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Random Walking

Can Large Insertions and Deletions between Genes Affect Development?

In a "News and Views" article published in Nature, Bruce Alberts and Rolf Sternglanz (1990) address the matter of inhibition of gene transcription through compaction of deoxyribonucleoprotein. This has for a long time been a recurrent theme in developmental molecular biology. It was first suggested by cytologists that in regards to compaction different sectors of the genome behave differently in different cells and at different times of development (Gersh 1973). The potential importance in this respect of sectorial variations in DNA sequence motifs and base composition has been pointed out (Zuckerkandl 1988). The data in particular of Daneholt and his associates (Björkroth et al. 1988) on Balbiani rings, the oversized puffs in polytene chromosomes of *Chironomus*, signal a probable inverse correlation between rates of transcription and degrees of compaction.

Compaction of chromatin has been implicated in the mechanism of position effect variegation. In a fraction of the cells in a tissue, said to be variegating, a group of genes can be inhibited after being transposed into the immediate neighborhood of heterochromatin, a particularly compacted form of chromatin. There has been a recently regained interest in this process of variegated gene expression. The process is characterized by a directional "spreading effect" of transcriptional inhibition, in which genes proximal to the insertion point in a translocation are more frequently inactivated than more distal genes (Spofford 1976). According to Alberts and Sternglanz (1990), position effect variegation may hinge upon a highly cooperative "crystallization" event, "which is nucleated from special chromosomal sites and then spreads along the chromosome to take in hundreds of kilobases of DNA." This concept dates back many years, since it was pointed out in 1974 that "a heterochromatic region . . . is considered to be a center of nucleation of its own . . . structure, something like a crystal seed that is able to induce adjacent regions of euchromain to co-crystallize" (Zuckerkandl 1974). The co-crystallization was then attributed to the action of unknown locking molecules that lock in a high-order chromatin structure. The nature of some such molecules has been determined (Blumenfeld et al. 1978; Hsieh and Brutlag 1979; Moore et al. 1979; Strauss and Varshavsky 1984; James and Elgin 1986; Eissenberg 1989). One of them possesses five specially spaced zinc fingers (Reuter et al. 1990). The observed correlation between the amount of cellular heterochromatin and the extent of variegation was explained by a competition between heterochromatic regions for the locking macromolecules, namely by mass action effects played out between heterochromatin and protein species binding to it (Zuckerkandl 1974). Recently, this concept was further developed by Locke et al. (1988). There appear to be a number of factors (Locke et al. 1988; Wustman et al. 1989) that contribute to heterochromatization.

Position effect variegation was conceived in 1974 (Zuckerkandl 1974) as being brought about by the spreading of certain DNA-binding proteins, starting from a presumably heterochromatic center of nucleation, as a function of DNA replication and therefore of developmental time. Alternatively, the initiator site may be a mobile element not related to satellite DNA (Tartof et al. 1984). Such a molecular spreading effect appeared potentially applicable to gene complexes whose member genes are transcriptionally activated or inactivated in the order of their occurrence on the chromosome, such as mammalian globin gene complexes, the bithorax complex in *Drosophila*, or the "constant" exons in complexes of mammalian immunoglobulin heavy chain genes. The concept implied directionally travelling DNA-bound proteins or protein modifications. As the physical progression of the macromolecular agent was presumed to be limited to the time of DNA replication, the concept offered in principle the basis for a *developmental molecular clock*. Essentially the same concept, minus perhaps the titration of the

"spreading" protein(s) by competing DNA, was later elaborated independently and was published by Stubblefield (1986).

In our discussions regarding the bithorax complex, which took place in 1978–1979 and again in 1983, Ed Lewis (1978) remained unconvinced of the above mechanisms, primarily because rearrangements that break up the complex do not necessarily alter the sequential activation of the genes. This was, however, not a compelling reason to abandon the hypothesis, as pointed out by Gary Struhl (personal communication; Struhl 1984), because the complex could include more than a single sector of origin of directionally spreading "transconformational" proteins (bringing about conformational change). The application of the concepts to globin gene complexes—an application for which I have been attempting to gather evidence since 1980 and recently again in collaboration with Morris Goodman, Dan Tagle, and Teni Boulikas—has so far not been convincing, because of the proportion of apparent exceptions to the case that we attempted to make. Nonetheless, the latest data bearing on the mechanism of hemoglobin switching in humans (Enver et al. 1990) appear to be compatible with the developmental clock hypothesis. The evidence can be taken to suggest that in the cell the upstream "locus activating region" (LAR) will activate promptly any proximal gene and will activate more distal genes only at later developmental stages—the later, the greater the distance from the LAR—irrespective of which globin genes are used in the LAR/globin gene constructs.

Further consideration may then be granted to the hypothesis stating that transcription in successive genes of a gene complex could in certain cases occur at developmental time intervals correlated with the physical distance between the genes along the chromosome. If such were the case, pseudogenes located within a gene complex would affect the developmental clock of the complex by increasing the distance between functional genes. Sizable insertions and deletions of noncoding sequences in the neighborhood of genes could play an evolutionary role in affecting the developmental timing of gene expression.

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