Evidence for Intrinsic Differences in the Formation of Chromatin Domains in *Drosophila melanogaster*

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ABSTRACT

The results of an investigation into intrinsic differences in the formation of two different heterochromatic domains are presented. The study utilized two different position effect variegation mutants in *Drosophila melanogaster* for investigating the process of compacting different stretches of DNA into heterochromatin. Each stretch of DNA encodes for a gene that affects different aspects of bristle morphology. The expression of each gene is prevented when it is compacted into heterochromatin thus the genes serve as effective reporter systems to monitor the spread of heterochromatin. Both variegating mutants are scored in the same cell such that environmental and genetic background differences are unambiguously eliminated. Any differences observed in the repression of the two genes must therefore be the result of intrinsic differences in the heterochromatic compaction process for the two stretches of DNA. Studies of the effects different enhancers of variegation have upon the compaction of the two genes indicate each compaction event occurs independently of the other, and that different components are involved in the two processes. These results are discussed with regard to spreading heterochromatin and the role this process may play in regulating gene expression.

THE assemblage of DNA and protein into chromatin plays a central role in regulating gene expression. One dramatic example of this is the effect compacting genes into heterochromatin has upon their ability to be expressed.

Heterochromatin is a highly condensed form of chromatin that fails to decondense during the interphase portion of the cell cycle. In many instances, compacting genes into heterochromatin effectively shuts off their expression. Examples of this include *X* chromosome inactivation in mammals with more than one *X* chromosome, repression of the paternal set of chromosomes in the mealy bug, and precocious *X* chromosome inactivation during spermatogenesis. The compaction into heterochromatin, once achieved, tends to be perpetuated to all daughter cells in a clonal fashion.

The formation of heterochromatin appears to require an initial nucleation event from which compaction can spread to include adjacent sequences. Sequences normally destined to be compacted into euchromatin may instead be compacted into heterochromatin if, through a chromosomal rearrangement, they are juxtaposed near a site of heterochromatic nucleation. Heterochromatic compaction will thus spread from the initial nucleation event to include adjacent, normally euchromatic DNA sequences. This spreading of heterochromatic compaction has been observed in *X* to autosome translocations in mammals [see EISCHER (1970) and GARTLER and RIGGS (1983)] as well as numerous rearrangements that bring together normally separated euchromatic sequences with heterochromatin in Drosophila [see SPOFFORD (1976), EISSENBERG (1989) and HENIKOFF (1990)]. Spreading of heterochromatin in these systems can be monitored by the repression of the genes being compacted into heterochromatin. As such, both the *X* autosome translocations in mammals as well as the Drosophila mutants, referred to as position effect variegation mutants, provide an excellent model system for studying the compaction of DNA into heterochromatin.

It has long been thought that all heterochromatin within an organism is formed by the same mechanism. Observations of position effect variegation mutants in Drosophila, however, are not entirely consistent with this idea. SPOFFORD (1976), citing unpublished results, indicated the genetic modifier of variegation *Su(var)* suppressed certain position effect variegation mutants (affecting the *white* gene in both $Dp(1;3)N^{264\text{-}58}$ and $In(1)w^{m4}$; and the *roughest* gene in both $Dp(1;3)N^{265\text{-}58}$ and $In(1)rst^{3}$) while enhancing other ones (affecting the *yellow* gene in $In(1)y^{3P}$ and the *scute* gene in $In(1)sc^{4}$). Additionally, SINCLAIR, MOTTUS and GRIGLIATTI (1983) published results suggesting modifiers may exert a stronger affect upon some variegating mutants than upon others. These results, involving different variegating genes, are difficult to evaluate. One difficulty in making comparisons between different variegating mutants is that they often involve genes expressed at different times in development or in different tissues. Even when the same gene is ex-

amined in different variegating rearrangements it is nearly impossible to ensure the strains do not have important differences in their genetic backgrounds.

