bral cortex (K_M 0.021 μM, V_{max} 320 fmole/min-mg protein) (*11*). For this type of deiodinase sulfation does not appear to enhance deiodination. It remains to be established to what extent sulfation determines the hepatic deiodination and clearance of iodothyronines in vivo. Our results support the concept that sulfation is not merely a means to facilitate biliary and urinary excretion of hydrophobic aglycons (*12*).

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15. Supported by grant 13-34-110 from the Division for Health Research TNO. We thank G. Hennemann, R. Docter, and E. P. Krenning for advice; H. F. Bernard, J. Blom, and M. A. C. van Loon for technical assistance; and Y. J. van Dodewaard for secretarial assistance.

4 April 1983

A Gene Controlling Condensation of Heterochromatin in *Drosophila melanogaster*

Abstract. *A temperature-sensitive lethal mutant of* Drosophila melanogaster *was used to identify an essential cell cycle function that is necessary for the mitotic condensation of heterochromatic but not of euchromatic portions of the genome. This mutant is an allele at a locus (*mus-101*) identified earlier by the use of mutagen-sensitive mutants. The data suggest that the mutagen-sensitive and repair-defective phenotypes of viable* mus-101 *mutants result from a disruption in chromosome organization.*

The DNA of eukaryotic cells is condensed some 7000-fold into metaphase chromosomes with such exquisite precision that each cell cycle reproduces chromosomes of constant size, arm ratio, and features of longitudinal differentiation such as primary and secondary constrictions, differential condensation of euchromatin and heterochromatin, and chromosome banding. Although the early steps of DNA packaging (initial coiling into 100-Å filaments and folding into 250-Å fibers) are relatively well defined, the subsequent steps in chromatin condensation are much less well understood (*1*). One approach to deciphering the processes of chromosome packaging is the analysis of mutants affecting chromosome condensation. Mutants in genes necessary for the occurrence of many steps in the cell cycle have been known in yeast and in mammalian tissue culture cells for some time [for reviews see (*2*)]. More recently, we have identified mutants defective in a variety of processes necessary for normal mitotic chromosome behavior in *Drosophila melanogaster* (*3–8*). In this report, we describe mutants at one of these loci whose wild-type product is necessary for the mitotic condensation of heterochromatin but not euchromatin.

One of our methods for identifying mutants in genes specifying essential mitotic functions has been to isolate temperature-sensitive (ts) lethal mutants and then screen them under semirestrictive conditions for those that increase the frequency of chromosome instability (*2, 3*). From a collection of 168 X-linked ts lethal mutants, we recovered 15 that affect mitotic chromosome behavior. Details of the isolation and preliminary characterization of all 15 mutants will be given elsewhere (*7*).

One of the X-linked ts lethal mutants is *mus-101*^{ts1}; adults homozygous or hemizygous for this mutation do not survive at temperatures above 22°C. Chromosome instability, detected both genetically and cytologically, is also temperature-sensitive [see (*7*) and below]. Conventional mapping placed *mus-101*^{ts1} at 41.6 on the X chromosome, and deficiency mapping showed that both the phenotypes of ts lethality and increased chromosome instability are uncovered by the deficiency *Df(1)HA92* (12A6,7 to 12D3) (*4*). These findings provide strong evidence that lethality and chromosomal effects result from the same mutation.

The mutagen-sensitive locus *mus-101*, previously identified by use of two homozygous viable mutants exhibiting hypersensitivity to killing by mutagens (*9*) and a defect in postreplication repair (*10*), is also located in the region uncovered by *Df(1)HA92* (*4, 11*). Complementation tests based on the phenotypes of methyl methanesulfonate sensitivity, female sterility, and mitotic chromosome instability all showed *mus-101*^{ts1} to be an allele at the *mus-101* locus (*7*).

To characterize the effect of *mus-101*^{ts1} cytologically, we shifted third-instar larvae reared at 18°C to the restrictive temperature of 29°C for various periods of time and made metaphase chromosome preparations by squashing ganglia in acetic orcein (*12*). When *mus-101*^{ts1} cells are kept at 18°C, they are cytologically normal and are indistinguishable from wild-type control cells. However, after 2 hours at 29°C there is a dramatic effect on the heterochromatic Y chromosome (Fig. 1 and Table 1). In more than two-thirds of metaphase preparations, the Y chromosome appears elongated and, in many cases, it exhibits one or more stretched areas of variable extension (Fig. 1, b to d versus a). After longer treatments, nearly all metaphases exhibit a clear undercondensation of the Y chromosome. After 2 hours at 29°C, a few cells with undercondensed autosomal centric heterochromatin are also observed. In many cases, thin threads of chromatin connecting the two autosomal arms are visible (Fig. 1, e and f), whereas in others the undercondensation of heterochromatin is so drastic that it mimics a chromosome break (Fig. 1d). In most affected cells exposed for 2 or 4 hours to the restrictive temperature, only one autosome (Fig. 1d) or a pair of autosomes (Fig. 1e) are affected; however, at subsequent times, cells showing undercondensation of the heterochromatin of all chromosomes are also observed (Fig. 1, f to i). The proportion of cells with a complete failure of heterochromatin condensation increases with the treatment time and at 12 and 24 hours is about one-third of the affected cells.

