Mini-Review

Transvection, Nuclear Structure, and Chromatin Proteins

C.-ting Wu

Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, Massachusetts 02115

RESEARCHERS are inventing and honing the technologies to provide increasingly clear pictures of the organization of the nucleus. One driving issue is the role of chromosome arrangement in gene expression. The intractable nature of this question lies in the fact that it is not yet possible to extract a "chromosome arrangement" and assay it for function. Instead, it must be observed in situ and correlated with patterns of gene expression. Today, as exemplified by Hiraoka et al. (1993) in this issue of the *Journal of Cell Biology*, as well as by others (for example, Selig et al., 1992; reviewed by Manuelidis, 1990; Trask, 1991; Jackson, 1991), we are equipped with a level of resolution that allows the positioning of each gene on a temporal and spatial map of the nucleus.

Hiraoka et al. (1993) focus on that magical moment in *Drosophila* development when the syncytial blastoderm undergoes cellularization and the embryo launches itself full force into zygotic gene expression. By following the two histone loci situated on their homologous chromosomes, the authors demonstrate two striking changes in chromosome arrangement: (*a*) the loci pair, implying the onset of pairing for all homologous chromosomes, and (*b*) the loci move from the center of the nucleus toward the apically positioned centromeres, apparently due to the condensation of heterochromatin flanking the centromere. These observations beckon us to that phenomenon called transvection and the possibility that chromosome structure can drive developmental decisions.

Transvection

For nearly 50 years after homologous chromosomes were found to be paired in the somatic cells of *Dipteran* insects such as *Drosophila*, researchers mused over the possibility that pairing influences gene expression. In 1954, Lewis crystallized a large body of work into the term "transvection," implying that yes, gene expression can be altered depending upon whether or not genes are paired with their homologue. He illustrated transvection by showing how complementation between two alleles of the bithorax gene complex can be antagonized by disruptions of somatic pairing. Since then, the number of loci exhibiting transvection effects in *Drosophila* has grown significantly. Keeping pace with the number of loci, is the number of models evoked to explain transvection (reviewed by Judd, 1988; Ashburner, 1989; Wu and Goldberg, 1989; Pirrotta, 1990; Pirrotta, 1991; Tartof and Henikoff, 1991; also Micol et al., 1990; Kassis et al., 1991; Hazelrigg and Petersen, 1992). As has been frequently noted, the mechanism of transvection may differ from locus to locus. Only two examples of transvection and only a subset of the models are mentioned here.

Fig. 1 illustrates transvection at the yellow gene of *Drosophila* (Geyer et al., 1990). The y^2 and y^{59b} mutations cause abnormal yellow pigmentation. y^2 is caused by the insertion of a transposable element between the promoter and two enhancers, and y^{59b} is a derivative of y^2 that lacks part of the transposable element as well as the promoter and first exon. Surprisingly, y^2 is complemented by y^{59b}. Furthermore, substantial amounts of wild-type sized transcripts can be detected in y^2/y^{59b} pupae while this is not the case for y^2/y^2 or y^{59b}/y^{59b} pupae. The authors implicate transvection by showing that complementation is negated if the two alleles are not allowed to pair. They propose that transcription factors that have been attracted to the y^{59b} enhancers act upon the y^2 promoter by tracking or DNA looping. Similar models evoking looping and/or the intermolecular action of bound or free regulatory factors have been raised to explain transvection at other loci (see reviews).

Transvection is also involved in the interaction of the *zeste*^{1} (z^1)[1] mutation and the white gene (reviewed by Wu and Goldberg, 1989; Pirrotta, 1991; also Chen et al., 1992; Laney and Biggin, 1992). The white gene is important for the red pigmentation of the eyes of the fly. When a white gene is paired with another white gene, it can be repressed in a mutant z^1 background such that the eyes become yellow. An unpaired white gene escapes repression. The zeste protein can bind DNA, alter transcription, as well as self-aggregate. This has led to the suggestion that the mutant z^1 protein represses by binding white and then aggregating to an excessive degree, perhaps doing so more efficiently when white genes are paired.