To investigate the question of whether or not the spreading of heterochromatin always occurs by the same mechanism and is independent of either the particular euchromatic or heterochromatic sequences involved, a system for evaluating two separate instances of this phenomenon in the same cell is required. Toward this end, a stock containing two separate variegating rearrangements was established– *In(1)y^{3P}; T(2;3)Sb^{V}/+; TM2*. Each rearrangement affects a different aspect of the phenotype of the large bristles in *Drosophila melanogaster*. The *yellow* gene in *In(1)y^{3P}* affects the color of the bristle while the *Stubble* gene in *T(2;3)Sb^{V}* affects the bristle's length. Each trait can be scored independently of the other within the same cell. This approach unambiguously eliminates both environmental and genetic background differences allowing intrinsic differences between variegating mutants to be assayed. The results indicate that, in general, the two genes are compacted independently of each other and that they can exhibit fundamental differences in their response to specific genetic modifiers. These results are discussed in regard to the formation of separate chromatin domains and the role this may play in regulating gene expression.

MATERIALS AND METHODS

Stocks: The stocks used are described in LINDSLEY and GRELL (1968), LOCKE, KOTARSKI and TARTOF (1988), TARTOF *et al.* (1989) or WUSTMANN *et al.* (1989).

***In(1)y^{3P}; T(2;3)Sb^{V}* system:** *In(1)y^{3P}* is an inversion of the *X* chromosome that has placed the euchromatic *yellow* gene proximal to the rDNA genes in *X* heterochromatin. The euchromatic breakpoint is either within or very close to the *yellow* gene itself (CAMPUZANO *et al.* 1985 and BIESSMANN 1985). This mutant variegates for the expression of *yellow* producing a yellow bristle when the gene is inactivated. *T(2;3)Sb^{V}* is a reciprocal translocation between the second and third chromosomes resulting in the juxtaposition of the third chromosome dominant *Stubble* to centric second chromosome heterochromatin. In this instance, heterochromatin-induced gene inactivation prevents the expression of the dominant *Stubble*, whose phenotype is a shortened bristle, producing a phenotypically wild-type bristle.

Scoring *In(1)y^{3P}* and *T(2;3)Sb^{V}*: Fourteen macrocheates were scored per fly for the yellow, Stubble or wild-type phenotypes. The fourteen bristles were: four scutellars, four post-alars, anterior pair of dorso-centrals and the four larger sternopleurals. A minimum of 20 and a maximum of 50 flies were scored for the genetic modifier experiments. Each experiment was repeated at least one time with comparable results obtained in each instance. A bristle was scored as yellow if it was brown or lighter and as non-Stubble if it was over half the length of a corresponding wild-type bristle. Macrocheates were scored and counted in one of four categories: Stubble, yellow Stubble, yellow and wild type. Since the genetic crosses to examine the affects of genetic modifiers on *In(1)y^{3P}* and *T(2;3)Sb^{V}* produced only males with the correct genotype, to be consistent, only males were scored in all experiments.

Effects of genetic modifiers: In order to examine the effects of different genetic modifiers upon both *In(1)y^{3P}* and *T(2;3)Sb^{V}* within the same cell, the following general crossing scheme was used:

♀♀ *In(1)y^{3P}; T(2;3)Sb^{V}/TM2, Ubx* × *In(1)w^{m4}; Genetic Modifier/Balancer* ♂♂

Males that were genotypically *In(1)y^{3P}; T(2;3)Sb^{V}/Genetic Modifier* (experimental) were scored and compared to *In(1)y^{3P}; T(2;3)Sb^{V}/Balancer* (control) sibling males. This scheme eliminates any differences between *In(1)y^{3P}* and *T(2;3)Sb^{V}* due to maternal or paternal effects since both position effect variegation mutations are maternally derived and since male siblings from the same cross were compared, both control and experimental males carried the same *Y* chromosomes. Other chromosomes should segregate evenly between control and experimental flies. Parental stocks and experimental crosses were raised at 24–25° unless otherwise noted.

Data obtained from the genetic modifier experiments with *In(l)y^{3P}; T(2;3)Sb^{V}/Balancer* or *Enhancer* males were analyzed for independence of the inactivation event for the two variegating mutants. A 2 × 2 χ^2 analysis of the four possible bristle phenotypes, Stubble, yellow Stubble, yellow and wild type showed that the inactivation of *In(l)y^{3P}* within the same cell occurs independently of the inactivation of *T(2;3)Sb^{V}* (Table 2).

