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Temperature-induced alteration of the polytene X chromosome structure in male larvae of the strain *In(1)B^{M2}* (*reinverted*) of *Drosophila melanogaster*

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Summary

In *Drosophila melanogaster*, the polytene X chromosome of male third instar larva appears twice as wide as an unpaired female X chromosome or an autosome. This characteristic morphology of the male X chromosome is correlated with the increased rate of transcription of the sex-linked genes, which ensures gene dosage compensation. In male third instar larvae of the strain *In(1)B^{M2}* (*reinverted*), polytene nuclei manifest unusually puffy X chromosomes at 18±1 °C. Such 'puffy X' chromosomes are pompons, that is, despite the increased width of the chromosome, transcription remains at the wild-type level. This characteristic is a caveat to the invariable correlation between polytene chromosome puffs and transcription, and suggests that the mutant X chromosomes arise due to perturbation of a pathway that controls the structure but not the transcription of the polytene X chromosome. In this report we present evidence that the pompons of *In(1)B^{M2}* (*reinverted*) arise due to spiralization of the male X chromosome, which results in condensing of the chromosome. This unusual structural alteration can be induced only in male larvae of this strain, at the third instar larval stage, through temperature shifts from 24±1 °C to 18±1 °C and during recovery from cold shock. Furthermore, extract from male adult, pupae and third instar larvae can induce chromosome condensation in wild-type larvae *in vitro*. This new evidence not only explains the absence of correlation between chromosome width and transcription of the pompons of *In(1)B^{M2}* (*reinverted*), but also suggests that the chromosomal rearrangement perturbs a pathway that regulates the condensation of chromosomes.

1. Introduction

Polytene chromosomes of *Drosophila* show qualitative and quantitative variation in puffing pattern during development (Ashburner, 1972). Various mutant strains of *Drosophila* are known in which the polytene chromosomes undergo gross alterations in morphology, resulting in atypical chromosome structures (reviewed in Ashburner, 1972; Zhimulev, 1996). For example, in the strain *lethal* (*3*) *tumorous larvae* (*l(3)tl*), the length and width of all polytene chromosomes are altered, resulting in chromosomes that have increased widths and reduced lengths. The structural alteration is most remarkable for the hemizygous male X chromosome, which resembles a giant puff. Some of these unusually puffy polytene chromosomes are termed pompons (reviewed in Zhimulev, 1996). Unlike polytene chromosome puffs, which represent sites of enhanced transcription, pompons do not show any increase in transcription despite the fact that the chromosome resembles a giant puff. The reason for absence of correlation of structure and transcription of pompons is unknown (Zhimulev, 1996).

The strain *In(1)B^{M2}* (*reinverted*) of *Drosophila melanogaster* was recovered as a spontaneous reinversion in the strain *In(1)B^{M2}* (Mazumdar *et al.*, 1978; Mukherjee & Ghosh, 1986). Adult flies of this strain do not manifest any obvious abnormalities. However, polytene chromosome preparations from third instar larvae show that the male X chromosome undergoes a global alteration in structure, appearing unusually puffy, in about 25% of salivary gland nuclei (Fig. 1*a*). The morphology of the female X chromosome and the autosomes remain unaltered.