Mutations of zeste affect three other loci that exhibit transvection effects, although these loci differ in their responses to the different kinds of zeste mutations (reviewed by Tartof and Henikoff, 1991). Zeste protein has therefore been proposed to act generally, holding DNA segments together either intramolecularly during looping or intermolecularly during transvection. These ideas are being tested (reviewed by Pirotta, 1991; also Qian et al., 1992).

Is it possible that the paired state of genes plays a dynamic role in regulation? For example, a gene might be regulated by being switched between two states (Fig. 2): (*a*) the linearly locked state where the accessibility of DNA to

1. *Abbreviations used in this paper*: Psc, Posterior sex comb; z^1, *zeste*^{1}.

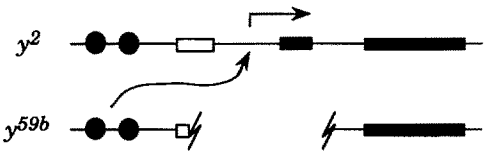

Figure 1. Transvection at yellow. The intact transposable element (□) of y^2 inhibits the action of the enhancers (●) on the promoter *in cis*. The y^2 promoter can be activated (*curved arrow*) *in trans* by a paired y^{59b} which, because of a deletion of its promoter and an exon (■), cannot produce transcripts on its own (not to scale; adapted from Geyer et al., 1990).

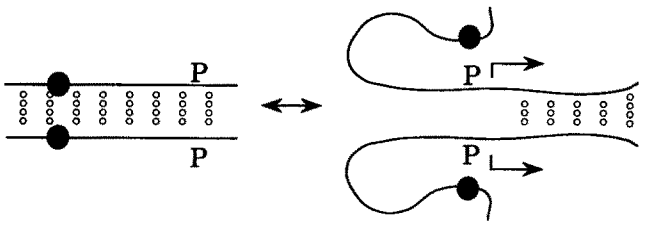

Figure 2. Linear locking and looping. Here, pairing (○) locks enhancers (●) away from promoters (*P*), while looping brings enhancers to promoters.

regulatory factors is reduced and enhancers are locked away from the promoter, and (*b*) the looped state where intramolecular looping can stimulate expression. In this view, mutant z^1 protein represses paired white genes by restraining them in the linearly locked state, while an unpaired white gene escapes repression because it cannot be linearly locked. The different responses of loci to the various types of zeste mutations might imply that the consequences of the linear and looped states differ from locus to locus. In some cases, linearity might correspond to the gene-activated state. Such a model could also apply intramolecularly.

Does Transvection Occur Outside Drosophila?

The larger phenomenon that transvection represents, communication between alleles including autoregulation, transsensing, and some epigenetic events, is not unique to *Drosophila* (for example, see Jorgensen, 1990; Monk, 1990; Tartof and Henikoff, 1991). Whether the specific mechanism of transvection, which involves gene pairing, occurs elsewhere remains a tantalizing possibility (Tartof and Henikoff, 1991). Most recently, Sabl and Laird (1992), Tsai and Silver (1991), and Bollmann et al. (1991) have proposed transvection-like bases for Huntington's chorea in humans, expression of the T-associated maternal effect locus in mouse, and the semi-dominance of a mutant nivea allele in snapdragon, respectively. Evidence for somatic pairing outside Diptera, including plants and vertebrates, also exists (reviewed by Grell, 1969). For example, Arnoldus et al. (1991) have found evidence for tissue specific somatic pairing in humans, suggesting that pairing is not only developmentally regulated but a form of regulation in itself.

Is Transvection Essential?

Transvection may not be essential for viability since chromosome rearrangements in *Drosophila*, including deletions, generally do not result in lethality, and haploid patches of tissue survive to adulthood (Santamaria, 1983). In fact, it is possible that transvection is not only nonessential, it is undesirable, and *Drosophila* has evolved mechanisms to prevent rampant communication between paired homologues. This view is consistent with the benign effects of rearrangements and predicts that transvection will not be apparent unless the blockade is removed by, for example, specific kinds of mutations. Transvection at yellow is consistent with this proposal. Geyer et al. (1990) found that alleles with a dysfunctional promoter are able to complement y^2 while those that disrupt yellow posttranscriptionally fail. Apparently, *cis* activity of yellow enhancers precludes their action *in trans*.

