairing during meiosis. At the stage when homologs pair, the partially condensed chromosomes are transcriptionally active. Each chromosome in the haploid set will then possess a unique array of active transcription units organized into a unique string of specialized factories and associated clouds running from telomere to telomere; only the homolog possesses a similar array. [If a human cell contains only 10 different types of specialized factory and 8000 factories, a string of 4 can be enough to specify position uniquely within the genome (as $10^4 > 8000$).] Here, only one of the many loops associated with a factory is shown. (i) The orange promoter on maternal chromosome 2 (2^m) is unlikely to bind to the green factory on maternal chromosome 1 (1^m). (ii) Just as a specialized factory facilitates pairing between minichromosomes bearing similar transcription units, correct pairing begins when the orange (2^m) promoter binds to the orange factory organized mainly by its homolog (2^p) – and/or *vice versa*. (iii) Once transcription of the orange promoter on 2^m begins in the factory in the 2^p string (and/or *vice versa*), 2^m and 2^p become temporarily tethered together through one of the tightest non-covalent interactions known — the association of an engaged polymerase with its template. (iv) This increases the chances that the grey promoter on 2^m will initiate in the grey factory on 2^p. (v) As the brown promoter initiates in the homologous brown factory, and the pink promoter in the homologous pink factory, 2^m and 2^p will become zipped together (grey arrowhead). Homologous arrays are shown as identical but will differ slightly, as loops attach and detach and factories split and fuse. Modified from [7].

this will make it both less likely that Brownian motion will separate the two clouds and more likely the two arrays will be zipped together.

7. Transvection

We have seen how transcription drives pairing, but pairing can occasionally affect transcription. In 1954, Lewis applied the term "transvection" to the complementation seen when two alleles of the bithorax complex of *Drosophila* were paired, but which was lost when they were separated [56]. Fig. 4 illustrates how we imagine that enhancers work, and how an enhancer on one chromosome might act upon a promoter on a homolog — but only when homologs are paired [23,57].

8. Concluding remarks

We have argued that interactions between promoters and the appropriate transcription factories drive pairing between homologs. This model has several advantages. First, the number of sites to be scanned for homology is reduced. For example, an initial scan of the human genome with a base-pairing probe requires $>10^9$ interactions, but here a few tens of thousands of promoters must scan $\sim 10^4$ factories (i.e., the number of nucleoplasmic factories; [28]). Moreover, the homology scan is not defeated by repeats, which are often packaged into inert heterochromatin. Second, the proposed interactions – between transcription units and the polymerizing machinery – are well-characterized. Third, it is a general but economic model. Fourth, it is testable; for example, pairing sequences must be transcribed in the same factory, and point mutations in the relevant promoters should disrupt pairing. Of course, other interactions will augment the ones discussed here. Some of these may be non-specific — for example involving entropic forces between heterochromatic clumps [58]; others may be specific — for example, involving DNA:DNA or RNA:RNA kissing, or particular pairing sites (e.g., telomeres in yeast, the homolog recognition region in the worm, and centromeric heterochromatin in the fly) and/or proteins [10].

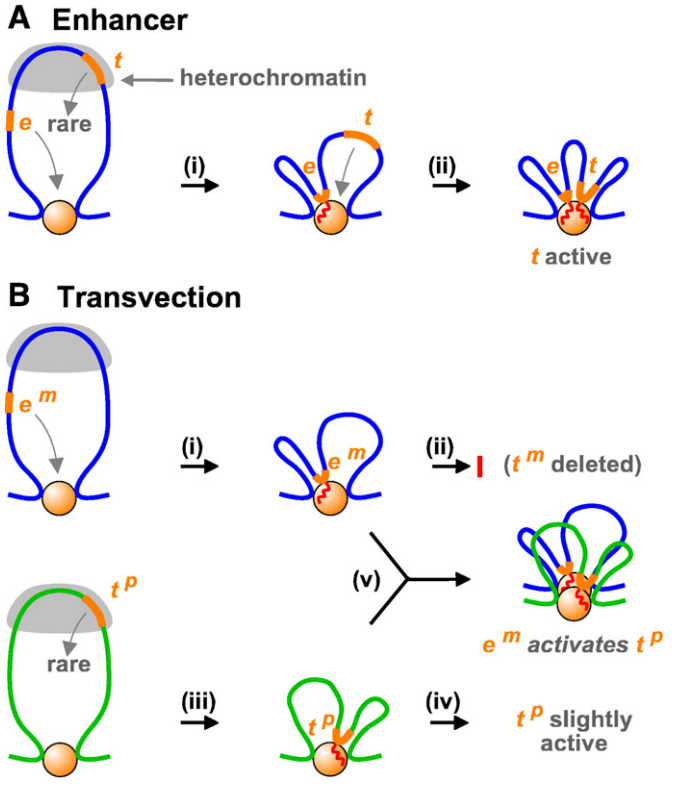

Fig. 4. Action at a distance. (A) An enhancer and target gene (embedded in heterochromatin) on the same chromosome. An enhancer (*e*) is tethered closer to a factory than its target (*t*), and so is more likely to contact the factory. [See [61] for how position in a loop affects contacts with a factory.] (i) Like many enhancers, this one encodes a promoter; when this initiates, *t* is inevitably dragged out of heterochromatin to increase its chances of contacting the factory. (ii) Both *e* and *t* are now in molecular contact in the factory, enhancing transcription of *t*. Modified from [60] with permission. (B) Transvection between homologs. The maternal homolog (above) encodes an enhancer (e^m), but has lost the gene (t^m). (i) e^m can still initiate in the factory, but (ii) as t^m is deleted no transcripts can ever be produced. The paternal homolog (below) has lost the enhancer (e^p). Distant t^p rarely contacts the factory to (iii) initiate, and so (iv) is transcribed rarely. (v) If homologs pair (middle), e^m can contact t^p in the same (fused) factory; and so enhance transcription of t^p. Modified from [7].

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