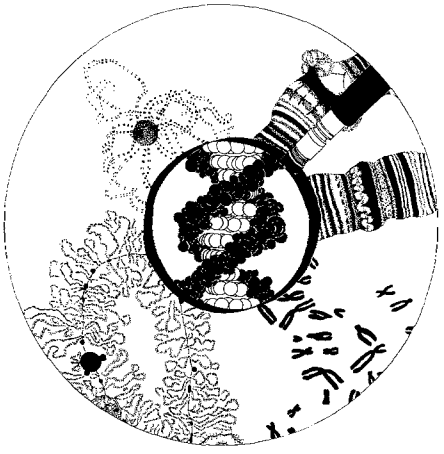
Chromosoma (1990) 100:3–7

CHROMOSOMA

Chromosoma Focus



Heterochromatin: junk or collectors item?

Mary Lou Pardue [1] **and Wolfgang Hennig** [2]

The First EMBO International Workshop on Drosophila Heterochromatin (May 9–13, 1990) brought some 70 geneticists, cytologists and molecular biologist to the lovely village of Alberobello in the Italian countryside near Bari. The meeting, organized by S. Pimpinelli and S. Gatti, was a lively one; considerable progress is being made in sorting out the differences and the similarities in the multiple elements that are too often lumped under the single heading of heterochromatin. Although the work presented was limited to studies of Drosophila, the findings should apply to heterochromatin in all organisms. The varieties of questions considered can be illustrated by the session topics, which we will use as a framework for our thoughts on the discussions. This commentary reflects our personal views and is not an attempt to give a balanced review of the subject. Where specific presentations at the Workshop are mentioned, the name and institution of the speaker are given. Where possible, we have cited a recent relevant reference as an entry to the literature for the interested reader.

[1] Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139-4391, USA
[2] Department of Molecular and Developmental Genetics, Catholic University, Toernooiveld, NL-6525 ED Nijmegen, The Netherlands

Offprint requests to either author

Heterochromatin: junk or collectors item?

Like the blind men, who investigated the elephant with apparently contradictory results, different biologists have arrived at different pictures of heterochromatin. *Cytologists* have found that there are some chromosome regions that show properties quite different from those of the rest of the genome – a more intense staining (positive heteropyknosis), late replication (even underreplication in polytene tissues), and differential decondensation in stages where other chromosome regions are condensed (negative heteropyknosis). *Geneticists* find that heterochromatin has few, if any, genes that can be detected by conventional mutation analysis. Heterochromatin also does not participate in meiotic recombination. In spite of the apparent failure of heterochromatin in the standard genetic assays of mutation and recombination, other types of studies show that heterochromatin does have some significant genetic effects. It can markedly influence the level of activity of genes which, by chromosome rearrangement, are transferred into, or close to, heterochromatic regions (position-effect variegation). In addition, a number of euchromatic genes have been identified whose expression can be modified when extra copies of certain regions of heterochromatin are present in the genome. *Molecular biologists* know that most of the DNA sequences that have been associated with heterochromatic regions are repetitive sequences, many of

them highly repeated, and few have any evidence of protein coding. Such sequences do not fit easily into current paradigms for genetic activity, raising the possibility that heterochromatin may be junk. (For a recent review on heterochromatin, see Hennig 1986.)

One reason for the differing views on heterochromatin is that most systems where heterochromatin is studied by cytology or genetics are not easy to study molecularly and vice versa. Furthermore, the genetic effects that have been ascribed to heterochromatin tend to be more quantitative than qualitative and are therefore less easily analyzed. Perhaps most important, neither heterochromatin nor repeated DNA is found in the prokaryotic systems that have been so important in defining basic concepts of molecular genetics.

