

Commentary

A rosy future for heterochromatin

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Perhaps the most striking and enigmatic aspect of genome organization in eukaryotes is the division of chromosomes into euchromatic and heterochromatic regions. Zhang and Spradling recently described in the *Proceedings* (1) a method for recovering marked transposable elements inserted in heterochromatic regions. Coupled with other current approaches, this method makes the molecular-genetic dissection of many heterochromatic regions and functions an attainable goal.

Heterochromatin Is Important

Approximately 15% of the human genome and 30% of the *Drosophila melanogaster* genome is heterochromatic (2). In *Drosophila*, the centric one-quarter to one-half of every major chromosome consists of heterochromatin, and the Y and fourth chromosomes are largely heterochromatic (3). Heterochromatin is distinguished from euchromatin by its paucity of genes, tightly compacted structure throughout the cell cycle, replication late in S phase, and high content of repetitive sequences (2). Furthermore, in dipteran polytene chromosomes, heterochromatic DNA copy number is underrepresented 64- to 1000-fold with respect to the euchromatin (4). The heterochromatin may be further subdivided into α - and β -heterochromatin. The "deep" or α -heterochromatin contains highly repeated satellite DNA, as well as "islands" of complex DNA (5, 6), but contains no known genes. β -Heterochromatin contains genes in addition to middle-repetitive DNA (7).

The repetitive nature of heterochromatin has led some to suggest that heterochromatin is merely "junk" DNA having no utility to the cell. However, many essential functions reside in heterochromatic regions, including the ribosomal genes. In *Drosophila*, genes required for viability (e.g., lethal mutable genes) and fertility (e.g., the Y-chromosome male fertility factors) are in heterochromatin (3). Furthermore, heterochromatic DNA is necessary for normal chromosome inheritance. In multicellular eukaryotes, centromeres are generally placed deep in α -heterochromatin (8). Likewise, heterochromatic sequences may be necessary for sister chromatid adhesion in mitotic and meiotic chromosomes (9). A number

of other inheritance functions defined in *Drosophila* also require heterochromatic sequences. In female meiosis, one system that ensures the disjunction of achiasmatic chromosomes utilizes centric heterochromatic sequences (ref. 10; H. Le and G.H.K., unpublished results), and in males, the meiotic pairing of the X and Y chromosomes requires the intergenic spacer of the ribosomal genes (11, 12). Heterochromatic sequences play critical roles in meiotic drive systems (Segregation Distortion and X-Y drive), characterized by the preferential, nonmendelian recovery of one chromosome over its homologue (13).

Removal of some genes [e.g., *light* (*lt*) and *rolled* (*rl*)] from their usual heterochromatic location prevents their normal expression (14, 15). Likewise, genes usually occupying a euchromatic position are repressed when placed in heterochromatin. The variable inactivation of both classes of genes that occurs when euchromatin and heterochromatin are abnormally juxtaposed is called position-effect variegation. Gene function may require the particular chromatin environment or nuclear position provided by the normal chromosomal location (16). An intriguing possibility is that position-effect variegation may be a consequence of the elimination of heterochromatic chromosomal regions from the cell during development (17).

Molecular-Genetic Analysis of Heterochromatin Is Difficult

Analysis of the structure and function of heterochromatic regions presents special problems not usually encountered in the study of euchromatin. The structure and function of a few single-copy heterochromatic genes [e.g., *lt* (18) and suppressor of forked *su(f)* (19)] have been investigated, but intensive molecular analysis of whole heterochromatic regions has only recently been attempted. It is difficult to dissect heterochromatic regions by classical genetic methods, because complete deletion of dispersed or clustered repeated arrays is required to observe mutant phenotypes. Furthermore, cytogenetic maps of heterochromatin lack the resolution of most euchromatic maps (3). The sequences of satellite DNA repeats and their proportions in the genome have

been well characterized, but their organization in heterochromatin is only roughly known (20).

The molecular organization of specific regions of centric heterochromatin can be analyzed by restriction mapping heterochromatic regions immediately adjacent to single-copy DNA. Entry points are provided by chromosome rearrangements that juxtapose cloned euchromatic sequences with heterochromatin. This approach has been used successfully to restriction map 1 megabase (Mb) of X centric α -heterochromatin in the minichromosome *Dp1187*, capitalizing on pulsed-field Southern analysis to expand the size of the region analyzed (refs. 5 and 6; G.H.K. and H. Le, unpublished results). Functional analyses of *Dp1187* deletion derivatives have also elucidated the size and composition of heterochromatic inheritance elements, including the centromere (T. Murphy, H. Le, and G.H.K., unpublished results). Similar approaches are being applied to map the centric heterochromatin of other *Drosophila* chromosomes (B. Wakimoto, personal communication; D. Wines and S. Henikoff, personal communication), but the general application of this method is limited by the availability of suitable chromosomal rearrangements and cloned euchromatic sequences.