RESULTS

Effects of genetic modifiers upon *In(l)y^{3P}; T(2;3)Sb^{V}/TM2, Ubx* males: The effects of genetic modifiers upon both *In(l)y^{3P}* and *T(2;3)Sb^{V}* within the same cell were examined (Table 1). When the region of four of the genetic modifiers was duplicated (*Dp(2;2)E19* and *Dp(2;2)E39A* for 28D-29C and *Dp(3;3)E8* and *Dp(3;3)E11* for 88D) or deleted or mutated (*Df(2L)cl-h3*, *Df(2L)E66* and *Df(2L)clot-7* for 25F-26A and *Df(3R)E40* for 100CF) the effect upon both *In(l)y^{3P}* and *T(2;3)Sb^{V}* was similar, either enhancing or suppressing both. Two of these regions, 25F-26A and 88D, exert a stronger effect; compared to their control chromosomes, upon *yellow* inactivation than upon that of *Stubble*. Duplications of the 28D-29C region gave mixed results while the modifier at 100CF appears to enhance *Stubble* inactivation more so than that of *yellow*.

Most interesting are the examples where there are differences in response to a modifier. One region (24A-25E), when duplicated, acted to suppress *In(l)y^{3P}* while slightly enhancing or having no effect on *T(2;3)Sb^{V}*. The 24A-25E duplications neither enhanced nor suppressed *In(l)w^{m4}* (LOCKE, KOTARSKI and TARTOF 1988). The 42E-43C deficiency had the opposite effect, enhancing *In(l)y^{3P}* inactivation (and also enhancing inactivation of *white* in *In(1)w^{m4}*) (WUSTMANN *et al.* 1989) while slightly suppressing that of *T(2;3)Sb^{V}*. *Df(2L)H2O* may cause a weak suppression of *In(l)y^{3P}* inactivation while having no effect on *T(2;3)Sb^{V}*. This same deficiency was reported by WUSTMANN *et al.* (1989), and confirmed by us (C. P.

TABLE 1

Effects of genetic modifiers on *In(1)y^{3P}* compared to their effects on *T(2;3)Sb^{V}*

Chromosome	Modifier	n^a	yellow^{Inactivated} Ave^b	SE	Stubble^{Inactivated} Ave^b	SE
Dp(2;2)AM3	24A-25E	420	0.2	0.1	5.1	0.3
CyO	Control	322	0.7	0.1	4.4	0.4
Ratio, *AM3/CyO*			**0.3**		**1.2**	
Dp(2;2)AM17	24A-25E	700	0.2	0.1	4.1	0.3
Cy, bw	Control	700	1.6	0.2	3.2	0.3
Ratio, *AM17/Cy*			**0.1**		**1.3**	
Df(2L)cl-h3	25F-26A	504	9.3	0.4	7.4	0.4
CyO	Control	700	1.5	0.2	4.1	0.6
Ratio, *cl-h3/CyO*			**6.2**		**1.8**	
Df(2L)E66	25F-26A	686	10.4	0.3	5.1	0.3
CyO	Control	574	1.9	0.2	3	0.4
Ratio, *E66/CyO*			**5.5**		**1.7**	
Df(2L)clot-7	25F-26A	700	5.4	0.4	8.2	0.3
CyO	Control	448	1.1	0.1	4.5	0.1
Ratio, *clot-7/CyO*			**4.9**		**1.8**	
Dp(2;2)E19	28D-29C	420	5.5	0.3	7.4	0.4
Cy, bw	Control	448	1.5	0.2	1.7	0.2
Ratio, *E19/Cy*			**3.7**		**4.4**	
Dp(2;2)E39A	28D-29C	672	6.1	0.3	11.1	0.2
CyO	Control	280	1.2	0.2	4.3	0.3
Ratio, *E39A/CyO*			**5.1**		**2.6**	
Df(2L)H2O	36A-36E	700	1.3	0.1	2.8	0.3
CyO	Control	700	1.9	0.2	2.8	0.3
Ratio, *Df(2L)H2O/CyO*			**0.7**		**1.0**	
Df(2R)pK78K	42E-43C	700	4.9	0.3	2.7	0.3
CyO	Control	700	2.3	0.2	4.2	0.3
Ratio, *Df(2R)pK78K/CyO*			**2.1**		**0.6**	
Dp(3;3)E8	88D	686	4.6	0.2	7.1	0.3
TM2	Control	616	1	0.1	2.3	0.2
Ratio, *E8/TM2*			**4.6**		**3.1**	
Dp(3;3)E11	88D	420	7.4	0.3	6.8	0.5
TM2	Control	294	1.2	0.2	2.6	0.3
Ratio, *E11/TM2*			**6.2**		**2.6**	
Df(3R)E40	100CF	700	5.8	0.3	9.1	0.3
TM6	Control	700	3.3	0.2	3.3	0.3
Ratio, *E40/TM6*			**1.8**		**2.8**	

[a] n = number of bristles scored, scoring 14 bristles/fly.
[b] Ave = average number of bristles inactivated (yellow or Stubble$^+$)/fly.