Recombinant DNA technology has made it possible to circumvent many of the old problems to the study of heterochromatin and has opened the way to a reconsideration of some very interesting questions. Several features make *Drosophila* especially favorable for these analyses. The rich background of genetic studies on *Drosophila* has provided many rearranged chromosomes which move genes into the orbit of influence of heterochromatin. Other chromosome constructions can be used to change the dosage of particular regions of heterochromatin. (Many genes have been identified that are sensitive to modulation by heterochromatin.) Other genes have been identified that can modulate the activity of heterochromatin. The giant polytene chromosomes of *Drosophila* allow a cytological division of heterochromatin into two types, α and β. The two types cannot be distinguished on metaphase chromosomes of *Drosophila* and thus may be hidden in the metaphase chromosomes of other organisms which do not have polytene chromosomes to reveal them.

The multiple facettes of heterochromatin

Structural aspects of heterochromatin

In most cells the majority of the heterochromatin is concentrated in the regions flanking the centromeres and the telomeres. In all species studied the pericentric heterochromatin has contained tandemly repeated simple sequence "satellite" DNA. In *D. melanogaster* four major and several minor satellites have been identified. Most have the sequence $(RRN)_mRN$ where R is usually A. In situ hybridization to polytene chromosomes shows no evidence for these sequences outside the pericentric regions (α-heterochromatin), although there are very few euchromatic regions that show hybridization at reduced stringency (Lohe, Case Western Reserve; Lohe and Roberts 1988). The consistent association of satellite-type repeats with pericentric region makes it tempting to propose a structural role for the repeats, but it is not easy to test such predictions. Hawley (Albert Einstein) reported evidence showing that homologous pairing in heterochromatic intervals is an important component of meiotic segregation and that the gene *Axs* mediates this association. An important, but difficult question is whether satellite DNA is the component of heterochromatin acted on by *Axs*. The question is especially interesting in light of a recent study of meiotic pairing of the *X* and *Y*. McKee and Karpen (1990) have found that a single copy of the gene for ribosomal RNA (rRNA) restores part of the *X–Y* meiotic pairing that is lost when the *X* heterochromatin is deleted (McKee, University of Wisconsin-Eau Claire). It thus appears that rDNA has more than one function. Can satellite and rDNA also have overlapping functions? Or do the repeats in the non-transcribed spacer of the rDNA mediate the meiotic pairing? The further characterization of several genes involved in the control of meiotic segregation in males (Endow, Duke University) will shed more light on the sequences involved in pairing.

The β-heterochromatin of polytene chromosomes shows that not all pericentric heterochromatin is composed of short tandem repeats. The β-heterochromatin shows homology to many transposable elements that are also present at many euchromatic sites. Recently, a major family of repeated DNA, HeT DNA, has been characterized which appears to be totally specific for β-heterochromatin and telomeric regions. The repeats have an irregular, mosaic character and contain elements with features reminiscent of various types of transposable elements (Valgeirsdottir et al. 1990). Studies of "healed" telomeres on terminally deleted chromosomes prove that at least one of the subfamilies of HeT DNA elements can actually transpose (Biessmann et al. 1990). Pardue (MIT) proposed that cells may use mechanisms of transposition for chromosomal maintenance, such as healing broken telomeres, and that the transposable elements found in euchromatic sites may have appropriated parts of these mechanism for their own uses. Pellison (Universite Blaise Pascal, Aubiere) reported that all species of the melanogaster subgroup have sequences homologous to the I transposable element in β-heterochromatin; however, only *D. melanogaster* has I elements that move around in the euchromatin, and the mobile elements are thought to be the result of a recent invasion (Vaury et al. 1989). An alternative interpretation is that at least some of the heterochromatic I element sequences are true cellular components while the euchromatic elements are sequences that have managed to escape the cellular control.

