Overexpression of the *SuUR* gene induces reversible modifications at pericentric, telomeric and intercalary heterochromatin of *Drosophila melanogaster* polytene chromosomes

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Accepted 27 September 2002
Journal of Cell Science 116, 169-176 © 2003 The Company of Biologists Ltd
doi:10.1242/jcs.00196

Summary

The *SuUR* (suppressor of underreplication) gene controls late replication and underreplication of DNA in *Drosophila melanogaster* polytene chromosomes: its mutation suppresses DNA underreplication whereas additional doses of the normal allele strongly enhances underreplication. The SuUR protein is localized in late replicating and underreplicating regions. The N-terminal part of the SuUR protein shares modest similarity with the ATPase/helicase domain of SWI2/SNF2 chromatin remodeling factors, suggesting a role in modification of chromatin structure.

Here we describe novel structural modifications of polytene chromosomes (swellings) and show that *SuUR* controls chromatin organization in polytene chromosomes. The swellings develop as the result of *SuUR* ectopic expression in the transgene system Sgs3-GAL4; UAS-SuUR$^+$. They are reminiscent of chromosome puffs and appear in ~190 regions of intercalary, pericentric and telomeric heterochromatin; some of them attain tremendous size. The swellings are temperature sensitive: they are maximal at 29°C and are barely visible at 18°C. Shifting from 29°C to 18°C results in the complete recovery of the normal structure of chromosomes. The swellings are transcriptionally inactive, since they do not incorporate [^{3}H]uridine. The SuUR protein is not visualized in regions of maximally developed swellings. Regular ecdysone-inducible puffs are not induced in cells where these swellings are apparent.

Key words: *SuUR* gene, Heterochromatin, Silencing, Polytene chromosomes, *Drosophila*

Introduction

Three types of heterochromatin in polytene chromosomes of *Drosophila melanogaster* are known: pericentric (PH), telomeric (TH) and intercalary (IH). They differ in DNA sequences and location, yet demonstrate a set of common characteristics such as special packing of chromatin (solid compact bands of IH, α-heterochromatin and net-like granular β-heterochromatin pack in pericentric regions and specific net-like material packs into some telomeric ends of chromosomes), late DNA replication in S phase, DNA underreplication during polytenization cycles (resulting in weak point or breaks) and non-homologous (ectopic) pairing of chromosome regions (Zhimulev, 1998; Richards and Elgin, 2002). At the molecular level all three types of heterochromatin show additional similarities: regions of pericentric and telomeric heterochromatin contain at least one specific protein (Heterochromatic protein 1, HP1) (James et al., 1989; Eissenberg et al., 1995). Many sites of IH contain specific silencer proteins of the *Polycomb* Group of genes (*PcG*) (Zhimulev et al., 2002). Both types of proteins, PcG and HP1, share structural and functional similarity: they have chromodomains and are present in specific protein complexes, which seem to participate in the formation of silencing (PcG) or heterochromatic (HP1) domains (Eissenberg et al., 1995; Wallrath, 1998; Cavalli and Paro, 1998). New approaches to the study of heterochromatin are provided by the discovery of the *SuUR* gene. Mutation of this gene results in the complete suppression of underreplication in intercalary heterochromatin and partial suppression of underreplication in pericentric heterochromatin (Belyaeva et al., 1998; Moshkin et al., 2001; Semeshin et al., 2001). The mutation shifts the time of completion of DNA replication to later in S phase for regions of IH. These regions complete DNA replication later than euchromatic regions but generally terminate replication earlier than the wildtype; that is, closer to the stage of continuous replication in chromosomes (Zhimulev et al., 2002). Antibodies against the SuUR protein are localized almost completely in regions of late replication of polytene chromosomes, namely in regions of IH and TH. Especially strong binding of antibodies was found in pericentric heterochromatin (Makunin et al., 2002).

No homology with full-length SuUR protein was found in databases when a BLAST search was used (Altschul et al., 1997). However, the first 250 amino acids from the N-terminus show a moderate similarity to the N-terminal part of the ATPase/helicase domain found in the SWI2/SNF2 family of proteins (Makunin et al., 2002).