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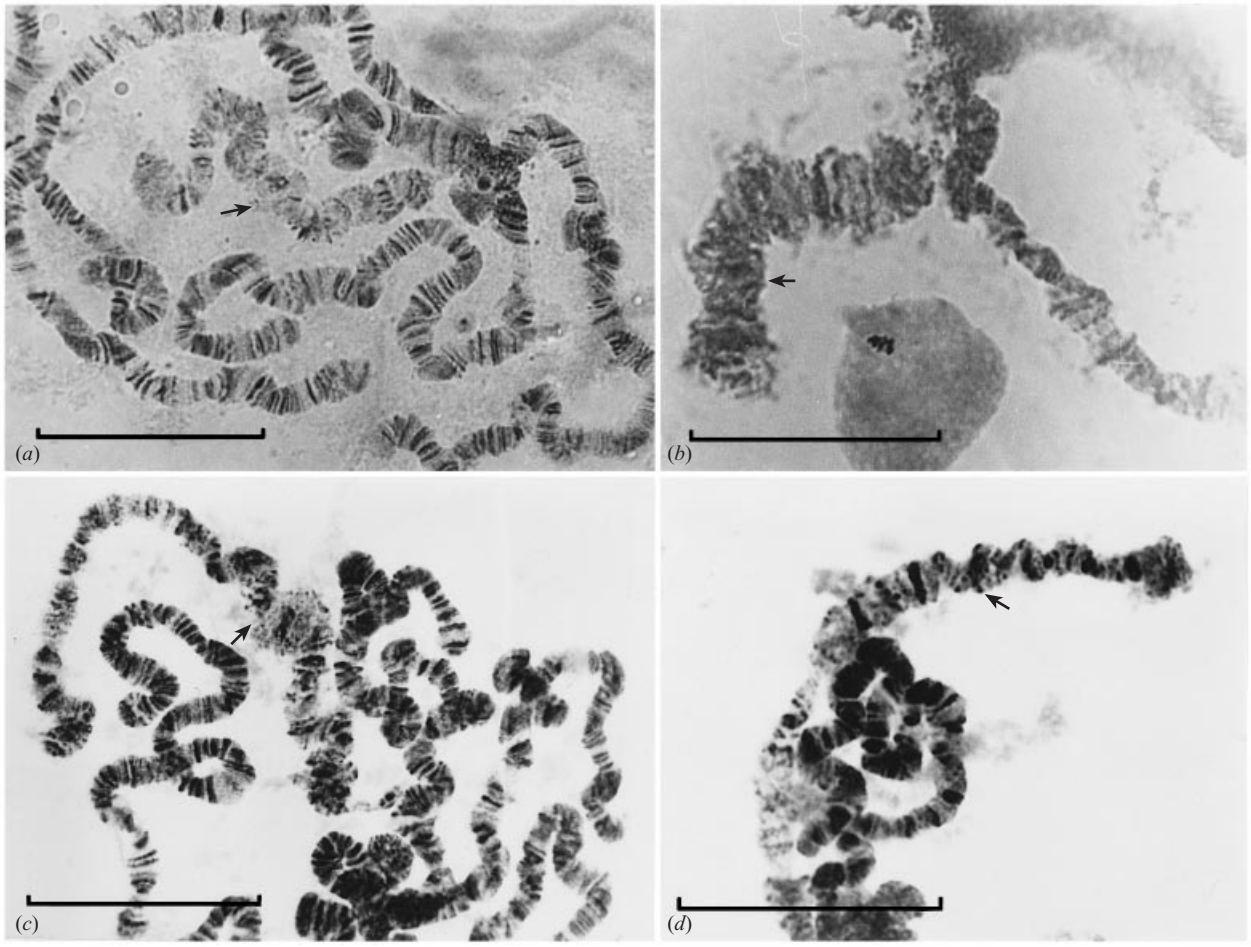

Fig. 1. Structural alteration of the male X chromosome of *In(1)B*^{M2} (*reinverted*). (*a*) Puffy male X chromosome (arrow) of larva reared uninterruptedly at 18 ± 1 °C. (*b*)–(*d*) X chromosome morphology of temperature-shifted male third instar larvae of *In(1)B*^{M2} (*reinverted*). (*b*) Highly condensed X chromosome (arrow) after cold shock at 12 ± 1 °C followed by recovery of larva at 18 ± 1 °C. (*c*, *d*) X chromosomes showing spiralization (arrows), leading to increased width of the chromosome. Scale bars in this and subsequent figures represent 10 μm.

The mutant X chromosomes, termed puffy Xs, are pompons, since the transcription of such chromosomes is not enhanced relative to that of male X chromosomes of wild-type larvae (Kar & Pal, 1995). The sex and chromosome specificity of this alteration in the X chromosome morphology in *In(1)B*^{M2} (*reinverted*) is of significance, since dosage compensation in *Drosophila*, the phenomenon of equalization of the difference in the X-linked gene doses between males and females, is brought about through a twofold increase in the level of X chromosome transcription in males, relative to a single female X chromosome (Mukherjee & Beermann, 1965).