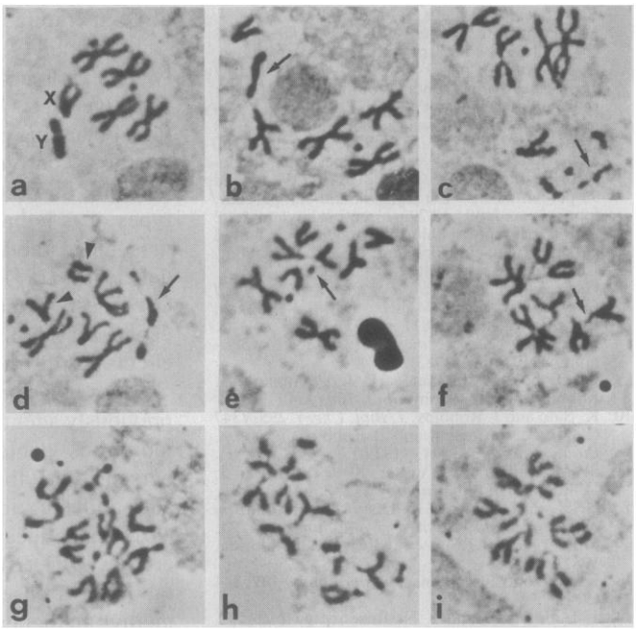

Fig. 1. Effects of temperature shifts on larval neuroblasts of *mus-101*^{ts1}. (a) *mus-101*^{ts1} grown at 18°C; the main heterochromatic regions are the entire Y chromosome, centric 20 percent of the major autosomes, and proximal 40 percent of the X chromosome. (b) Cell with elongated Y (arrow). (c and d) Cells showing very long Y chromosomes with stretched areas (arrows); in (d), drastic undercondensation of part of the heterochromatin of one autosome mimics an isochromatid break (arrowhead). (e) Cell showing one pair of autosomes with undercondensed heterochromatin (arrow). (f) Cell with both pairs of autosomes affected and the proximal X heterochromatin undercondensed (arrow). (g to i) Cells in which most, if not all, heterochromatin is undercondensed.

Uncondensed heterochromatin was also consistently observed in prophase chromosomes; no effects on euchromatin were detected at any phase of mitosis of any treatment time. These results indicate that the cytological picture observed in *mus-101*^{ts1} is due to a specific failure of heterochromatin condensation and not to the decondensation of already contracted heterochromatic material.

These data show that the condensation of all major heterochromatic regions of the *Drosophila* genome can be affected in *mus-101*^{ts1}. However, it is also evident that even after long times at the restrictive temperature, a substantial fraction of cells do not exhibit any cytological defect or only show uncondensed Y chromosomes, as though *mus-101*^{ts1} might be leaky even at 29°C. To test this, we examined chromosome condensation in female larvae carrying only one copy of *mus-101*^{ts1} [*mus-101*^{ts1}/*Df(1)HA92*] after a shift from 18° to 29°C. The percentage of affected cells in *mus-101*^{ts1}/*Df(1)HA92* females was always higher than that observed in *mus-101*^{ts1}/*mus-101*^{ts1} females (Table 1). After 12 and 24 hours at 29°C, 79 percent and 86 percent, respectively, of the metaphases were affected in the hemizygous females. Moreover, most of these metaphases exhibit undercondensation of all heterochromatic regions. These results demonstrate that the less severe effect seen in *mus-101*^{ts1} males and *mus-101*^{ts1}/*mus-101*^{ts1} females is the result of residual wild-type activity in the mutant even at the restrictive temperature.