In lines containing two to six additional transgenic doses of

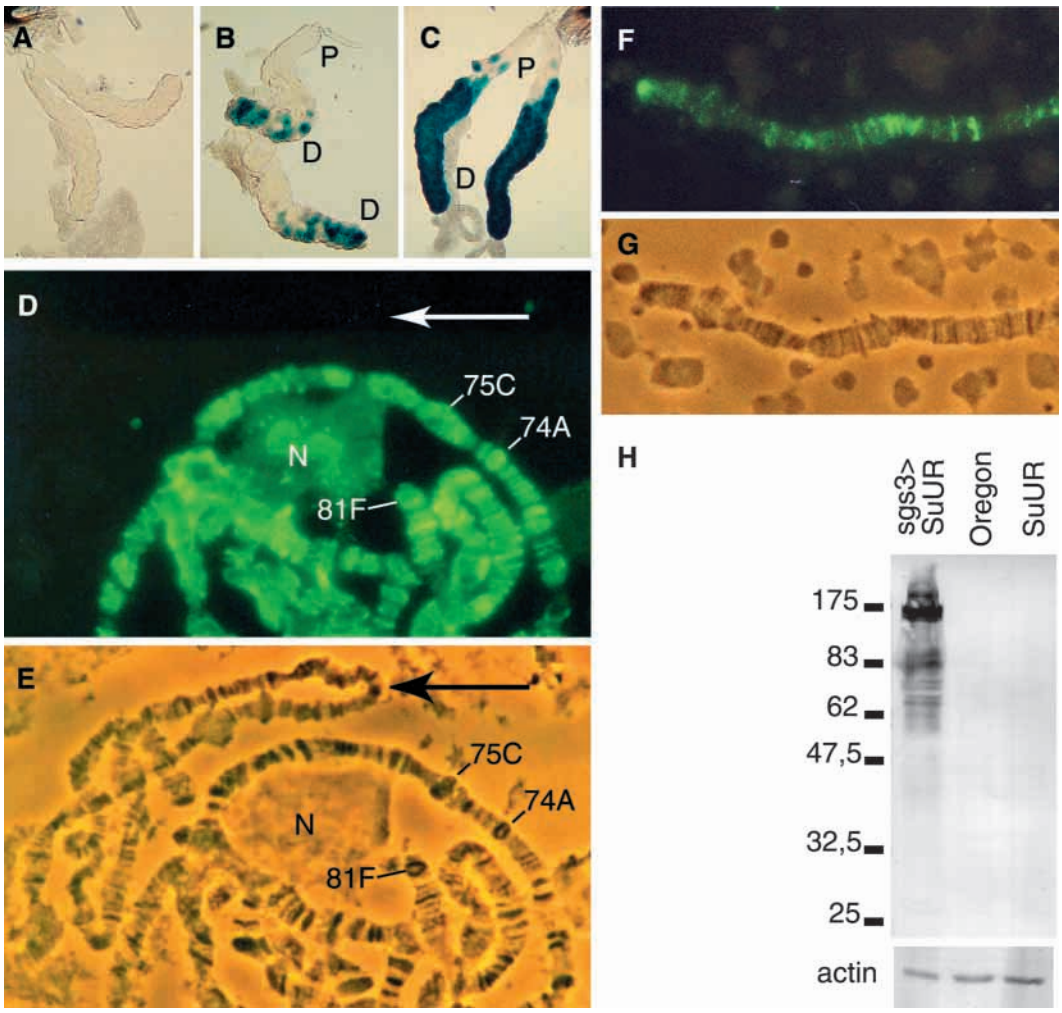

Fig. 1. Cell specificity of expression and overexpression of the *Sgs3* promoter in the salivary gland cells. Staining in the distal (D) parts and absence of staining in the proximal (P) parts of the salivary gland in *Sgs3-Gal4 UAS-lacZ* larvae (A-C). No expression was seen in young (about 100 hours) larvae (A). The beginning of expression was seen in the salivary glands of older (B) and late (C) larvae. Binding of antibodies against SuUR protein in polytene chromosomes of distal part of salivary gland of young *SuUR/SuUR* Sgs3-Gal4 UAS-SuUR$^+$ larvae: immunofluorescence (D) and phase contrast (E). Arrows point to a smaller polytene chromosome in the proximal part of the salivary gland that does not bind the antibodies. Binding of antibodies against SuUR protein in polytene chromosomes of the distal part of salivary gland of wildtype (F,G**)** shown by immunofluorescence (F) and phase contrast (G). Recognition of an additional band in the *SuUR/SuUR* Sgs3-Gal4 UAS-SuUR$^+$ larvae by the SuUR antibodies on western blots (H)

the *SuUR*$^+$ gene the degree of DNA underreplication and ectopic pairing in regions of IH is sharply enhanced; that is, the *SuUR* gene functions as an enhancer of underreplication causing many late replication sites to become underreplicated. Overexpression of *SuUR*$^+$ under an ubiquitously active promotor is lethal for the organism, whereas overexpression of the gene under a promotor that is continuously active in the salivary gland cells results in development of tiny salivary glands (E.I.V. and I.V.M., unpublished). In this paper we describe visible modifications of polytene chromosome structure and morphology resulting from ectopic expression of UAS-SuUR under the control of the Sgs3-Gal4 driver. It contains the promoter region from the tissue-specific gene *Sgs3* of *D. melanogaster* and a coding sequence for the yeast transcription activator GAL4 (Do et al., 2002). The *Sgs-3* gene is active only in cells of larval salivary glands and only during the second part of the third larval instar (Biyasheva et al., 2001). This peculiarity of the driver permits us to analyze the overexpression of *SuUR* in a single larval organ, which normally histolyses soon; this overexpression presumably will not damage the normal development of the whole organism. We expected that strong overexpression of the *SuUR*$^+$ gene under the Sgs3-*Gal4* driver would result in a further enhancement of underreplication and ectopic pairing. However, by contrast, in the polytene chromosomes of the Sgs3-Gal4; UAS-SuUR$^+$ larvae and prepupae unexpected and unusual swellings appeared in the regions of IH, PH and in some telomeric regions. The most interesting characteristics of these swellings are described in this paper.

Materials and Methods

Drosophila stocks

The *Sgs3-Gal4* transposon was constructed by L. Cherbas and A. Andres, and the transformed stock was received from L. Cherbas. The insertion is located in the third chromosome (Cherbas et al., 2002; Do et al., 2002).