In *Drosophila melanogaster*, the increased transcription of the male X chromosome has been correlated with the morphology of the polytene X chromosome, which appears diffuse and puffy and twice as wide as that of a single female X chromosome or autosome (Offermann, 1936; Aronson *et al.*, 1954; Dobzhansky, 1957). An invariable correlation has been observed between the structure of the male X chromosome and the function of the dosage compensation genes (Belote & Lucchesi, 1980; Lucchesi & Skripsky, 1981). The sex and chromosome specificity of the puffy Xs, and the fact that global alteration in chromatin structure is associated with the process of dosage compensation in other organisms, suggested that the chromosomal rearrangement in this strain perturbs the functions of a known or unknown regulator of the dosage compensation pathway (Mukherjee & Ghosh, 1986; Lucchesi & Manning, 1987; Baker *et al.*, 1994; Mukherjee & Basu, 1997).

Although *In(1)B*^{M2} (*reinverted*) was recovered in 1978 (Mazumdar *et al.*, 1978), the molecular basis of alteration of the architecture of the male X chromosome is unknown. In this report we show that the width increase of the pompons of *In(1)B*^{M2} (*reinverted*) is brought about by a topological alteration that results in condensing the male X chromosome, thereby causing an increase in width and reduction in length

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of the chromosome. This alteration can be induced at the third instar larval stage through temperature shifts. The evidence not only provides an explanation for the absence of correlation between structure and transcription of the puffy Xs, but also suggests that the chromosomal rearrangement of *In(1)B^{M2}* (*reinverted*) affects a pathway that controls the higher-order organization of the polytene chromosomes.

2. Materials and methods

(i) *Strain*

The strain *In(1)B^{M2}* (*reinverted*) was recovered as a spontaneous reinversion in the strain *In(1)B^{M2}* (Mazumdar *et al*., 1978). The characteristics of this strain have been described in Mukherjee & Ghosh (1986). The isolate of the strain used in the experiments described here was recovered in 1991 through pair-mating flies from the original strain. A preliminary characterization of this strain has been reported earlier (Kar & Pal, 1995; Kulkarni-Shukla & Kar, 1999). Flies were routinely maintained at either 18±1 °C or 24±1 °C on standard cornmeal–molasses–agar medium, containing *n*-methylparaben as mould inhibitor.

(ii) *Temperature shift experiments*

For temperature downshift experiments, flies were allowed to lay at 24±1 °C for 4 h, after which adults were removed. Downshifts from this temperature to 18±1 °C were performed at the time points indicated in Section 3. Reciprocal experiments were performed by temperature-shifting cultures that had been reared at 18±1 °C to 24±1 °C. Cold shock and recovery experiments were performed by temperature downshift from 24±1 °C to 12±1 °C for 4 h, followed by recovery at room temperature (24±1 °C) or 18±1 °C for the time periods indicated below. In all experiments, rearing was continued at the specified temperature until the third instar larval stage, after which polytene chromosomes were prepared using routine methods. Chromosomes were photographed using an Olympus CH-2 photomicroscope.

3. Results and discussion

The structural alteration of the male X chromosome of *In(1)B^{M2}* (*reinverted*) arises due to position effect variegation (PEV) (Bose & Duttaroy, 1986; Kar, unpublished observations). Evidence from earlier studies suggested that the chromosomal rearrangement of *In(1)B^{M2}* (*reinverted*) affects the structure but not the transcription of the male X chromosome (Kar & Pal, 1995). The evidence is: (i) Absence of correlation between structure and transcription of the male X chromosome. (ii) Heat shock results in the selective alteration of the male X chromosome morphology. About 10% of these chromosomes appear narrow with a reduction in chromosome width. (iii) Upon recovery from heat shock, the number of puffy Xs increases compared with control heat-shocked salivary glands that have not been allowed to recover from heat stress. These results imply *de novo* formation of mutant X chromosomes during recovery from temperature shock. In the present report we describe further experimental evidence that shows that the puffy Xs arise due to perturbation in a mechanism that controls the condensation of the polytene male X chromosome.