Y chromosomes and fertility factors

The *Y* chromosome is distinguished from the other chromosomes by its heterochromatic appearance and by its lack of conventional genes. In spite of this, the chromosome is absolutely necessary for the development of functional sperm (Hess, Düsseldorf), so the *Y* chromosome presents a clear association of heterochromatin with defined genetic activity. The molecular structure of the genetic elements of the *Y* has been studied extensively in *D. hydei* and, more recently, in *D. melanogaster*. Although very little DNA sequence is conserved, the remarkable structure of these genetic elements is very

similar in the two species. The *Y* chromosome carries a small number of genetic elements called fertility factors. Deficiency mapping indicated, in agreement with earlier ultrastructural studies, that each of these is very large, 1000–4000 kb, and the very high mutation rates support the idea that the elements are very large. These elements are active only in the primary spermatocyte where some of them form large lampbrush loops covered with RNA and protein. Each loop has its own distinctive morphology and these structures have intrigued and puzzled cytologists since they were first discovered by Meyer et al. (1961). The first analyses of DNA sequences from the loops provided the surprise that these actively transcribed regions do not appear to contain functional protein-coding sequences. Instead, they contain sequences that share many characteristics with other heterochromatin. For instance, the noose loops of *D. hydei* contains relatively short, tandemly arranged clusters of noose-specific DNA sequences, interspersed with longer, more heterogeneous DNA. At least part of the heterogeneous DNA has homology with transposable elements (Hennig, Nijmegen). On the *D. melanogaster Y*, three of the satellite sequences are found within loops and contribute to the loop transcripts (Bonaccorsi et al. 1990). These same satellites are limited to the pericentric heterochromatin in the other chromosomes. The novel features of the loops suggest that they have novel functions. Studies of proteins bound to the loops led to the suggestion that they bind site-specific nuclear proteins. In addition, many data suggest that the loop function is more complex, and it has been proposed that parts of the loop transcripts or the proteins bound to them may serve in regulatory events at the post transcriptional level later in the program for spermiogenesis (Hennig 1990). Bonaccorsi (Roma) reported that the Kl-3 loop of *D. melanogaster* binds antibody to tectin, a structural component of the sperm tail.

Position effect variegation

The most obvious characteristic common to different kinds of heterochromatin is the ability to form differentially condensed chromatin, at least in some cell types. The condensation is usually associated with lack of active transcription. The differential condensation must be directed by DNA sequences carried within the heterochromatic segment, although such sequences have not been identified. Apparently, sequences determining the structure of heterochromatin can act over some distance since euchromatic genes brought near a break in heterochromatin by chromosomal rearrangement can come under the influence of the heterochromatin. This phenomenon is known as position-effect variegation; gene expression is reduced by an effect that apparently spreads in a gradient for variable distances out from the adjacent heterochromatin. In some cases the effect seems to be on the chromatin structure of the affected euchromatin (Reuter, Martin-Luther Universität Halle-Wittenberg; Grigliatti, University of Vancouver). In other cases, the euchromatic genes are found to be underrepresented in the tissue (Karpen, Carnegie Institution of Washington); in these cases it appears that the euchromatin has taken on another of the characteristics of heterochromatin, tissue-specific underreplication or somatic excision (Karpen and Spradling 1990).

The interactions between heterochromatin and euchromatin that result in position-effect variegation can be modulated by a variety of factors, including temperature and the amount of some kinds of heterochromatin within the genome. In addition, a growing number of genes have been identified that either suppress or enhance the degree to which the activity of a gene will be decreased by adjacent heterochromatin (Reuter, Martin-Luther-Universität; Tartof, Fox Chase Cancer Center; Grigliatti, University of Vancouver). One of the genes with suppressor functions, *Su(var) 205,* codes for a heterochromatin-specific protein (Elgin, University of Washington). Other members of this set of genes appear to have a variety of functions. *Su var(3)7* encodes a zinc finger protein (Garzino, University of Geneva; Reuter et al. 1990). *Su(var) 216* has 72% amino acid homology to *cdc2* (Grigliatti, University of Vancouver; Hayashi et al. 1990). For many of these genes it appears that a duplication of the gene will suppress the effect of heterochromatin on euchromatic genes while a deficiency will enhance the effect (or vice versa), suggesting that the spread of the heterochromatic influence is very sensitive to the level of certain gene products. The theoretical background, especially at a quantitative level, is considered by models of the counting mechanism (Tartof, Fox Chase Cancer Center; Locke et al. 1988). These considerations show that quantitative effects are not trivial for our understanding, especially of several components are involved, since assembly reactions, which must be involved in such mechanisms, display an exponential character. Similar considerations must be taken into account for models, explaining *X* inactivation in mammalian females.