Transgenic larvae *Sgs3-Gal4/+ UAS-SuUR*$^+$*/+* obtained from mating of lines *Sgs3-Gal4* and *UAS-SuUR*$^+$ were raised on standard medium at 18, 25 or 29°C. Both backgrounds, *SuUR* and *SuUR*$^+$, were used for transgene expression. Polytene chromosomes of larvae and prepupae obtained from mating of these strains were analyzed.

The *Drosophila* strain containing UAS-lacZ was given by F. Karch.

Cytology

Preparations of salivary gland polytene chromosomes stained with acetic orcein were made by the standard method and analyzed under a phase-contrast microscope. Polytene chromosome maps were taken from a previous paper (Bridges, 1935).

For autoradiography, salivary glands were dissected in Ephrussi and Beadle solution (Ephrussi and Beadle, 1936) and transferred to the same medium containing [^{3}H]uridine (25 mCi/ml, specific activity 38 Ci/mM, Amersham) for 30 minutes. They were then fixed in alcohol-acetic acid (3:1) mixture, covered with liquid emulsion Illford L4, exposed for two weeks and then developed (for details, see Zhimulev, 1999).

Squashes for EM purposes were prepared as described earlier (Semeshin et al., 2001). Sections 120-150 nm thick were cut with an LKB-IV ultratome and examined under the JEM-100C electron microscope at 80 kV. Immunostaining of polytene chromosome was performed according to a previous paper (Elgin, 1996) with minor modifications.

Constructs for transformations

The clone f27 contains the full ORF and 3′UTR of the *SuUR* transcript cloned into *pBluescript SK*$^+$ between *Pst*I and *Xho*I sites (for details, see Makunin et al., 2002). The insert of the f27 clone was excised with *Not*I and *Acc*65.I and subcloned into *pUAST* (Brand and Perrimon, 1993) that had been digested with *Not*I and *Acc*65.I. The resulting U6 clone contains the *SuUR* ORF and 3′UTR under the control of the UAS-containing minimal *Hsp70* promoter in the *P*-element vector. For transformation 8 μg of the U6 DNA were mixed with 2 μg of DNA of helper plasmid *pUChspi delta 2-3 turbo* in a total volume 20 μl, and this mixture was injected in *y w* embryos by standard procedures, and several independent transformed lines were established. Two of the insertions of the UAS-SuUR$^+$ constructs were localized in polytene chromosome regions 59DE and 47A, respectively (V.S., unpublished) [UAS(59DE) and UAS(47A)]. The main part of the work was done using the *UAS*(59DE) line. Experiments on reversibility of the swellings, antibodies localization and [^{3}H]uridine incorporation were performed on the UAS(47A) line.

Results

The salivary gland is differentiated in two parts, distal and proximal; the first synthesize the salivary gland secretion, one component of which is the SGS3 protein (for a review, see Berendes and Ashburner, 1978). Analysis of the expression of the *Sgs3* promoter in transgenic larvae Sgs3-Gal4 UAS-lacZ shows that it is expressed only in the distal part of the salivary gland (Fig. 1A-C). In *SuUR*, Sgs3-Gal4/+ UAS-SuUR$^+$/+ larvae (which have no other source of the SuUR protein except that from the transgene), antibodies against the SuUR protein stained chromosomes only in the distal part of the salivary gland but not the proximal (Fig. 1D,E). These data can be considered, in turn, as an additional confirmation of the high specificity of the antibodies.

The *Sgs3* transgenic promoter, as well as the genomic *Sgs3* gene, are expressed only in salivary gland cells of the third instar larvae, beginning at mid instar (about 100 hours after oviposition) and continuing until pupariation (120 hours of larval development or 0 hours prepupa) (for a review, see Biyasheva et al., 2001). Specific changes appear in polytene chromosomes during the period of activity of the *Sgs3* promoter and, as a consequence, there is the period of *SuUR*$^+$ ectopic expression in the Sgs3-GAL4/+ UAS-SuUR$^+$/+ larvae and prepupae. In young larvae, actively feeding and moving in the media (this developmental stage corresponds to 100-114 hour larvae), characteristic capsules appear in polytene chromosome bands (Fig. 2A). In older larvae migrating on tube walls (114-120 hours) some of these capsules convert into swellings of tremendous sizes (Fig. 2B). The size and number