Although it is clear that condensation of all heterochromatin is ultimately affected in *mus-101*^{ts1}, not all regions are equally sensitive to the mutation. For example, after 2 or 4 hours at 29°C only the Y chromosome, a pair of autosomes and, less frequently, the X chromosome are affected. This situation is reminiscent of the effect of the compound Hoechst 33258 on living *Drosophila* neuroblasts; after short incubations with this compound, only portions of the Y, X, and chromosome 3 heterochromatin are uncondensed (*13*). To examine whether the autosomal regions that are Hoechst-sensitive are also the ones preferentially affected by *mus-101*^{ts1}, we constructed *mus-101*^{ts1} males heterozygous for *T(2,3)bw*^{vDe4}, a genotype in which all chromosome arms are readily recognized (*6*, *13*). The analysis of larval neuroblasts of these males exposed to 29°C for 4 hours showed that *mus-101*^{ts1} preferentially affects chromosome 2 heterochromatin and thus induces a different pattern of decondensation than does Hoechst 33258.

Table 1. Effects of temperature shifts on larval neuroblasts of *mus-101*^{ts1}. Abbreviations: *m*, *mus-101*^{ts1}; *Df*, *Df(1)HA92*; *FM7* (*15*), a balancer chromosome carrying *mus-101*$^{+}$.

Genome tested	Time at 29°C (hours)	Number of cells scored	Percentage of cells showing uncondensed chromosomes	
			Y	At least one X or autosome
m/Y	0	300	0	0
m/m	0	698		0
m/Df	0	329		0
m/Y	2	462	68.0	5.0
m/m	2	417		3.1
m/Df	2	637		13.0
m/Y	4	485	92.8	13.6
m/m	4	528		13.8
m/Df	4	477		41.3
m/Y	6	478	96.5	21.0
m/m	6	568		16.7
m/Df	6	337		40.9
m/Y	8	881	96.7	27.0
m/m	8	842		33.5
m/Df	8	484		70.0
m/Y	12	485	97.3	32.0
m/m	12	424		33.3
m/Df	12	481		79.0
m/Y	24	765	96.1	38.8
m/m	24	874		37.6
m/Df	24	858		86.2
m/FM7	24	800		0

To ascertain whether the cytologically detectable effects of *mus-101*^{ts1} are the result of gross defects in DNA synthesis in heterochromatic portions of the genome, we asked whether a temperature shift could induce a failure of condensation in post–S phase (G_2) cells. Neural ganglia of mature *mus-101*^{ts1}/*Df(1)HA92* larvae grown at 18°C were shifted to 29°C in saline containing tritiated thymidine (5 μCi/ml) and fixed after 2 and 4 hours. The slides were then autoradiographed (*14*) and scored for the labeling pattern of both uncondensed and normal cells. After 4 hours of treatment, nearly all metaphases were heavily labeled, and grains were often observed on the thin heterochromatic filaments connecting autosomal arms. Of the 274 cells scored after 2 hours at 29°C, there were 125 unlabeled normal cells, 124 normal labeled cells, 14 unlabeled affected cells, and 17 labeled affected cells. These results demonstrate that uncondensed heterochromatic regions incorporate tritiated thymidine during the S phase and that the failure of condensation can be induced in G_2 cells. Although these data do not rule out a subtle effect of *mus-101*^{ts1} on DNA replication indirectly affecting condensation, they suggest that the normal wild-type *mus-101*$^{+}$ product is directly involved in chromosome packaging.

The above data establish that the *mus-101*$^{+}$ locus encodes an essential cell cycle function that is specifically required for heterochromatin condensation. This suggests that the mutagen-sensitive (*9*) and repair-defective (*10*) phenotypes shown by the viable *mus-101* alleles are secondary consequences of a primary effect on chromosome condensation that either renders chromatin more susceptible to mutagen damage or less available to repair. Moreover, our findings that

mus-101$^+$ as well as two other *mus* loci (*mus-105*$^+$ and *mus-109*$^+$) (*6*) encode essential functions indicate that a substantial fraction of eukaryotic mutagen-sensitive and repair-defective mutants may be leaky mutants in essential loci.

The cytological analysis of *mus-101* mutants demonstrates that the condensation of euchromatic and heterochromatic portions of the genome is, at least in part, under separate genetic control. That other functions required for mitotic contraction of both euchromatin and heterochromatin can be identified is indicated by a cytological screening of a large collection of lethal mutants, which has led to the isolation of six other mutants affecting chromosome condensation (*8*). Thus, a cytogenetic approach to identifying genes whose products are responsible for the condensation and longitudinal differentiation of chromosomes is feasible. Mutations in these genes should provide probes that will aid in deciphering the organization and function of the eukaryotic chromosome.

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16. Supported by Euratom grants BIO-E-400 and BIO-E-450, by Public Health Service grant GM23345, by Italy–United States cooperative research grant INT-8203086, and by NATO grant 134.82.