For genes normally located near heterochromatic regions (such as *light* and *rolled*), movement away from the heterochromatin can produce position-effect variegation (Wakimoto, University of Washington; Hilliker, University of Guelph; Devlin et al. 1990). This variation can also be affected by the suppressors and enhancers discussed above. It was proposed that the repetitive sequences of the heterochromatin are functionally important for the adjacent genes and act to compartmentalize proteins required for the expression of the heterochromatic genes (Wakimoto and Hearn 1990).

Interaction between euchromatic and heterochromatic elements

Several euchromatic genes have been found to be affected in quite specific ways by certain regions of heterochromatin. The *abnormal oocyte (abo)* gene is a recessive maternal-effect mutation located in the euchromatin of chromosome *2*. Increases in the amount of certain discreet regions of heterochromatin (called *ABOs)* from *X*, *Y*, and chromosome *2* in the genome of the zygotes

from such mothers can increase the probability of survival (Pimpinelli, University of Bari; Pimpinelli et al. 1985). This rescue seems to occur in the period between cellular blastoderm and cuticle formation. At least two suppressors of position-effect variegation, *Su(var)205* and *Su(var)208*, also increase the viability of *abo*-derived progeny, suggesting that the interaction of *abo* with heterochromatin may be analogous to that of variegating genes (Tomkiel, University of Washington).

daughterless-abo-like (dal) is a maternal effect semilethal mutation that is closely assoiated with *abo*. In embryos from homozygous *dal* females, nuclear divisions begin to become abnormal at cycle 11 but can be partly rescued by paternally derived heterochromatin. Analyses of the mutant embryos has shown that the defect is in the centrosomes that replicate but fail to separate (Sullivan, UCSF).

A third euchromatic gene with a remarkable interaction with specific regions of the heterochromatin is *Segregation Distorter, Sd,* which is located in the euchromatin of chromosome *2L* (Sandler and Golic 1985). The heterochromatic elements of this system included *Responder, Rsp*, in the pericentric heterochromatin of chromosome *2R* and *Enhancer of Sd, E(Sd)*, in the heterochromatin of *2R*. The *SD* locus is capable of causing the preferential loss of spermatids bearing a sensitive allele of *Rsp*. Thus, if *Sd* and the sensitive *Rsp* allele are on homologous chromosomes, most functional sperm will carry the *Sd* chromosome. The *Rsp* locus sensitivity of a chromosome can be correlated with the number of copies of a 120 bp AT-rich repeat sequence that can be mapped to the base of chromosome *2R* (Lyttle, University of Hawaii; Wu et al. 1988). The *Rsp* locus is expected to contain repeated elements since chromosome breaks could reduce the sensitivity without eliminating it (Lyttle 1990). The system is, however, more complex because of the *E(Sd)* locus (Temin, University of Wisconsin). An addition, a new element, *supp-X(SD)*, on the *X* chromosome (Hiraizumi, University of Texas) can severely suppress the segregation distortion. It may not be solely the DNA sequence of *Sd* that governs the function of *Rsp*. A 12 kb fragment has been isolated containing a 5 kb tandem duplication specific for the *Sd* allele (Powers, University of Wisconsin). This 12 kb DNA fragment has been used to transform *Drosophila*. None of the six insertion lines that were obtained have shown ability to distort transmission of a sensitive relative to an insensitive chromosome, suggesting that, if the 12 kb sequence is, in fact, the determiner of *Sd* activity, it must be placed in a very special chromosomal environment.