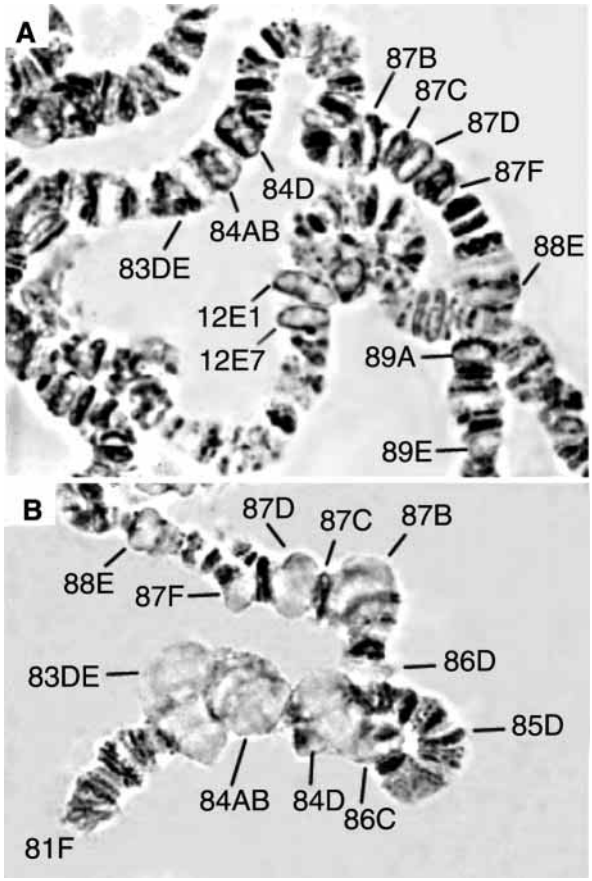

Fig. 2. Proximal part of the polytene 3R chromosome of *SuUR*$^+$/*SuUR*$^+$ Sgs3-GAL4 UAS-SuUR$^+$ young larva (A) and late prepupa (B): small capsules and swellings in regions 83DE, 84AB, 84D, 86C, 86D, 87B, 87D, 87F and 88E are marked.

of the swellings are maximal in 4-8 hour prepupae. In the *SuUR* Sgs3-GAL4 UAS-SuUR$^+$ larvae and prepupae the swellings are bigger than in the transgenic strain with normal endogenous *SuUR* genes. The localization of the capsules and swellings in chromosomes is very specific (see below) and highly reproducible (see mapping in Figs 2 and 3). In total, about 190 bands demonstrate capsule or swelling formation in polytene chromosomes (Table 1). These swellings look like the puffs known in polytene chromosomes for decades (for a review, see Zhimulev, 1999). Nevertheless they strongly differ from puffs, in at least, four aspects, which are listed below.

1. The capsules and swellings arise in chromosome regions where puffs never appear; that is, the sites of their formation are tightly condensed solid bands. In the distal part of chromosome 2R these swellings arise in six regions (Fig. 3), five of which belong to regions of IH and one (60F) is a region of TH. Several regions are of special interest, particularly 84AB (Fig. 2) and 89E1-4 where the genes of the *Antennapedia* complex and the *Bithorax* complex are located. These two are classic examples of silenced regions in IH. Especially impressive is the swelling of the PH (Fig. 4). The heterochromatic material visible in the chromocenter of normal polytene chromosomes gradually converts, first into bubble-like mass, and eventually into a light transparent cloud (Fig. 4). Analysis of all 190 polytene chromosome bands where

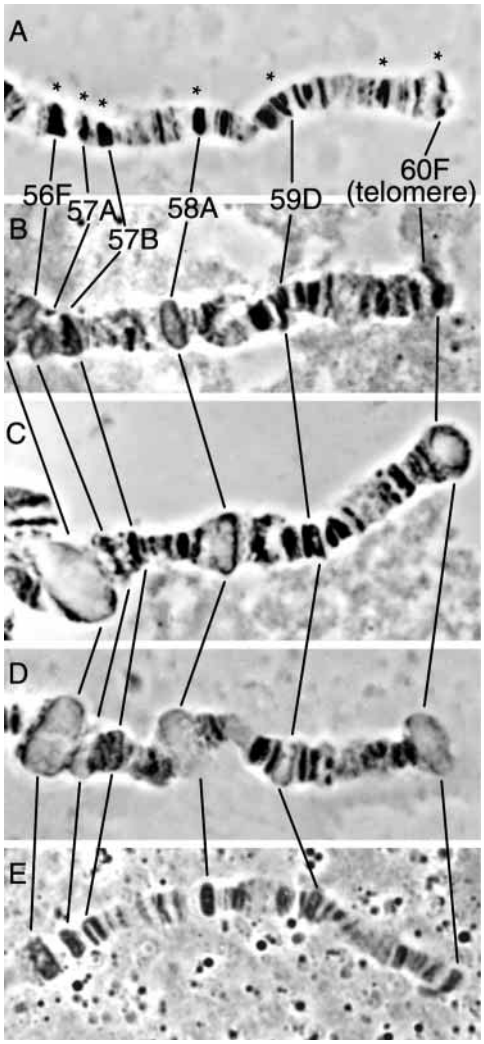

Fig. 3. Swellings in the distal part of the chromosome 2R of Oregon R control stock (A) and *SuUR*$^+$/*SuUR*$^+$ Sgs3-GAL4 UAS-SuUR$^+$ young larvae (B), late larva (C) and late prepupa (D). The chromosome shown in E was taken from prepupa that developed till the 0 hours prepupal stage at 29°C followed by a 15 hour development at 18°C. Asterisks in A mark regions of late replication.

capsules or swellings appear shows that they represent the regions of IH characterized by late replication, underreplication and localization of the SuUR protein and, in many cases, PcG proteins localization. As is seen in Fig. 5, almost all regions bind antibodies against the SuUR protein, and regions of DNA underreplication (weak points) form these swellings. A somewhat lower proportion of swelling-forming regions was found in late replicating regions, although in the tip of the chromosome arm 2R shown on Fig. 3 the correlation between late replication and swelling formation sites is almost complete. The swellings appears in 47% of PcG protein-binding sites (Fig. 5).