This train of thought leads us to ask why transvection exists at all. The trivial response is that we are mistaken and have failed to find the circumstances under which it is required. Alternatively, transvection may exist because it confers advantages by, for example, permitting complementation that is otherwise not possible (also Zachar et al., 1985; Monk, 1990). Finally, transvection may exist because it uses factors that participate in a mechanism that is essential and also involves gene pairing. In fact, all genes of all organisms experience pairing at least once per cell cycle. This occurs when they pass through the replication fork and are not only near their replicated sister but are exchanging information with it. Such proximity has also been proposed to mediate replication control (Roberts and Weintraub, 1986; Abeles and Austin, 1991; Kittell and Helinski, 1991). Is it possible that some molecules of the replication machinery moonlight as modulators of transvection outside the confines of the replication fork?

Gene pairing also occurs in *Drosophila* tissues that harbor polytene chromosomes or undergo gene amplification. As proposed by Ashburner (1977), transvection could be the reenactment between homologues of the essential process that assures the uniform activation of genes within a single polytenized chromosome.

When and Where Does the Critical Pairing Event Occur?

While many models propose the crucial pairing event to occur at transcription, other possibilities should be kept in mind when new cases of transvection are being considered. For instance, the evidence for nuclear compartmentalization (for example, see Carter et al., 1991; Leonhardt et al., 1992 and references within; reviewed by Manuelidis, 1990; Jackson, 1991) and the restriction of transcripts to nuclear "tracks" (Xing and Lawrence, 1991) lend plausibility to a proposal that transvection can occur posttranscriptionally by *trans*-splicing (Judd, 1979). If transcripts are clustered in the nucleus, they will enter the cytoplasm as a cluster, and subsequent translation may then result in a local high concentration of product in the cytoplasm, or in the nucleus if the proteins are shuttled back. If the activity of the products is concentration dependent, the consequence of homologue pairing may be realized only posttranslationally.

The critical pairing event may also occur before transcription if the paired, or unpaired, state can be imprinted such that when genes express themselves later, they do so with the memory of having been paired or not. For example, transvection may occur at the time of replication. If pairing of homologous genes results in paired replication forks, then the side by side arrangement of two replication machineries may

permit the cross-feeding of information from one replication fork to another in a way that allows factors, base modifications, or chromatin structure to be transferred from one replicating gene to its homologue, or to be coordinately determined by them. Because imprinting, however accomplished, could endure cycles of replication, the consequences of transvection might persist long after the critical pairing event, as has been proposed by Sabl and Laird (1992).

Zachar et al. (1985) also focus on events before transcription and propose that pairing functions to colocalize genes to the proper nuclear compartment. If diploidy or multigene families impose constraints on compartmentalization, somatic pairing certainly would be an elegant solution. With the technologies pioneered by Hiraoka et al. (1993) and others, it should now be possible to determine whether, in the absence of somatic pairing, homologous genes are drawn to the same compartment by other means and are therefore effectively paired, or whether compartments are duplicated. Alternatively, genes may be imprinted by passage through a compartment so that there is no need for homologues to reside simultaneously in one.

Chromatin Proteins

Because transvection causes genes to be modulated by the proximity of their homologues, it can be used to identify loci in *Drosophila* that establish chromatin structure. Thus far, two genes that can be mutated to become modifiers of z^1 eye color have been found to encode chromatin proteins. These are Posterior sex combs (Psc) and Suppressor 2 of zeste [Su(z)2]. The two genes share a region of homology which is also conserved in mammals, highlighting the implication of these studies for a general understanding of chromatin (reviewed by Pirrotta, 1991; van Lohuizen et al., 1991; Brunk et al., 1991; Martin and Adler, 1993).