In principle, transposon insertions could also be used as single copy entry points into heterochromatin. *P* element insertions have facilitated mapping of repetitive DNA (6), and useful heterochromatic deficiencies could be recovered by scoring for loss of the *P* element marker gene after mutagenesis (21). The barrier to using *P* elements in the analysis of centric heterochromatin has been the low frequency of recovery of heterochromatic insertions. Furthermore, the rare and sporadic heterochromatic insertions reported in the literature appear to be restricted to β -heterochromatic sites. Individual *P* elements have been localized to the fourth chromosome (22, 23) and to the telomeric region of the third chromosome (24, 25) after *P* element germ-line transformation, and, in a few cases, *P* insertions have been identified by non-complementation of existing heterochromatic mutations after mobilization of endogenous *P* elements (18, 19, 26). Genetic screens using single marked *P*

elements have generally failed to produce *P* insertions in centric heterochromatin. In a screen designed to recover transpositions from the X chromosome into the heterochromatin of *Dp1187*, a substantial number of *P* insertions displayed position-effect variegation of the *ry*⁺ (*ry*⁺) marker gene; however, all of the heterochromatic *P* insertions were in subtelomeric (β -like) heterochromatin (6). Two screens examining intrachromosomal or "local" transpositions within *Dp1187* demonstrated that *P* elements transpose to closely linked sites at an elevated frequency, but no insertions into the 1 Mb of centric heterochromatin were recovered (27, 28).

Two factors could cause low recovery of *P* insertions in centric heterochromatin: a low rate of transposition into heterochromatic sites or the failure of marker genes carried by the *P* elements to be expressed due to very strong position effects. In previous screens, it was impossible to determine the contribution of each factor. Low rates of insertion could be due to the scarcity of appropriate target sites in heterochromatin, reflecting the repetitive nature of heterochromatin. However, the discovery that "islands" of complex DNA are present in α -heterochromatin (5, 6) indicates that possible target sites for *P* insertion do exist within heterochromatin. Since *P* elements seem to prefer insertion near actively transcribed genes (29), the rarity of transcription units in heterochromatin may also contribute to low insertion rates.

Although the frequency of *P* insertion into heterochromatin may be low, position effects undoubtedly contribute to low recovery. This was suggested by the marker gene phenotypes of minichromosome *P* insertions in subtelomeric heterochromatin (6). The *ry*⁺ eye color gene showed reduced expression that was alleviated when a known suppressor of position-effect variegation, an extra Y chromosome, was added to the genotype. Silent insertions were recognized among some *ry*⁻ lines by the gain of *ry*⁺ expression when an extra Y chromosome was added to the genotype (27), but none mapped to the centric heterochromatin. However, the primary role of marker gene repression in the low recovery of centric heterochromatic insertions was called into doubt by the fact that one of the *Dp1187* "local hopping" screens did not rely on marker gene expression, since new insertions were detected by Southern hybridization (28).

A New Method for Recovering Heterochromatic *P* Insertions

The work of Zhang and Spradling (1) provides the first evidence that heterochromatic *P* element insertions do occur at a reasonable frequency and that

screening for such insertions is practical. Their results demonstrate that position effects on marker gene expression have contributed significantly to low recovery of heterochromatic *P* element insertions in previous screens, and that partially alleviating position effects often allows recovery.

The Zhang and Spradling screen employed a novel strategy: new *P* element insertions were recovered in a genetic background (an extra Y chromosome) that suppressed position-effect variegation of the *ry*⁺ eye color gene carried by the *P* element. In the first set of crosses, an X chromosome *P* element was mobilized in XY males, and transpositions from the X chromosome to other chromosomes were recovered in *ry*⁺ XXY female progeny. In the second set of crosses, they used one of the few known heterochromatic *P* elements, an unexpressed Y chromosome insertion called 95-2, as the starting element. Transpositions within the Y chromosome, or transpositions to other chromosomes that allowed *ry*⁺ expression, were recovered in progeny bearing an extra Y chromosome. From both screens new insertions that lost *ry*⁺ expression when the extra Y chromosome was removed ("Y chromosome-dependent insertions") were kept as candidate heterochromatic insertions and were cytogenetically mapped on mitotic metaphase chromosomes by *in situ* hybridization.