2. The regular puffs are regions of very intensive transcriptional activity, this can be demonstrated by variety of techniques, including [^{3}H]uridine incorporation or binding of antibodies against different proteins of transcription complex, RNA polymerase II or transcriptional factors etc. (for a review, see Zhimulev, 1999). The swellings are completely inactive in the incorporation of the RNA precursor, although other chromosome loci are heavily labelled (Fig. 6A).

3. The swellings show a very specific structure (Fig. 7). Even at the low magnification of the light microscope swelling formation can be seen to start with the central part of the polytene chromosome band. The band becomes diffuse and light; however, its borders still contain condensed material, as in the bands 87C, 87D, 87F, 88E and 89A in Fig. 2a, or 57A

Table 1. Localization of sites of swellings and capsule formation in polytene chromosomes of the *SuUR/SuUR*, Sgs3-GAL4; UAS-SuUR$^+$

X chromosome	2L chromosome	2R chromosome	3L chromosome	3R chromosome
1B	21D	41A	61A	81F
1E	22A	41D	61F	83D4-5
3C	22B	41F	62C	84A1-2
4B	22F	42A	62D	84A4-5
4C	23A	42B	63A	84B1-2
4D	24D	43A	63E	84D
4E	25A	44A	64C1-2	85A
5D	25E	44C	64C4-5	86C
6A	25F	44D	64D	86D
7A	26A	44F	65A	86E
7B	26C	45A	65B	87B
7C	30A	47A	65D	87C
7E	32A	47D	65E1-4	87D
8B	32F	48A	66A	87E
8E	33A	48C	67A	87F
9A	33D	50A	67D	88A
9B	34A1-2	50C	67E	88E
10A	34EF	53B	67F	89A
10B	35B	54AB	68A	89D
11A	35C	55A	68E	89E
12A	35D	56AB	69D	90A
12E1-2	35E	56F	69F	90D
12E7-8	35F	57A	70A	90E
13A	36B	57B	70C	91B
13B	36C	58A	70D	91D
14B1-2	36D	59D	70E	91E
16F	36E	60F	71A	92A
17A	37D		71C	92B
17C	38A		72A	92D
17D	38C		73A	92E
18A	38E		74A	93A
19A	39A		75A	93E
19E	39D-E		75C	93F1
20A	40A		76B	93F9-10
20B-F			77A	94A
			77E	94D
			79D	95A
			79E	96A
			80C	96C
				96D
	4			96E
	chromosome			97B
	101DF			97F
				98A
				98B
				98C
				98D
				98E
				99A
				100A
				100B1-2
				100B4-5
				100C5

Regions are given according to 13.

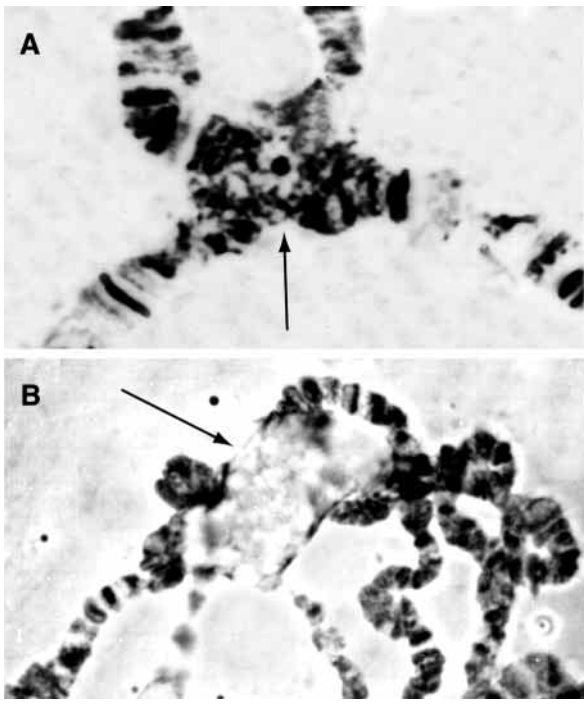

Fig. 4. Decondensation and swelling of pericentric heterochromatin regions in *SuUR*$^{+}$/*SuUR*$^{+}$ Sgs3-GAL4 UAS-SuUR^{+} (B) in comparison with the chromocenter of the Oregon-R control line (A). Arrows show chromocentral regions.

and 58A in Fig. 3b. Subsequently, when the capsules reach their maximal sizes and convert into the swellings, thin envelopes of material remain (indicated in Fig. 7 by arrows with crosses). The swellings are not empty inside; they contain abundant condensed electron-dense material (they are more dense than the neighboring interbands). The chromosome material within the swellings looks like foam (see 58A in Fig. 7A). When the chromosomes are stained with the fluorescent dye Hoechst 33258, abundant staining material is seen within the swellings but not in regular polytene chromosome puffs (Fig. 7C,D). This is evidence that a large amount of incompletely decondensed DNA is present in the swellings.

4. Swelling formation is temperature sensitive and reversible. The largest swellings occur when larvae develop at 29°C throughout, at least, for the last 12 hours of the third larval instar. Only capsules of minimal size, if any, appeared at 18°C. This permitted us to show that the formation of the swellings is reversible. As a result of development of larvae from mid third instar till 0 hour prepupae at 29°C, the swellings reach their maximal size. After a shift to 18°C the swellings condense and revert into almost normal polytene chromosome bands, sometimes with only small capsules in places of the former swellings (see 56F, 59D and 58A in Fig. 3E).