Psc also belongs to a class of homeotic genes called the Polycomb group, which regulates two gene complexes, bithorax and Antennapedia. Both these complexes are graced with transvection (Lewis, 1954; Pattatucci and Kaufman, 1991). Two Polycomb group genes in addition to Psc encode proteins that act at the level of chromatin. These are Polycomb (Pc) and polyhomeotic (ph). It now appears that the products of Psc, Su(z)2, Pc, and ph work in concert to establish chromatin structure (Franke et al., 1992 and references within; Martin and Adler, 1993).

Enhancer of zeste [E(z)] also falls in the overlap of the modifiers of z^1 eye color and the Polycomb group (Jones and Gelbart, 1990; Phillips and Shearn, 1990). E(z) mutant tissues that should be undergoing cell proliferation show few and abnormal mitotic figures, underlining the possibility that proteins mediating transvection may participate in other whole chromosome processes at the level of chromatin. These processes might include chromosome condensation, segregation, replication, recombination, stability, amplification, and dosage compensation.

The Heterochromatin Connection

When euchromatic genes are rearranged to be near heterochromatin, they frequently show variegated expression. The opposite is also true. Heterochromatic genes can variegate when rearranged to lie near euchromatin (reviewed by Spradling and Karpen, 1990; Reuter and Spierer, 1992). Explanations for this position-effect variegation have invoked the spreading of chromatin or other structures *in cis* across the heterochromatin/euchromatin boundary. Reminiscent of transvection, spreading *in trans* between paired homologues (reviewed by Tartof and Henikoff, 1991), or even between nonhomologous chromosome segments (Wakimoto and Hearn, 1990) has also been invoked.

Numerous enhancers and suppressors of position-effect variegation exist, and several identify members of the Polycomb group or another class of homeotic genes called the trithorax group (reviewed by Paro, 1990; Reuter and Spierer, 1992). Once again, Psc falls into the overlap (D. A. Sinclair, N. J. Clegg, T. A. Grigliatti, and H. W. Brock, manuscript submitted for publication). The similarities between the modifiers of position-effect variegation and the Polycomb group genes are further emphasized by a region of homology that is shared between the products of Pc and Su(var)205, a modifier encoding the heterochromatin-associated protein HP1. This homology is conserved in plants and mammals as well (Franke et al., 1992 and references within).

The biologies of transvection, chromatin, and position-effect variegation are drawing together. In this light, it is exciting that Hiraoka et al. (1993) find homologous chromosomes initiating pairing at the same time that heterochromatin becomes condensed. The appearance of heterochromatin at the syncytial/cellular blastoderm transition has prompted researchers to ask whether such a structural change may play a role in gene regulation (Pimpinelli et al., 1985). Now researchers can further muse over the potential implications of the coincidence of two major changes in chromosome structure at this critical time in development.

The author thanks her generous colleagues, in particular George Church and Vince Pirrotta, for years of inspirational conversations on transvection. In the interest of brevity, many primary articles were not cited, and apologies are extended to the authors.

Received for publication 6 November 1992 and in revised form 24 November 1992.

References

Abeles, A. L., and S. J. Austin. 1991. Antiparallel plasmid-plasmid pairing may control P1 plasmid replication. *Proc. Natl. Acad. Sci. USA.* 88: 9011–9015.

Arnoldus, E. P. J., I. A. Noordermeer, A. C. B. Peters, A. K. Raap, and M. van der Ploeg. 1991. Interphase cytogenetics reveals somatic pairing of chromosome 17 centromeres in normal human brain tissues, but no trisomy 7 or sex-chromosome loss. *Cytogenet. Cell Genet.* 56:214–216.

Ashburner, M. 1977. Happy birthday - puffs! *Chromosomes Today.* 6:213–222.

Ashburner, M. 1989. *Drosophila: A Laboratory Handbook.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 915–929.

Bollmann, J., R. Carpenter, and E.S. Coen. 1991. Allelic interactions at the nivea locus of Antirrhinum. *Plant Cell.* 3:1327–1336.