The expression of the puffy Xs is temperature dependent. Puffy Xs are observed in about 25% of polytene nuclei of larvae reared uninterruptedly at 18±1 °C until the third instar larval stage (Fig. 2*a*, BM2 18C). At 24±1 °C, all Xs show normal morphology (Fig. 2*a*, BM2 24C) identical to that of wild-type larvae reared either at 18±1 °C (Fig. 2*a*, WT 18C) or at 24±1 °C (data not shown). To determine whether temperature shifts from 24±1 °C to 18±1 °C could result in the induction of puffy Xs, larvae at the third instar larval stage (120±4 h of development), were subjected to temperature shift (Fig. 2*b*). The results showed that temperature downshift could induce structural alteration of the male X chromosome, such that a small number of puffy Xs could be scored in salivary gland nuclei of temperature-downshifted third instar larvae (Fig. 2*b*, 20–120 h). However, the number of puffy Xs that could be induced through temperature shifts was significantly less than that observed in preparations from larvae reared uninterruptedly at 18±1 °C (Fig. 2*b*, con). Interestingly, puffy Xs could also be induced when cultures at various stages of development were shifted from 24±1 °C to 18±1 °C (Fig. 2*b*). The structure of the female X chromosome and autosomes remained unaltered. Puffy Xs were not induced when cultures were shifted from 18±1 °C to 24±1 °C at any time during development (data not shown). These results indicated that the topological alteration was independent of the process of polytenization, and could be induced when this process was nearly completed.

Temperature downshifts from 24±1 °C to 12±1 °C also resulted in induction of puffy Xs (Fig. 3*a*, 0 h). Downshift of third instar larvae from 24±1 °C to either 12±1 °C (Fig. 3*a*, *b*) or 18±1 °C (Fig. 3*c*) followed by recovery at either 18±1 °C (Fig. 3*a*) or 24±1 °C (Fig. 3*b*, *c*) for 1, 2 and 4 h revealed the following. When cultures were shifted from 24±1 °C to 12±1 °C and allowed to recover at 18±1 °C or 24±1 °C, the number of puffy Xs increased during the

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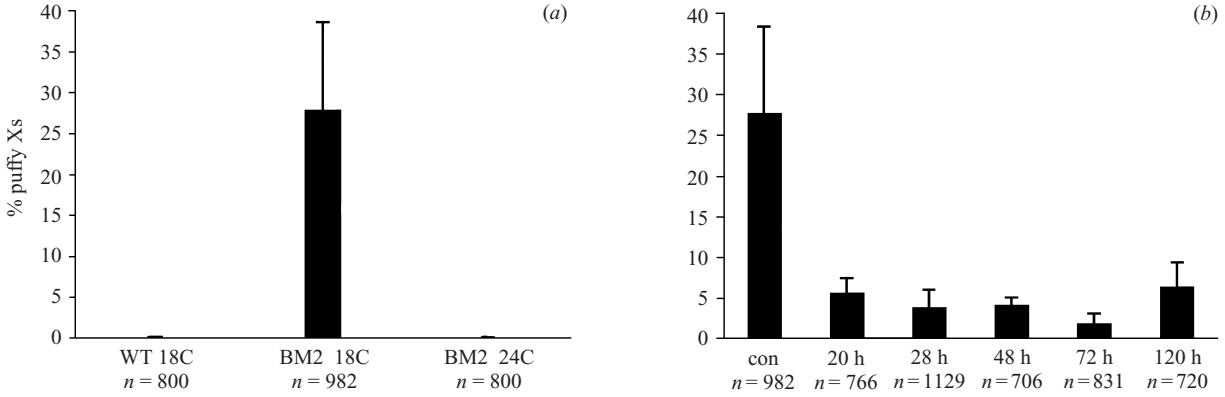

Fig. 2. (*a*) Mean ± SD of the percentage of puffy X chromosomes (puffy Xs) in male third instar larvae of $In(1)B^{M2}$ (*reinverted*) reared at 18 ± 1 °C (BM2 18C). Larvae of the same strain reared at 24 ± 1 °C (BM2 24C) show the normal male X chromosome morphology to that of the wild-type reared either at 18 ± 1 °C (WT 18C) or at 24 ± 1 °C (not shown). (*b*) Mean ± SD of the percentage of puffy Xs induced in third instar male larvae upon temperature downshift from 24 ± 1 °C to 18 ± 1 °C at various developmental stages (20 h, 28 h, 48 h, 72 h and 120 h of development); con represents the percentage of puffy Xs seen in squash preparations of larvae reared at 18 ± 1 °C. *n*, number of salivary gland nuclei scored.