Such an important change in the structure of numerous bands may result in changes in polytene chromosome function. In normal larvae more than 120 puffs are activating and inactivating during this period in a cascade of changing gene activity (Ashburner et al., 1974). Among many thousands of salivary gland nuclei analyzed after *SuUR* overexpression we could not find ecdysone-inducible puffs. The exceptions were

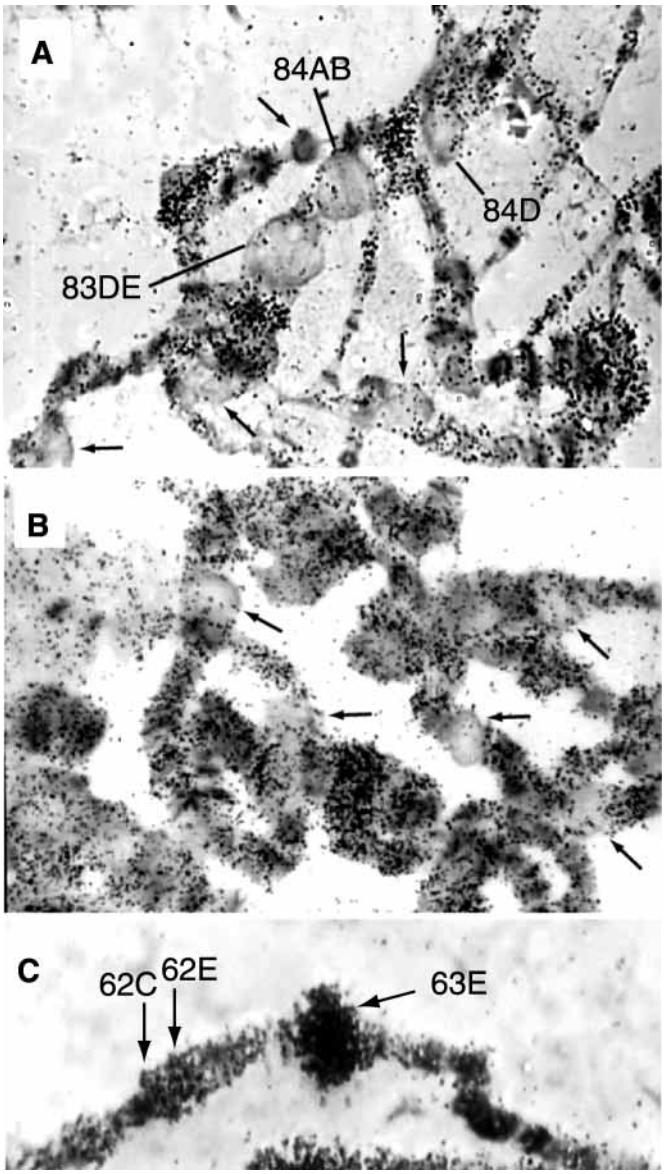

Fig. 6. Failure to incorporate [^{3}H]uridine into swellings in 83DE, 84AB and 84D regions as well as in some unidentified regions (indicated by arrows in A) in chromosomes of *SuUR/SuUR* Sgs3-GAL4 UAS-SuUR^{+} prepupae. (C) Incorporation of the [^{3}H]uridine into regular puffs 62C, 62E and 63E in wild-type polytene chromosomes.

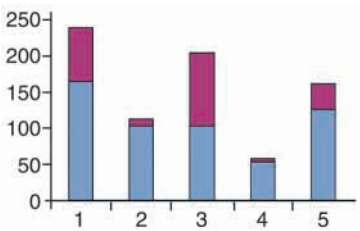

Fig. 5. Swelling and capsule formation in regions demonstrating different characteristics of heterochromatin. Abscissa: regions of late replication (1), SuUR (2) and PcG (3) protein localization, weak points in wildtype (2×*SuUR*$^{+}$) (4) and in line with extra copies of *SuUR* [(4-8) x *SuUR*$^{+}$] (5). Ordinate: number of regions demonstrating different characteristics (total height of columns) of intercalary heterochromatin and number of swellings or capsule formation regions among them (blue part of bars).