Conclusion: heterochromatin is a collector's item

Anyone who has visited a flea market knows that what appears to be junk can be a collector's item to those who know and understand it. We think we are at a point where it is evident that heterochromatin can be placed in the category of collectors' items, although important questions remain. Several questions, however, are obvious to us now.

1. The identification of heterochromatin begins with its cytological compaction. Clearly, all of the sequences in heterochromatin have some capacity to adopt this conformation. The capacity may well be related to the repetitive nature of the sequences. On the other hand, it should be emphasized that most if not all of these sequences are not in the heterochromatic state in some cells. Therefore, the compaction requires more than simply the sequence. What is the basis of this compaction?

This question just asked applies to all of the regions of what is called "constitutive" heterochromatin. There is also a class of heterochromatin that is called "facultative" (for example, the *X* chromosome in female mammals). This term is applied to regions, or chromosomes, of which one copy is compacted and the other extended in the same cell. Here there is evidence that the cell can discriminate between what must be basically the same sequence. A second characteristic of facultative heterochromatin is that it is rich in genes of the type found in euchromatin. Is facultative heterochromatin basically different from constitutive heterochromatin, or are the genes of facultative heterochromatin embedded in sequences that use mechanisms of condensation similar to those of constitutive heterochromatin? One clear example of interspersed sequences affecting chromatin structure occurs in the *X* chromosome of *Drosophila* where there is a chromosome-specific increased content of long CA, CT, and poly C sequences (Lowenhaupt et al. 1989). In polytene nuclei of males the *X* chromosome shows a significantly less compact structure than do the autosomes. Mutations in single genes (*msl* genes) can cause the structure of the *X* to return to that of the autosomes (Belote and Lucchesi 1980).

2. Another characteristic frequently associated with heterochromatin is underreplication or somatic elimination of DNA sequences in these regions. Is the tendency for underreplication or loss a consequence of the high degree of compaction of such regions which might impede the access of the replication machinery?

3. Heterochromatin is associated with centromeres and telomeres in almost all eukaryotic chromosomes. This association suggests that the heterochromatin may play a structural role and there are multiple roles that can be suggested. Could the structures be important for nucleating mitotic chromosome condensation? Could they be involved in associations between sister chromatids or between homologous chromosomes? In addition, could the intranuclear organization of the chromatin in interphase nuclei be directed by association of these structures with one another or with the nuclear membranes?

4. The phenomenon of position effect variegation emphasizes the ability of heterochromatin to influence adjacent regions of DNA. The molecular basis for this may involve either of at least two mechanisms, either inacti-

vation by a change in chromatin structure or loss of the sequence by failure to replicate. As suggested above, either of these mechanisms could be derived from the differential compaction of heterochromatin. An interesting feature of position effect is its imprecise nature. Even within the same tissue the influence of the heterochromatin extends for different distances along the chromosome and affects the expression of different numbers of genes. Is this imprecise border a characteristic of heterochromatin in undisrupted chromosomes or is it a consequence of the loss of a natural border?

5. The many genes that have already been found to enhance or suppress position-effect variegation appear to encode proteins involved in both chromatin structure and the transcription machinery. How many of these proteins will be found to be specific for heterochromatin and how many will act on both euchromatin and heterochromatin?

6. Several regions of heterochromatin behave like genes in genetic analyses. These include the *Responder* locus, *ABO*, and the fertility factors on the *Y* chromosome. What is know about their molecular organization suggests that these genes differ significantly from what is considered the typical euchromatic gene. Are the organization and molecular mechanisms of these genetic units related to their location in regions of heterochromatin?

Discussions resulting from the Workshop have helped to clarify questions that can be approached on the basis of the new data. Rapidly evolving new methods for the study of *Drosophila* will help to provide for the answers.