Brunk, B. P., E. C. Martin, and P. N. Adler. 1991. Drosophila genes Posterior sex combs and Suppressor two of zeste encode proteins with homology to the murine bmi-1 oncogene. *Nature (Lond.).* 353:351–353.

Carter, K. C., K. L. Taneja, and J. B. Lawrence. 1991. Discrete nuclear domains of poly(A) RNA and their relationship to the functional organization of the nucleus. *J. Cell Biol.* 115:1191–1202.

Chen, J. D., C. S. Chan, and V. Pirrotta. 1992. Conserved DNA binding and self-association domains of the Drosophila zeste protein. *Mol. Cell. Biol.* 12:598–608.

Franke, A., M. DeCamillis, D. Zink, N. Cheng, H. W. Brock, and R. Paro. 1992. Polycomb and polyhomeotic are constituents of a multimeric protein complex in chromatin of *Drosophila melanogaster*. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:2941–2950.

Geyer, P. K., M. M. Green, and V. G. Corces. 1990. Tissue-specific transcriptional enhancers may act *in trans* on the gene located in the homologous chromosome: the molecular basis of transvection in Drosophila. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2247–2256.

Grell, R. F. 1969. Meiotic and somatic pairing. In *Genetic Organization*, E. W. Caspari, and A. W. Arnold, editors. Academic Press, New York, 361–492.

