Abnormal tissue-dependent polytenization of a block of chromosome 3 pericentric heterochromatin in *Drosophila melanogaster*

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Summary

Heterochromatic DNA sequences in the polytene chromosomes of *Drosophila melanogaster* salivary glands are under-replicated in wild-type strains. In salivary glands of *SuUR* and in the nurse cells of *otu* mutants, under-replication is partly suppressed and a banded structure appears within the centric heterochromatin of chromosome 3. This novel banded structure in salivary gland chromosomes was called Plato Atlantis. In order to characterize the heterochromatic component of Plato Atlantis, we constructed a fine-scale cytogenetic map of deletions with break points within centric heterochromatin (*Df(3L)1-16*, *Df(3L)2-66*, *Df(3R)10-65*, *Df(3R)4-75* and *Df(3L)6B-29 + Df(3R)6B-29*). Salivary gland chromosomes show that *Df(3L)1-16* removes the complete Plato Atlantis, while *Df(3L)2-66* deletes the most proximal 3L regions. These deletions therefore show a substantial cytological overlap. However, in the chromosomes of nurse cells, the same deficiencies remove distinct heterochromatic blocks, with the region of overlap being almost invisible. Satellite (AATAACATAG, AAGAG) and dodecasatellite DNAs mapped in a narrow interval in salivary glands but were found in three clearly distinguishable blocks in nurse cells. The 1.688 satellite was found at a single site in salivary glands but at two sites in nurse cells. We show that newly polytenized heterochromatic structures include blocks h47-h50d of mitotic heterochromatin in salivary glands, but the additional blocks h50p, h53 and h57 are also included in nurse cell chromosomes. Tissue specificity of the patterns of abnormal heterochromatic polytenization implies differential control of DNA replication in somatic and germline cells.

Key words: Heterochromatin, Polytenization, Chromosome 3, *Drosophila melanogaster*

Introduction

The term ‘heterochromatin’ denotes chromosomal regions that remain condensed throughout most of the cell cycle, show late replication during normal cell cycles, under-replication in polytene chromosomes, extremely low gene density and high density of repeated sequences. Heterochromatin is characterized by modified histones H3 and H4 and high regularity of nucleosomes (reviewed by Gatti and Pimpinelli, 1992; Lohe and Hilliker, 1995; Weiler and Wakimoto, 1995; Elgin, 1996; Wallrath, 1998; Zhimulev, 1998; Dillon and Festenstein, 2002; Richards and Elgin, 2002).

At the cytological level, heterochromatin in mitotic chromosomes of *Drosophila melanogaster* has been divided into 61 regions (designed h1-h61) according to H- and N-staining (Gatti et al., 1994), which reflects heterogeneous contents of DNA. Sequences forming heterochromatin are divided into highly repeated (satellites), moderately repeated (mainly transposable elements) and occasional unique (genes). These sequences occur in fixed positions within the heterochromatic regions (Lohe et al., 1993; Pimpinelli et al., 1995; Carmena and Gonzalez, 1995; Makunin et al., 1999). Large blocks of simple satellite DNAs are interrupted by ‘islands’ of complex sequences (Le et al., 1995). The few heterochromatic genes are clustered within dull or moderately fluorescent regions after H-staining (Dimitri, 1991; Dimitri et al., 2003; Koryakov et al., 2002).

Euchromatin and heterochromatin also differ in nucleosome and histone organization. Transcriptionally active euchromatin has long tracts without nucleosomes, which are hypersensitive for nucleases, whereas inactive heterochromatin has long stretches with regular nucleosomal pattern (Sun et al., 2001). Heterochromatic histones carry epigenetically heritable modifications including hypoacetylation of H3 and H4 histones and methylation of lysine-9 of H3 histone (reviewed by Dillon and Festenstein, 2002; Richards and Elgin, 2002).