BISHOP and A. KOUTOULAS, unpublished), to enhance inactivation of the *white* locus in *In(1)w^{m4}*.

χ^2 analysis of the independence of inactivation: One of the advantages of the *In(1)y^{3P}; T(2;3)Sb^{V}* system is that it allows an examination of whether or not the two inactivation events occur independently of each other. It is possible that the inactivation of *yellow* and *Stubble* are mutually exclusive such that inactivating one gene prevents the inactivation of the other. This might be anticipated if the inactivation events compete for a scarce resource such as the structural components of heterochromatin. One inactivation event may monopolize all of the available structural components of heterochromatin not leaving enough of the heterochromatic proteins to construct additional heterochromatin. Another possibility is that the two events may act in unison either both being inactivated or neither being inactivated. This could occur if there are fluctuations from cell to cell for the expression of a gene or genes involved in forming heterochromatin. In cells in which a given gene is expressed there may be more than enough material to construct a large amount of heterochromatin while in the cells where the gene is not expressed, there is insufficient material to inactivate either gene. Yet another possibility is that the two inactivation events occur independent of each other. If the two inactivation events are dissimilar, independence of inactivation would be anticipated.

The results presented in Table 2 indicate that, in general, the inactivation of *yellow* occurs independent of that for *Stubble*. One exception to independence, *Cy, bw* from the *Dp(2;2)AM17* cross, is most easily explained as a statistical fluctuation. This same chromosome in the *Dp(2;2)E19* cross (and in repetitions of the *Dp(2;2)AM17* experiment, data not shown) inactivated the two genes independently of each other. The

TABLE 2

A 2 × 2 χ^2 analysis for independence of inactivation of *In(1)y^{3P}* and *T(2;3)Sb^V*

Genotype	y,Sb[a,b]	y$^+$,Sb	y,Sb$^+$	y$^+$,Sb$^+$	n	χ^2	Probability
Dp(2;2)AM3	4	262	1	153	420	0.605	0.5 > P > 0.25
CyO	12	209	4	97	322	0.317	0.75 > P > 0.5
Dp-(2;2)AM17	8	485	2	205	700	0.446	0.75 > P > 0.5
Cy, bw	70	460	10	160	700	6.823	0.01 > P > 0.005
Df(2L)cl-h3	145	88	191	80	504	3.835	0.1 > P > 0.05
CyO	52	442	22	184	700	0.004	P = 0.95
Df(2L)E66	311	122	201	52	686	4.901	0.05 > P > 0.01
CyO	60	392	17	147	616	0.93	0.5 > P > 0.25
Df(2L)clot-7	102	154	135	225	616	0.347	0.75 > P > 0.5
CyO	33	273	10	132	448	1.565	0.25 > P > 0.1
Dp(2;2)E19	74	123	90	133	420	0.343	0.75 > P > 0.5
Cy, bw	42	351	7	48	448	0.164	0.75 > P > 0.5
Dp(2;2)E39A	61	79	233	299	672	0.002	0.975 > P > 0.95
CyO	18	176	6	80	280	0.403	0.75 > P > 0.5
Df(2L)H2O	42	446	22	190	700	0.154	0.75 > P > 0.5
CyO	75	483	24	118	700	1.059	0.5 > P > 0.25
Df(2R)pk78K	190	377	54	79	700	2.386	0.25 > P > 0.1
CyO	85	404	28	180	700	1.572	0.25 > P > 0.1
Dp(3;3)E8	103	233	124	226	686	1.801	0.5 > P > 0.25
TM2	37	465	5	109	616	1.48	0.25 > P > 0.1
Dp(3;3)E11	117	100	104	99	420	0.31	0.75 > P > 0.5
TM2	23	216	2	53	294	2.06	0.25 > P > 0.1
Df(3R)E40	98	149	190	289	726	0.339	0.75 > P > 0.5
TM6	124	409	38	129	700	0.019	0.9 > P > 0.75

[a] Number of bristles in the indicated χ^2 cell.