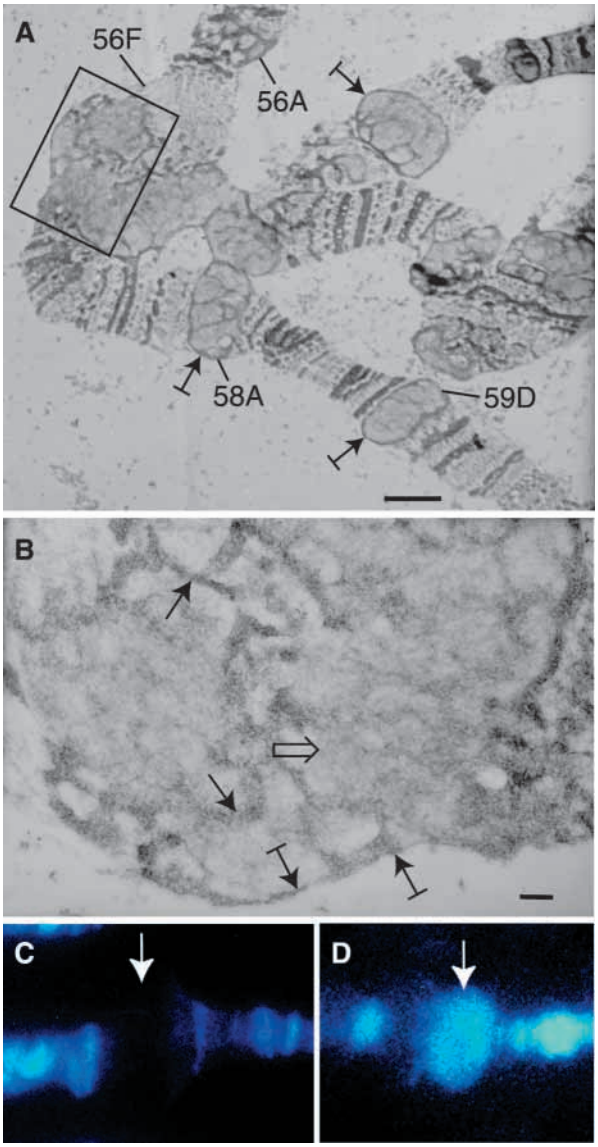

Fig. 7. Structure of swellings under an electron (A,B) and a fluorescent (C,D) microscope. Part of the swelling in 56F (rectangular in A) is shown at higher magnification in B. An external envelope of the bubble is indicated by arrows with crosses; inner envelopes around small bubbles are indicated by straight arrows; and open thick arrow indicate the matrix within the bubble. A fluorescent Hoechst-33258-stained regular chromosome puff 71CF of the Oregon R strain (C) and swellings in regions designated by arrows in chromosomes of *SuUR/SuUR* Sgs3-GAL4 UAS-SuUR^{+} prepupae (D) are shown. Scales represent 1 μm (A) and 0.1 μm (B).

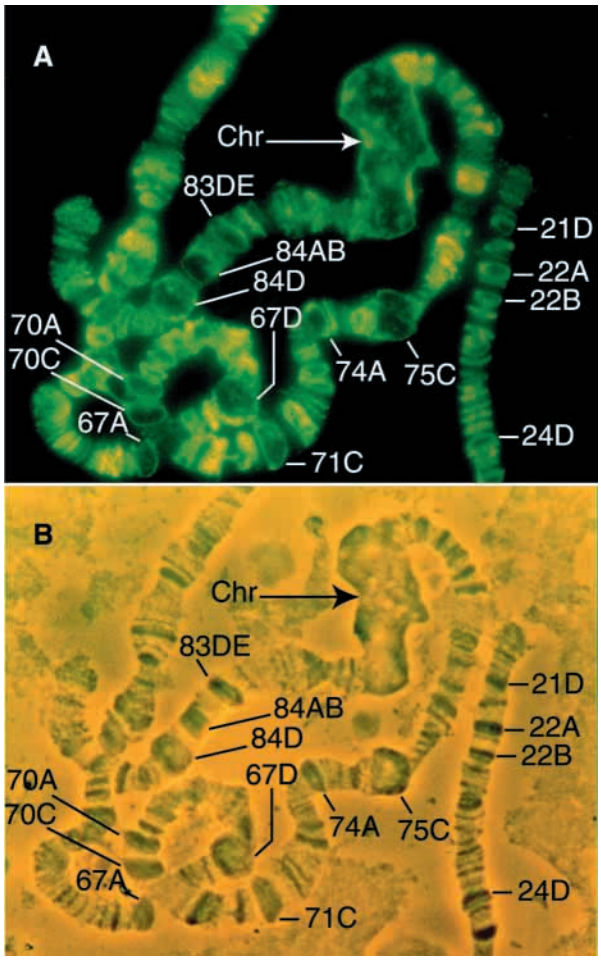

Fig. 8. Localization of SuUR antibodies in polytene chromosomes of the *SuUR*$^{+}$*/SuUR*$^{+}$ Sgs3-GAL4 UAS-SuUR^{+} prepupae after overexpression of the *SuUR*$^{+}$ gene. The swollen chromocenter (arrow), small capsules (21D, 22A, 22B and 24D) and big swellings (75C, 74A, 71C, 67D and others) are free of the antibodies.

a few nuclei in which the chromosomes contained very small puffs at the earliest ecdysone inducible sites 74E-75B.

The SuUR protein in wild-type larvae is localized at a limited (113) number of sites (Makunin et al., 2002). But after even a short period of overexpression in young larvae, it appeared in practically all visible polytene chromosome bands. (Fig. 1D,E). Swellings as a rule have not yet developed at this stage; however, in places where they will soon appear, small cavities free of the antibodies are visible (74A, 75C and 81F in Fig. 1D). At the stage when the swellings reach their maximal size, staining of IH and PH with antibodies is not revealed (Fig. 8A).

Discussion

Three types of heterochromatin in polytene nuclei, pericentric, telomeric and intercalary have been described, and they share several common characteristics: condensed chromosome structure, late replication and underreplication of DNA during polytenization cycles. The SuUR protein is located predominantly in these three types of heterochromatin, and the level of polytenization of these regions strongly depends on dosage of this gene (Zhimulev et al., 2002). The results described in this paper show that all three types of heterochromatin similarly react to strong overexpression of the *SuUR* gene by decondensation of chromosome material and visible swelling. Mechanisms of formation of swellings are not known. There are, at least, two possibilities, which we could discuss.