Hazelrigg, T., and S. Petersen. 1992. An unusual genomic position effect on Drosophila white gene expression: Pairing dependence, interactions with zeste, and molecular analysis of revertants. *Genetics.* 130:125–138.
Hiraoka, Y., A. F. Dernburg, S. J. Parmelee, M. C. Rykowski, D. A. Agard, and J. W. Sedat. 1993. The onset of homologous chromosome pairing during *Drosophila melanogaster* embryogenesis. *J. Cell Biol.* 120:591–600.
Jackson, D. A. 1991. Structure-function relationships in eukaryotic nuclei. *BioEssays.* 13:1–10.
Jones, R. S., and W. M. Gelbart. 1990. Genetic analysis of the Enhancer of zeste locus and its role in gene regulation in *Drosophila melanogaster*. *Genetics.* 126:185–199.
Jorgensen, R. 1990. Altered gene expression in plants due to trans interactions between homologous genes. *Trends Biotechnol.* 8:340–344.
Judd, B. H. 1979. Allelic complementation and transvection in *Drosophila melanogaster*. *ICN-UCLA Symp. Mol. Cell Biol.* 15:107–115.
Judd, B. H. 1988. Transvection: allelic cross talk. *Cell.* 53:841–843.
Kassis, J. A., E. P. VanSickle, and S. M. Sensabaugh. 1991. A fragment of engrailed regulatory DNA can mediate transvection of the white gene in Drosophila. *Genetics.* 128:751–761.
Kittell, B. L., and D. R. Helinski. 1991. Interon inhibition of plasmid RK2 replication *in vitro*: Evidence for intermolecular coupling of replication origins as a mechanism for RK2 replication control. *Proc. Natl. Acad. Sci. USA.* 88:1389–1393.
Laney, J. D., and M. D. Biggin. 1992. Zeste, a nonessential gene, potently activates Ultrabithorax transcription in the Drosophila embryo. *Genes Dev.* 6:1531–1541.
Leonhardt, H., A. W. Page, H. -U. Weier, and T. H. Bestor. 1992. A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. *Cell.* 71:865–873.
Lewis, E. B. 1954. The theory and application of a new method of detecting chromosomal rearrangements in *Drosophila melanogaster*. *Am. Nat.* 88:225–239.
Manuelidis, L. 1990. A view of interphase chromosomes. *Science (Wash. DC).* 250:1533–1540.
Martin, E. C., and P. N. Adler. 1993. The Polycomb group gene Posterior sex combs encodes a chromosomal protein. *Development (Camb.).* In press.
Micol, J. -L., J. E. Castelli-Gair, and A. García-Bellido. 1990. Genetic analysis of transvection effects involving *cis*-regulatory elements of the Drosophila Ultrabithorax gene. *Genetics.* 126:365–373.
Monk, M. 1990. Variation in epigenetic inheritance. *Trends Genet.* 6:110–114.
Paro, R. 1990. Imprinting a determined state into the chromatin of Drosophila. *Trends Genet.* 6:416–421.
Pattatucci, A., and T. C. Kaufman. 1991. The homeotic gene Sex combs reduced of *Drosophila melanogaster* is differentially regulated in the embryonic and imaginal stages of development. *Genetics.* 129:443–461.
Phillips, M. D., and A. Shearn. 1990. Mutations in polycombeotic, a Drosophila Polycomb-group gene, cause a wide range of maternal and zygotic phenotypes. *Genetics.* 125:91–101.
Pimpinelli, S., W. Sullivan, M. Prout, and L. Sandler. 1985. On biological functions mapping to the heterochromatin of *Drosophila melanogaster*. *Genetics.* 109:701–724.
Pirrotta, V. 1990. Transvection and long-distance gene regulation. *BioEssays.* 12:409–414.
Pirrotta, V. 1991. The genetics and molecular biology of zeste in *Drosophila melanogaster*. *Adv. Genet.* 29:301–348.
Qian, S., B. Varjavand, and V. Pirrotta. 1992. Molecular analysis of the zeste-white interaction reveals a promoter-proximal element essential for distant enhancer-promoter communication. *Genetics.* 131:79–90.
Reuter, G., and P. Spierer. 1992. Position effect variegation and chromatin proteins. *BioEssays.* 14:605–612.
Roberts, J. M., and H. Weintraub. 1986. Negative control of DNA replication in composite SV40-bovine papilloma virus plasmids. *Cell.* 46:741–752.
Sabl, J. F., and C. D. Laird. 1992. Epigene conversion: a proposal with implications for gene mapping in humans. *Am. J. Hum. Genet.* 50:1171–1177.
Santamaria, P. 1983. Analysis of haploid mosaics in Drosophila. *Dev. Biol.* 96:285–295.
Selig, S., K. Okumura, D. C. Ward, and H. Cedar. 1992. Delineation of DNA replication time zones by fluorescence in situ hybridization. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:1217–1225.
Spradling, A. C., and G. H. Karpen. 1990. Sixty years of mystery. *Genetics.* 126:779–784.
Tartof, K. D., and S. Henikoff. 1991. Trans-sensing effects from Drosophila to humans. *Cell.* 65:201–203.
Trask, B. J. 1991. Fluorescence *in situ* hybridization: applications in cytogenetics and gene mapping. *Trends Genet.* 7:149–154.
Tsai, J. -Y., and L. M. Silver. 1991. Escape from genomic imprinting at the mouse *T-associated maternal effect (Tme)* locus. *Genetics.* 129:1159–1166.
van Lohuizen, M., M. Frasch, E. Wientjens, and A. Berns. 1991. Sequence similarity between the mammalian bmi-1 proto-oncogene and the Drosophila regulatory genes Psc and Su(z)2. *Nature (Lond.).* 353:353–355.
Wakimoto, B. T., and M. G. Hearn. 1990. The effects of chromosome rearrangements on the expression of heterochromatic genes in chromosome 2L of *Drosophila melanogaster*. *Genetics.* 125:141–154.
Wu, C. -t., and M. L. Goldberg. 1989. The Drosophila zeste gene and transvection. *Trends Genet.* 5:189–194.
Xing, Y., and J. B. Lawrence. 1991. Preservation of specific RNA distribution within the chromatin-depleted nuclear substructure demonstrated by *in situ* hybridization coupled with biochemical fractionation. *J. Cell Biol.* 112:1055–1063.
Zachar, Z., C. H. Chapman, and P. M. Bingham. 1985. On the molecular basis of transvection effects and the regulation of transcription. *Cold Spring Harbor Symp. Quant. Biol.* 50:337–346.