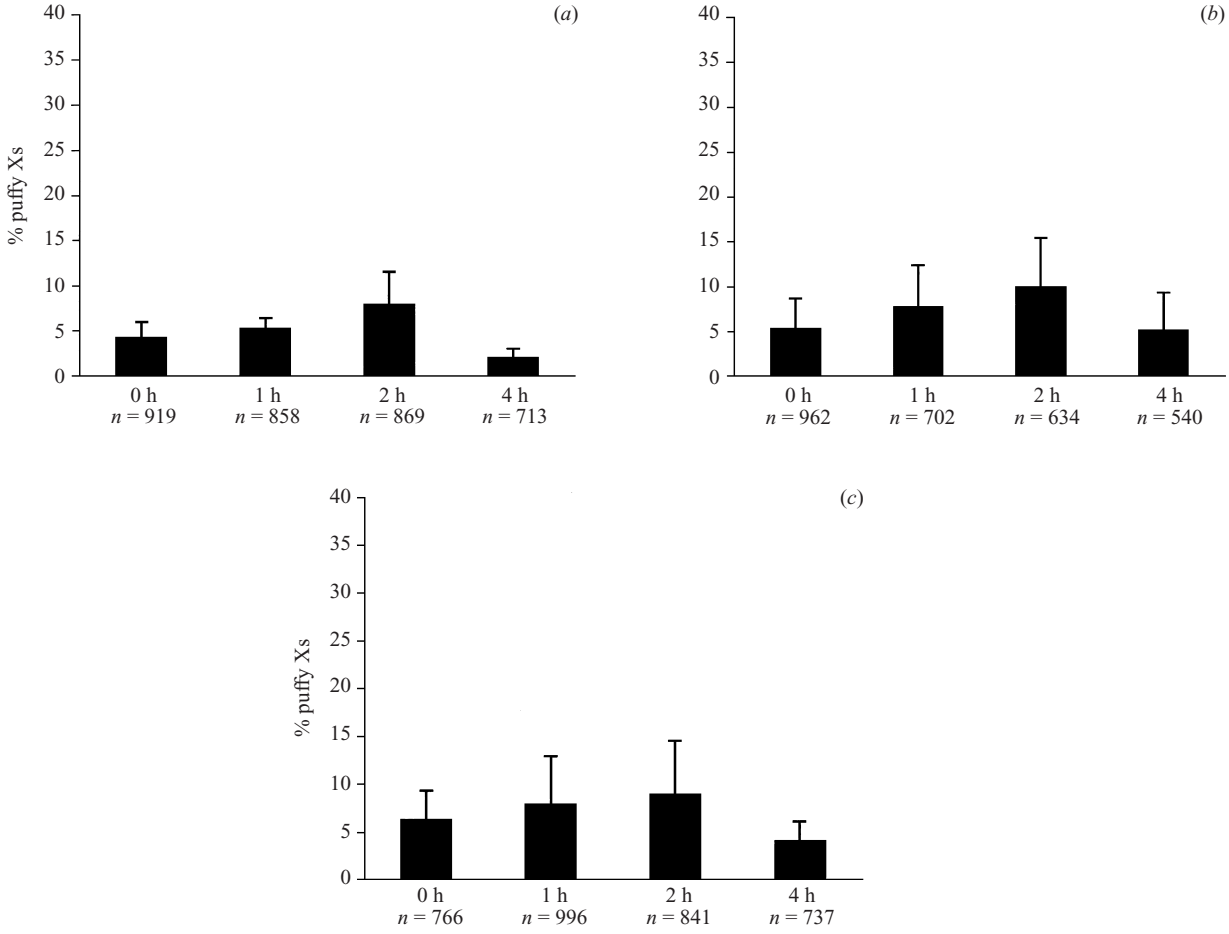

Fig. 3. Mean ± SD of the percentage of puffy X chromosomes induced in male third instar larvae of $In(1)B^{M2}$ (*reinverted*) after temperature downshift from 24 ± 1 °C to 12 ± 1 °C (*a*, *b*; 0 h) or 18 ± 1 °C (*c*; 0 h), followed by recovery at 18 ± 1 °C (*a*) or 24 ± 1 °C (*b*, *c*) for 1 h, 2 h and 4 h. *n*, number of salivary gland nuclei scored.