1. *SuUR* overexpression may act indirectly, blocking transcription of some locus required for maintaining a compact

chromatin structure. We find that in conditions of overexpression numerous ecdysone puffs do not appear in polytene chromosomes, meaning that ecdysone-inducible genes are not able to activate. The same can happen with other genes inducible during this period of development. Data on [^{3}H]uridine incorporation suggest that binding of overexpressed SuUR protein to all bands does not stop transcription in chromosome regions that are already active (they incorporate [^{3}H]uridine) but prevents induction of the ecdysone puffs. Binding of other overexpressed proteins to all polytene chromosome bands has been shown for HP1, Su(var)3-7 (Delattre et al., 2000), Su(z)2 and Psc (Rastelli et al., 1993) proteins and probably takes place as result of their affinity for DNA or chromatin, but in those papers there are no indications of the swellings and inhibition of puff development described here. At the same time as we see, the possibility of inhibition of transcription induction exists, and it has to be taken into consideration when interpreting the results of ectopic overexpression of genes.

2. The other possible mechanism for swelling formation is direct action of the SuUR protein on heterochromatic regions. As was indicated above, all the facts point to heterochromatic regions being targets for *SuUR* gene activity. There may be some common structural peculiarities in all types of heterochromatin, which are critical for binding the SuUR protein in the wildtype. It is not clear whether this would be specific protein complexes or a specific conformation of heterochromatin. When it is overexpressed the SuUR protein binds with all bands but swellings develop only in heterochromatic regions. Perhaps some structural specificity of heterochromatin is responsible for DNA underreplication when *SuUR* normally expresses and disintegration of chromosome material when this gene is overexpressed.

It is possible that the effects of additional doses of the SuUR protein are determined by the similarity of SuUR to SWI2/SNF2 (Makunin et al., 2002), a member of a protein family capable of remodelling chromatin complexes. For this, SWI2/SNF2 has an ATP hydrolysing function. The SWI/SNF complex can alter histone-DNA interactions in the nucleosome. High concentraions of SWI/SNF complex can disrupt a synthetic nucleosome core (Wolffe and Guschin, 2000). As shown recently, null mutation of the *ISWI* gene, a highly conserved member of the SWI2/SNF2 family, affects both cell viability and gene expression and causes striking alterations in the structure of the male X chromosome (Deuring et al., 2000). Mutations of other gene, *JIL-1*, coding for tandem chromosomal kinase, leads to dramatic changes in banding pattern (Wang et al., 2001). We could, therefore, propose that overexpression of *SuUR* results in changes of chromatin packaging specifically in all types of heterochromatin and results in swellings. These changes appear to be reversible, and after lowering the temperature, heterochromatic regions condense again, swellings disappear and chromosomes acquire an almost normal morphology. These effects are probably related to adaptation of the Gal4-UAS system to high temperature (Brand et al., 1994). This means that chromosomes are able to restore normal structure and functions and swelling formation does not cause irreversible changes in chromosome structure. Most interesting was the finding that after gene overexpression, the SuUR protein itself is not revealed within the swollen heterochromatic regions where it normally resides. At the same time DNA is easily visible in the swellings after staining with Hoechst 33258. It is possible that the SuUR protein and other proteins dissociate from chromatin. A case of such dissociation occurs when under the influence of the *E(z)* mutation, some of proteins of the PcG complex dissociate from chromosomes (Rastelli et al., 1993).

Other cases of global changes of properly heterochromatin structure are known as well. For example, specific puffs appeared in regions of PH in polytene chromosomes of *Glyptotendipes barbipes* (Chironomidae) larvae developing at 18°C (Keyl, 1963) or in *Chironomus thummi thummi* after long maintenance of larvae in a solution of Actinomycin D (Kiknadze, 1965; Valeyeva et al., 1979). Heterochromatin of *Drosophila melanogaster* mitotic chromosomes looks decondensed in a *mus-101^{ts}* mutant at 29°C (Gatti et al., 1983). The *mus-101* gene encodes a member of the superfamily of proteins containing the BRCT domain, which is implicated in DNA repair and cell checkpoint control (Yamamoto et al., 2000). It shares homology with human TopBP1 protein, which is associated in vitro with DNA topoisomerase IIβ and with the fission yeast Rad4/Cut5 protein required for repair, replication and checkpoint control. So this gene is probably involved in processes of chromatin reorganization, and its action can influence heterochromatin condensation.

However, structures resembling the swellings described in this paper were not found before. These modifications of chromosome structure specifically appear in chromosome regions binding SuUR protein and demonstrating late replication in the endocycle.

We thank Lucy Cherbas for the gift of the Sgs3-Gal4 strain and F. Karch for UAS-lacZ. Thanks also to Michael Ashburner, Lucy and Peter Cherbas, Yurii Moshkin, Vincenzo Pirrotta and Geoff Richards for critical comments on this manuscript and Dmitrii Koryakov for preparing some of the pictures. This work was supported by grants from the RFBR program (N 00-15-97984, 02-04-48222, 01-04-06509), from the Russian State Program ‘Frontiers in Genetics of Russian Federation’ (02-2PNG) and a grant to support young scientists (V.V.S.) from the Siberian Division of the Russian Academy of Sciences.

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