References

Belote JM, Lucchesi JC (1980) Control of X chromosome transcription by the maleless gene in *Drosophila*. Nature 285:573–577

Biessmann H, Mason JM, Ferry K, Dhulst M, Valgeirsdottir K, Traverse KL, Pardue ML (1990) Addition of telomere-associated Het DNA sequences heals broken chromosome ends in *Drosophila*. Cell 61:663–673

Bonaccorsi S, Gatti M, Pisano C, Lohe A (1990) Transcription of a satellite DNA on two Y chromosome loops of *Drosophila melanogaster*. Chromosoma 99:260–266

Devlin RH, Bingham B, Wakimoto BT (1990) The organization and expression of the light gene, a heterochromatic gene of *Drosophila melanogaster*. Genetics 125:129–140

Hayashi S, Rudell A, Sinclair D, Grigliatti T (1990) Chromosomal structure is altered by mutations that suppress or enhance position effect variegation. Chromosoma 99:391–400

Hennig W (1986) Heterochromatin and germ line-restricted DNA. In: Hennig W (ed) Results and problems in cell differentiation. Germ line – soma differentiation, vol 13. Springer, Berlin Heidelberg New York, pp 176–192

Hennig W (1990) The Y chromosome of *Drosophila*. In: Adolph KW (ed) Chromosomes: eukaryotic, procaryotic, and viral. CRC Press, Boca Raton, pp 213–238

Karpen GH, Spradling AC (1990) Reduced DNA polytenization of a minichromosome region undergoing position-effect variegation in *Drosophila*. Cell (in press)

Locke J, Kotarski MA, Tartof KD (1988) Dosage-dependent modifiers of position effect variegation in *Drosophila* and a mass action model that explains their effect. Genetics 120:181–198

Lohe A, Roberts P (1988) Evolution of satellite sequences in *Drosophila*. In: Verma RS (ed), Heterochromatin, Cambridge University Press, Cambridge, pp 148–186

Lowenhaupt K, Rich A, Pardue ML (1989) Nonrandom distribution of long mono- und dinucleotide repeats in *Drosophila*: correlation with dosage compensation, heterochromatin and recombination. Mol Cell Biol 9:1173–1182

Lyttle (1990) The effect of novel chromosome position and variable dose on the genetic behavior of the Responder (*Rsp*) element of the Segregation Distorter (*SD*) system of *Drosophila melanogaster*. Genetics 121:739–750

McKee BD, Karpen GH (1990) *Drosophila* ribosomal RNA genes function as an X–Y pairing site during male meiosis. Cell 61:61–72

Meyer GF, Hess O, Beermann W (1961) Phasenspezifische Funktionsstrukturen in Spermatocytenkernen von *Drosophila melanogaster* und ihre Abhängigkeit vom Y-Chromosom. Chromosoma 12:676–716

Pimpinelli S, Sullivan W, Prout M, Sandler L (1985) On biological functions mapping to the heterochromatin of *Drosophila melanogaster*. Genetics 109:701–724

Reuter G, Giarre M, Farah J, Gausz J, Spierer P (1990) Dependence of position-effect variegation in *Drosophila* on dose of a gene enconding an unusual zinc-finger protein. Nature 344:219–223

Sandler L, Golic K (1985) Segregation distortion in *Drosophila*. Trends Genet 1:181–185

Valgeirsdottir K, Traverse KL, Pardue ML (1990) HeT DNA: a family of mosaic repeated sequences specific for heterochromatin in *Drosophila melanogaster*. Proc Natl Acad Sci USA (in press)

Vaury C, Bucheton A, Pelisson A (1989) The β-heterochromatic sequences flanking the I elements are themselves defective transposable elements. Chromosoma 98:215–224

Wakimoto BT, Hearn MG (1990) The effects of chromosome rearrangements on the expression of heterochromatic genes in chromosome 2L of *Drosophila melanogaster*. Genetics 125:141–154

W C-I, Lyttle TW, Wu ML, Lin G-F (1988) Association between a satellite DNA sequence and the Responder of segregation distorter in *D. melanogaster*. Cell 54:179–189