The most significant result of Zhang and Spradling's screen was the demonstration that *P* elements inserted in heterochromatin can be recovered efficiently if position-effect variegation is suppressed (1). Expression of *ry*⁺ in 7% of the new insertions was Y chromosome dependent, and all of these insertions were heterochromatic as determined by *in situ* hybridization. Their modest screen produced 32 new heterochromatic *P* insertions, including many more centric heterochromatin insertions than existed previously.

The mobilization of the 95-2 *P* element indicates that a heterochromatic insertion site does not inactivate *P* transposition, even when the expression of the marker gene it carries is severely repressed. The frequency of Y-linked *P* insertions was higher when *P* elements transposed from the 95-2 Y chromosome starting site than when they transposed from the X chromosome starting site. This result shows that preferential "local hopping" of *P* elements holds true for transpositions from heterochromatic sites, as was demonstrated previously for euchromatic insertions (27, 28).

An intriguing observation is that new heterochromatic insertions may have been recovered more efficiently when the *P* element transposed from a heterochromatic site rather than from a euchromatic

site (1). When the effects of "local hopping" within the Y chromosome were discounted, there was still a 2-fold increase in the frequency of heterochromatic insertions recovered from the Y starting site versus the X. Does the apparent greater efficiency of transpositions between heterochromatic sites reflect similar nuclear positions in cells undergoing transposition? Additional data will be required to test the generality of this observation and may provide clues to the underlying mechanism.

It is still not clear whether *P* element insertion into all heterochromatic regions, such as α -heterochromatin, can be recovered, despite the existence of complex sequences close to the centromere (5, 6). The finding that most of the heterochromatic *P* insertions were not immediately adjacent to centromeres could be due to the fact that suppression of position effects on marker expression may be possible only in β -heterochromatin. A number of the new *P* element insertions, as well as the original Y-linked 95-2 *P* element, are in heterochromatic regions deficient in the known satellite DNA sequences (20). Zhang and Spradling's finding that the *P*-induced mutation rate for vital heterochromatic genes approximates the rate for vital euchromatic genes, despite the low density of heterochromatic genes, may indicate a bias for the insertion of *P* elements into actively transcribed genes within the heterochromatin. Molecular analysis of the heterochromatic insertion sites will yield important information about insertion site preferences. If insertions in the "deeper" α -heterochromatin do occur, they may not respond to position-effect suppression, or may require additional suppressors [e.g., *Su(var)* mutations or even more Y chromosomes].

The recent observation that specific sequences ("boundary elements") can insulate genes from position effects (30-32) may allow the construction of *P* elements to probe more repressed regions of heterochromatin. In fact, mobilization of a *P* element bearing a white⁺ (*w*⁺) marker gene flanked by suppressor of Hairy-wing [su(Hw)] protein binding regions has led to efficient recovery of heterochromatic insertions (R. Roseman, E. Johnson, C. Rodesch, R. Nagoshi, and P. K. Geyer, personal communication). Subtelomeric and centric *P* insertions were recovered; unlike the Zhang and Spradling screen, no unlinked suppressors of variegation were required. Perhaps future screens designed to recover centric heterochromatic insertions should incorporate markers protected by boundary elements and unlinked modifiers of variegation. Might the frequency of heterochromatic insertions also be increased by including modifiers of position-effect variegation at the time of transposition?

Summary

The demonstration by Zhang and Spradling (1) of efficient *P* element transposition into heterochromatic regions will aid ongoing studies of heterochromatin structure and function. *P* element insertions will provide entry points for further molecular analysis of heterochromatin and will allow the isolation of small and large heterochromatic deficiencies.

The generation of heterochromatic *P* insertions also will aid the study of heterochromatic genes. Of the heterochromatic insertions isolated by Zhang and Spradling, five were homozygous lethal, and one of these defined a lethal locus not previously uncovered by heterochromatic deficiencies. *P* elements have previously been used to mutagenize and clone specific heterochromatic genes (14, 19, 26). New methods, like those described here (1, 32), should allow the efficient identification and molecular isolation of other single-copy heterochromatic genes. Furthermore, since position-effect suppression allowed the recovery of heterochromatic *P* insertions, it may also allow the recovery of insertions in euchromatic regions previously refractory to *P* mutagenesis.

Studies of position-effect variegation show that genes normally found in heterochromatin require a heterochromatic context for normal expression and that heterochromatin is inhibitory to euchromatic gene expression (16). The physical basis of these related phenomena—chromatin assembly, nuclear positioning, and/or heterochromatin elimination—

can be resolved only with a more thorough understanding of heterochromatin structure and functions. Analyzing heterochromatin also will help define the chromosomal components responsible for inheritance processes such as chromosome pairing, sister chromatid adhesion, and centromere function. These efforts will be facilitated by the effective use of *P* elements combined with other current molecular-genetic approaches.

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