first 2 h of recovery but returned to the basal level by the fourth hour of recovery (Fig. 3*a*, *b*). Identical observations were made for cultures that were shifted from 24 ± 1 °C to 18 ± 1 °C and allowed to recover at 24 ± 1 °C (Fig. 3*c*). Since puffy Xs are not observed when cultures are reared at 24 ± 1 °C, the structural

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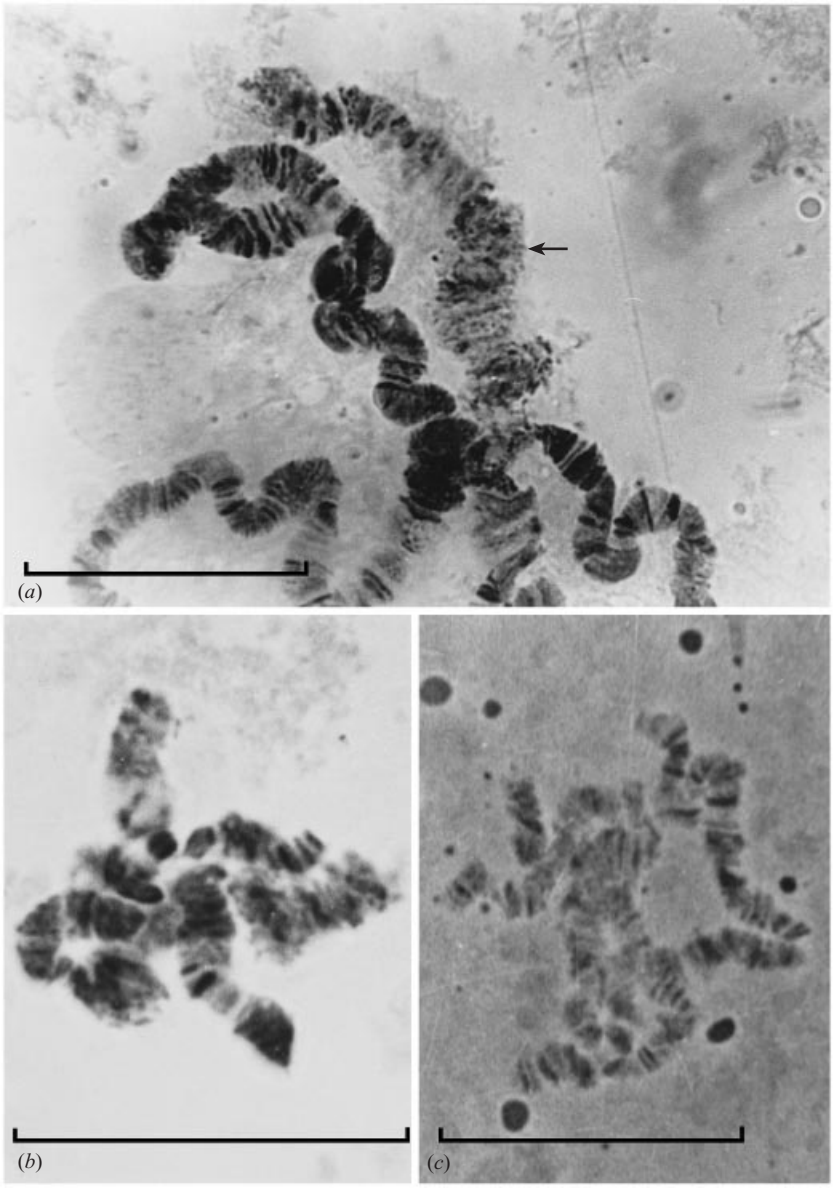

Fig. 4. Structural alteration of chromosome of Oregon R (wild-type) larvae, induced by *In(1)B*^{M2} (*reinverted*) extracts. (*a*) Alteration in the structure of the male X chromosome (arrow) using protein extracts from third instar male larvae. (*b*), (*c*) X non-specific chromosome condensation using extracts from adult males of the strain *In(1)B*^{M2} (*reinverted*).

alteration must occur through *de novo* synthesis of puffy Xs during temperature shifts.