[b] y = yellow inactivated (yellow bristle), y$^+$ = yellow expressed (wild-type coloration); Sb$^+$ = Stubble inactivated (wild-type bristle), Sb = Stubble expressed (Stubble bristle).

other two examples of non-independent inactivation involve deficiencies for the 25F-26A modifier. Two of the three deficiencies in this region had χ^2 values near to or greater than the critical value for 0.05 probability. The *Df(2L)E66* value of 4.901 is greater than the critical value of 3.841 while the number obtained for *Df(2L)cl-h3* was just below the critical value at 3.835. The third example, *Df(2L)clot-7* was well below these values at 0.347 (probability between 0.75 and 0.5). Observations on maternal effects associated with the *Df(2L)clot-7* chromosome suggest that there is another modifier located on this chromosome that may account for the differences between *Df(2L)clot-7* and the other two deficiencies in this region (C. JACKSON and C. P. BISHOP, manuscript in preparation).

An analysis of the individual cells' χ^2 values of *Df(2L)E66* and *Df(2L)cl-h3* indicated that no single cell can be rejected (Table 3). A comparison of the observed to the expected values, however, reveals that both experiments had more bristles than expected in which either both compaction events occurred (y; Sb$^+$) or in which neither event occurred (y$^+$; Sb) while there were less than the expected number in which only one of the two compaction events occurred (y, Sb and y$^+$, Sb$^+$).

DISCUSSION

Results from studies of variegating mutants have been difficult to compare because of differences in environmental and genetic backgrounds in the various experiments. In order to eliminate these complications, a stock (*In(1)y^{3P}; T(2;3)Sb^V/+; TM2*) was established where the spread of heterochromatin to inactivate two genes on different chromosomes could be monitored within the same cell. Because the inactivation is occurring within the same nucleus, both events are subjected to the same environmental and genetic influences.

The advantages of the *In(1)y^{3P}; T(2;3)Sb^V* system are: 1) *In(1)y^{3P}* and *T(2;3)Sb^V* each affect different aspects of bristle morphology, bristle coloration and length, respectively; 2) they involve different chromosomes and 3) one is sex-linked (*In(1)y^{3P}*) and the other (*T(2;3)Sb^V*) acts to suppress a dominant mutation. These three features permit the two variegating mutants to be brought together in the same nucleus and scored independently. Additionally, because one variegating mutant can be scored as a hemizygote and the other as a heterozygote, second and third chromosome genetic modifiers can easily be placed in the same genome. Since both inactivation events occur within the same nucleus, any differences observed must be due to intrinsic differences in the spreading to, or compaction of, the two reporter genes, *yellow* and *Stubble*.

If the process of compacting DNA into heterochromatin was always identical, we would anticipate that genetic modifiers would have a similar impact on the process for each example of heterochromatin formed. The results presented in this paper, however, are not consistent with this idea. Table 1 indicates that while some of the modifiers behave as anticipated, affecting both variegating genes in a similar manner, others only affect one of the two variegating genes. These results suggest that different components may be involved in the decision to compact separate stretches of DNA into heterochromatic domains.

The results in Table 1 suggest that there is a gene or genes contained in the 24A-25E region that act(s) to suppress the inactivation of *In(1)y^{3P}* while being neutral or perhaps enhancing *T(2;3)Sb^V* inactivation. The *Df(2R)pK78K* deficiency had the opposite effect, enhancing *In(1)y^{3P}* inactivation while being neutral or slightly suppressing that of *T(2;3)Sb^V*. Although *Df(2L)H2O* enhances the inactivation of the *white* gene

TABLE 3

χ^2 analysis of the individual cells for *Df(2L)cl-h3* and *Df(2L)E66*

Cells	y,Sb[a,b]	y⁺,Sb	y,Sb⁺	y⁺,Sb⁺	*n*
Df(2L)cl-h3					
Observed	145	88	191	80	504
Expected	157.83	79.76	176.98	89.44	
χ^2	1.043	0.852	1.111	0.996	
Probability	$0.5 > P > 0.25$	$0.5 > P > 0.25$	$0.5 > P > 0.25$	$0.5 > P > 0.25$	
Df(2L)E66					
Observed	311	122	201	52	686
Expected	323.97	112.13	185.66	64.25	
χ^2	0.519	0.871	1.268	2.337	
Probability	$0.5 > P > 0.25$	$0.5 > P > 0.25$	$0.5 > P > 0.25$	$0.25 > P > 0.1$	