10 March 1983

Inhibitory Conductance Changes at Synapses in the Lamprey Brainstem

Abstract. *Although the conductance and kinetic behavior of inhibitory synaptic channels have been studied in a number of nerve and muscle cells, there has been little if any detailed study of such channels at synapses in the vertebrate central nervous system or of the relation of such channels to natural synaptic events. In the experiments reported here, current noise measurements were used to obtain such information at synapses on Müller cells in the lamprey brainstem. Application of glycine to the cells activated synaptic channels with large conductances and relaxation time constants (70 picosiemens and 33 milliseconds, respectively, at 3° to 10°C). Spontaneous inhibitory synaptic currents had a mean conductance of 107 nanosiemens and decayed with the same time constant. In addition, the glycine responses and the spontaneous currents had the same reversal potential and both were abolished by strychnine. These results support the idea that glycine is the natural inhibitory transmitter at these synapses and suggest that one quantum of transmitter activates about 1500 elementary conductance channels.*

The characteristics of channels activated by various putative inhibitory synaptic transmitter substances have been studied in a number of nerve and muscle cells (*1*, *2*). However, except for a study of inhibitory voltage noise (*3*), we know of no detailed analysis of such channels at synapses in the vertebrate central nervous system. Conductance changes underlying inhibitory potentials at such synapses tend to be large and of long duration (*4*), and it would be of interest to know whether these properties reflect the characteristics of individual inhibitory channels. In addition, the characteristics of drug-activated channels can indicate whether the drug under consideration is the natural inhibitory neurotransmitter. Specifically, in addition to having the same pharmacological properties as the natural transmitter, the drug should activate channels with the same reversal potential as the naturally occurring inhibitory events and with compatible kinetic characteristics. Glycine has been shown to increase Cl^- conductance in neurons of the lamprey central nervous system and to mimic the natural inhibitory transmitter pharmacologically (*5*). We now report that in brainstem reticu-

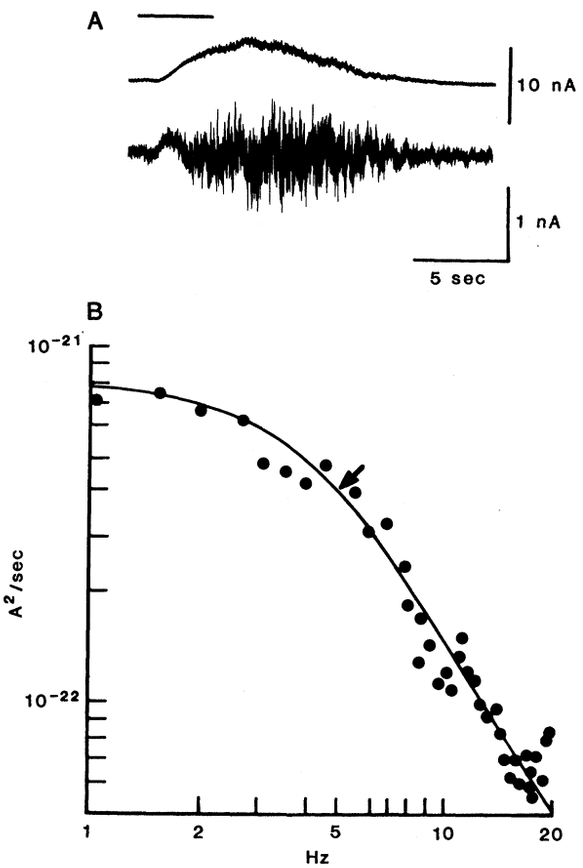

Fig. 1. (A) Current noise produced by a brief exposure of a Müller cell to glycine. Drug application (bar) produced a peak outward current of 5.6 nA (upper trace). The accompanying increase in baseline noise (lower trace) was obtained at a higher gain through an a-c amplifier with a bandpass response of 0.75 to 140 Hz (half-power points). (B) Spectral density distribution from the same cell as in (A). Data points represent the mean of 16 difference spectra, smoothed by the weighted averaging of adjacent points in groups of five. Smooth curve of the form $S(f) = S(0)[1 + (f/f_c)^2]^{-1}$ was fitted by eye with $S(0) = 8 \times 10^{-22}$ A^2/sec and $f_c = 5$ Hz. Holding potential, reversal potential, and mean current were −60 mV, −70 mV, and 6.6 nA, respectively. Calculated channel conductance was 95 pS and mean open time 31.8 msec (3°C).

1 JULY 1983