Temperature-shift-induced alteration in chromosome morphology was of two types: alteration of the global structure of the male X chromosome, resulting in typical (Fig. 1 *a*) or highly condensed puffy Xs (Fig. 1 *b*), or random regions of increased widths along the male X chromosome (Fig. 1 *c*, *d*). Further analysis of the latter revealed that the enhanced width was associated with regions of spiralization of the X chromosome (Fig. 1 *c*, *d*). This suggested that such a topological alteration results in increasing the chromosome width and reducing the chromosome length. The induction of a partial width increase suggests that this process occurs randomly along the chromosome, finally leading to global alteration and formation of the puffy X chromosome. In fact, during recovery experiments, spiralized regions are initially observed during the first hour of recovery, followed by the appearance of puffy Xs. These results indicate that the puffy Xs are generated due to alteration in the higher-order organization of the male X chromosome. This observation also provides an explanation for the absence of correlation between the transcription and

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the width of the puffy Xs of *In(1)B*^{M2} (*reinverted*). In this context, it is important to note that a similar spiralization is observed in condensed male X chromosomes, when third instar larvae of *In(1)B*^{M2} (*reinverted*) were subjected to heat shock for 20 min (Kar & Pal, 1995).

The induction of puffy Xs through temperature shifts suggests the synthesis/modification of a substance that can induce condensation of the structure of the male X chromosome. It is known that during development a process of spiralization of the homologous chromatids organizes the extended structure of the giant polytene chromosome (Beermann, 1952; referred to in Zhimulev, 1996; reviewed in Sorsa, 1988). The morphology of the spiralized Xs of temperature-shifted male larvae, as observed here, suggests that the puffy Xs of *In(1)B*^{M2} (*reinverted*) arise as a continuation of these processes, leading to further condensation of the chromosome structure. It may be possible that, whereas this process is terminated in a time- and stage-dependent manner during development, there has been a disruption in this process in *In(1)B*^{M2} (*reinverted*).

We have recently partially purified a heat-labile chromosome condensing activity from extracts of male third instar larvae, pupae and adults of *In(1)B*^{M2} (*reinverted*). Structural alteration of chromosomes can be induced when salivary glands of Oregon R larvae are incubated in this extract. At lower protein concentrations, the structure of the male X chromosome is altered (Fig. 4*a*), while at higher protein concentrations a dramatic sex- and chromosome-nonspecific alteration in the structure of the polytene chromosomes of either sex is observed (Fig. 4*b*, *c*). This chromosome condensing activity is being further purified from this strain and the details will be published elsewhere.

These results thus suggest that the chromosomal rearrangement in *In(1)B*^{M2} (*reinverted*) affects a pathway that regulates the condensation of the polytene chromosomes. The condensation is brought about by spiralization of the X chromosome, resulting in an increase in width, reduction in length and disruption of the banding pattern. The recovery of activity from adult males, however, implies that the condensing activity may not be specific to polytene chromosomes but could be a general chromosome condensation protein. This observation, together with the male specificity of the phenomenon, strongly suggests the involvement of this factor in the dosage compensation pathway. In *Caenorhabditis elegans*, the *dpy-27* dosage compensation gene product encodes a SMC chromosome condensation protein that alters chromatin structure (Chuang *et al*., 1994). The role of a generalized chromosome condensation system that is responsible for the distinct chromatin organization of the X chromosome and the autosomes of *Drosophila* has been speculated (Bashaw & Baker, 1996). It is possible that the condensing protein from *In(1)B*^{M2} (*reinverted*) could be a component of the general chromosome condensing protein, whose interaction with dosage compensation regulators brings about the appropriate chromatin environment that determines hypertranscription of the male X chromosome.

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