[a] Number of bristles observed or expected, expected = [(y%) (Sb%)/100] [*n*].
[b] y = yellow inactivated (yellow bristle), y⁺ = yellow expressed (wild type coloration); Sb⁺ = Stubble inactivated (wild-type bristle), Sb = Stubble expressed (Stubble bristle).

in *In(1)w^{m4}*, it has little effect on either the *yellow* or *Stubble* gene in the *In(1)y^{3P}; T(2;3)Sb^{V}* system.

Precisely how these modifiers exert their different influences on the two compaction events will depend upon the mechanism(s) employed to regulate the spread of heterochromatin. Control may reside in the heterochromatic component, such that heterochromatin will form and spread whenever the appropriate DNA binding proteins are present. In this instance the formation of heterochromatin will be controlled by the expression of the appropriate proteins. Both the timing of the formation of heterochromatin and the extent of spreading may be regulated by the appearance and amount of these proteins. If this is the correct mechanism, the response to the different genetic modifiers may be due to the involvement of different structural proteins in the formation or degradation of different heterochromatic domains. The initial formation of heterochromatic domains at the cellular blastoderm stage is probably regulated by the expression of at least some of these genetic modifiers.

In contrast, the ability of heterochromatin to form and spread may be dependent upon the existing chromatin (euchromatin). In this model, heterochromatin may act as an opportunistic invader spreading whenever conditions in euchromatin permit it to spread. The class II modifiers at 24A-25E, 36A-36E and 42E-43C may encode for a structural component of euchromatin that binds to DNA and prevents the spreading of heterochromatin. The dosage response of these class II modifiers is most consistent with encoding for a structural component of euchromatin (TARTOF *et al.* 1989). Although none of the class II dosage sensitive modifiers of variegation have been cloned and sequenced, the two class I modifiers that have been cloned, *Su(var)205* (EISSENBERG, ELGIN and JAMES 1987) and *Suvar(3)7* (REUTER *et al.* 1990) each encode for a structural component of heterochromatin and it is reasonable that most, if not all, modifiers of variegation encode for structural components of chromatin. Increasing the dosage of class II modifiers, as with *Dp(2;2)AM3* and *Dp(2;2)AM17*, should suppress the formation of heterochromatin–precisely the affect these two modifiers have upon the compaction of *yellow* in *In(1)y^{3P}* (Table 1). Conversely, a deletion of a structural component of euchromatin would enhance the compaction of DNA into heterochromatin, as with the affect *Df(2R)pK78K* had upon the compaction of *yellow* into heterochromatin in *In(1)y^{3P}* and of the affect that *Df(2L)H2O* has upon the *white* gene in *In(1)w^{m4}*.

This interpretation is consistent with recent results, summarized by EISSENBERG and ELGIN (1991), suggesting the existence of euchromatic domains that serve to functionally isolate genes from nearby chromosomal influences. The limits of such domains may be defined by the presence of scaffold attachment regions (SAR) as in the chicken lysozyme gene (STIEF *et al.* 1989 and BONIFER *et al.* 1990) or specialized chromatin structure (scs) elements as shown for the heat shock gene *hsp 70* in *D. melanogaster* (KELLUM and SCHEDL 1991). From these observations it is a logical step to hypothesize that separate euchromatic domains may be established and maintained by the attachment of different sequence-specific DNA binding proteins. The greater the amount of the specific DNA binding protein that is present, the greater the likelihood of maintaining the euchromatic domain in the presence of potentially spreading heterochromatin. The modifiers found at 24A-25E and 42E-43C may be involved in the establishment/maintenance of a euchromatic domain for the *yellow* gene (or perhaps a domain between that of the *yellow* reporter gene and the juxtaposed initiation site for the spreading of heterochromatin).

The two models presented here for regulating the compaction of DNA into either euchromatin or heterochromatin are not mutually exclusive. The deci-

sion to compact a given DNA sequence into either euchromatin or heterochromatin may involve both euchromatic and heterochromatic components. The formation of different heterochromatic domains might involve the expression of a specific set of gene products that recognize and bind to a specific initiation site. The propagation of heterochromatin into adjacent DNA sequences may subsequently be dependent upon both the presence of the appropriate heterochromatic proteins and upon the permissiveness of the chromatin encountered by the heterochromatin.

These mechanisms can explain the precise developmental control exerted over heterochromatic compaction of one *X* chromosome in *XX* females, inactivation of the *Y* chromosome in somatic Drosophila tissues, and the inactivation of the *X* chromosome in *XY* males during spermatogenesis. A single *X* chromosome could remain euchromatic, while additional ones become compacted into heterochromatin, by expressing a sequence-specific, euchromatic DNA binding protein that blocks the propagation of heterochromatin at sufficiently low concentrations that only one *X* chromosome receives this protection. All other *X* chromosomes would be vulnerable to compaction into heterochromatin. Compaction of the *Y* chromosome into heterochromatin in somatic tissues could be achieved by failure to express euchromatic proteins that recognize and bind to *Y* sequences, and similarly, compaction of the *X* into heterochromatin during spermatogenesis could occur by failure to express an *X* chromosome specific euchromatic protein. Alternatively, compaction of the *Y* in somatic cells and compaction of the *X* chromosome during spermatogenesis could be due to the expression of heterochromatic proteins that specifically bind to *Y* chromosomal or *X* chromosomal sequences. Thus, precise developmental control over where and when heterochromatin spreads may be achieved by regulating the expression of either euchromatic or heterochromatic, sequence-specific, DNA binding proteins.

Yet another way to regulate the spread of heterochromatin is to control the relative amount of both the euchromatic and heterochromatic DNA binding proteins. This model would not necessitate the existence of proteins which recognize specific DNA sequences. The distance that heterochromatin spreads would be controlled by the relative concentration of the competing, nonspecific euchromatic and heterochromatic proteins. Developmental control over genes adjacent to heterochromatin could be exerted in a crude fashion by this mechanism. The differences in response to the genetic modifiers presented in this study, to be consistent with this hypothesis, would require the *Stubble* gene be expressed prior to the expression of both the genetic modifiers and that *yellow* expression occur after the expression of the modifiers. The late expression of the class II modifiers would reverse the compaction into heterochromatin to allow for the *yellow* gene to be expressed. This mechanism would not, however, allow any control over where heterochromatin spreads and would result in all heterochromatin spreading when the concentration of heterochromatic proteins is high and retreating when it is low. This model, therefore, cannot account for the selective compaction into heterochromatin of one *X* chromosome in *XX* female mammals, the *Y* chromosome in somatic cells of Drosophila, nor the *X* chromosome during spermatogenesis in *XY* males. In contrast, regulating the expression of sequence-specific DNA binding proteins can account for all of the data.

If the compaction of separate stretches of DNA into heterochromatin involves different components, as the results presented here suggest, then we might expect the compaction events to occur independently of each other. The analysis of independence of the two compaction events (the inactivation of *yellow* in *In(1)y*^{3P} and *Stubble* in *T(2;3)Sb*^{V}) presented in Table 2 indicate that indeed, with one exception, the two events proceed independently of each other. These results are thus consistent with there being fundamental differences in the process of compacting different DNA sequences into heterochromatin. The likelihood of either event occurring, however, would be changed in the same fashion by altering the amount of any components the two systems have in common. This was observed with most of the genetic modifiers.

The only possible exception to this rule involves the modifier found in the 25F-26A region. The presence of this modifier region appears to act in an all or none fashion (Table 3), either causing both compaction events or not allowing either to occur. This might indicate that the 25F-26A gene is expressed to different extents in different cells. Those cells in which the gene is expressed at a high level produce sufficient product to increase the compaction of both genes while reduced or no expression would reduce the likelihood that either gene is inactivated.

The differences reported in the response to specific genetic modifiers of two different heterochromatic events, within the same nucleus, raises the possibility that these differences may be exploited to developmentally regulate gene expression. The expression of genes on the *Y* chromosome during spermatogenesis, contrasted to the heterochromatic nature of the *Y* in somatic tissue of Drosophila, precocious *X* chromosome inactivation (also during spermatogenesis), as well as the maintenance of both *X* chromosomes in the euchromatic state in certain tissues in female mammals while compacting all but one *X* chromosome in other tissues, may be the most obvious examples of a

general system for regulating gene expression by selective compaction into heterochromatin.

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