In addition to modified histones, *Drosophila* heterochromatin contains the non-histone proteins HP1, Su(var)3-7, Su(var)3-9 and SuUR. The HP1 protein is found mainly in pericentric heterochromatin, chromosome 4 and telomeres (James and Elgin, 1986), where it binds to methylated lysine-9 of H3 histone (Jacobs et al., 2001). This methylation is probably catalysed by Su(var)3-9 protein, as mammalian homologs of *Drosophila* Su(var)3-9 have been shown to have this activity (Aagaard et al., 1999; Rea et al., 2000). The Su(var)3-7 protein also interacts with HP1 (Cleard et al., 1997). The SuUR protein is present in pericentric and intercalary heterochromatin, but its function remains unclear (Makunin et al., 2002).

The phenomenon of position effect variegation is caused by inactivation of a euchromatic gene relocated to heterochromatin. Genetic inactivation results from chromatin changes in the relocated region, namely under-replication and compaction of DNA, histone modifications and the appearance of HP1 (Belyaeva et al., 1993) (reviewed by Elgin, 1996; Wallrath, 1998; Richards and Elgin, 2002). There appear to be specific centers of inactivation within heterochromatin as not all heterochromatic fragments can evoke position effect variegation [(Pokholkova et al., 1993) and references therein].

The specific content of DNA and proteins causes late replication of heterochromatin in the cell cycle in comparison with euchromatin. This attribute is common for chromosomes of many organisms (Lima-de-Faria and Jaworska, 1968), including *Drosophila melanogaster* (Ananiev et al., 1977; Steinemann, 1980). Heterochromatin replicates late only when it forms dense heavily stained blocks. In early embryogenesis, heterochromatin is indistinguishable in appearance from euchromatin and replicates simultaneously (Lima-de-Faria and Jaworska, 1968). However, centromeric regions in deep heterochromatin replicate at the same time as euchromatin despite the dense appearance (Ahmad and Henikoff, 2001).

One of the main attributes of heterochromatin in polytene chromosomes is its under-replication. For instance, euchromatin passes up to 10 replication cycles in salivary glands (SGs), whereas heterochromatin passes only 1-2 cycles (Rudkin, 1965; Berendes and Keyl, 1967; Mulder et al., 1968). However, the number of cycles can vary significantly in different heterochromatic regions. For example, the *light* gene, situated in the most distal region h35 of the 2L arm, polytenizes to the level of euchromatin (Devlin et al., 1990). The *rolled* gene, located in the most proximal part of 2R heterochromatin, also polytenizes almost to the level of euchromatin, whereas repeated sequences flanking this gene (Bari-1 and AAGAC) are extremely under-replicated (Berghella and Dimitri, 1996). Insertions of P-transposons in different blocks of heterochromatin also display highly polytenized regions (Zhang and Spradling, 1995).

Different factors can influence the level of polytenization. In follicle and nurse cells (NCs), the quantity of DNA was greater in strains containing attached X and Y chromosomes compared with that in strains containing free and Y chromosomes (Freed and Schultz, 1956). Relocating of a fragment of the X chromosome right arm, containing the AAGAG satellite, to euchromatin by the inversion *In(1LR)pn2b* increases the level of the satellite polytenization considerably (Koryakov et al., 1999). Some genes also influence the polytenization level of heterochromatic regions. The *SuUR* mutation suppresses under-replication of pericentric and intercalary heterochromatin in SG chromosomes, which results in full polytenization of intercalary heterochromatin sites and the appearance of a new structure with reproducible banding pattern in the pericentric heterochromatin of chromosome 3, called Plato Atlantis (Belyaeva et al., 1998; Moshkin et al., 2001; Semeshin et al., 2001). Mutation of the other gene, $cycE^{1672}$, increases the polytenization level of satellite DNAs in ovaries (Leach et al., 2000).

The level of heterochromatin polytenization is higher in chromosomes of germline cells than in somatic cells. These differences probably reflect the fact that, in SG chromosomes, under-replication begins from the first endocycle whereas the genome of NCs fully replicates during the first four cycles (Dej and Spradling, 1999).

In *D. virilis*, satellite sequences are more highly replicated in ovaries than in SGs and Malpighian tubes (Endow and Gall, 1975). In *D. melanogaster*, mutation *otu* causes formation of polytene chromosomes in NCs (King et al., 1981), which show greater polytenization of heterochromatic sequences than in SGs (Mal'ceva and Zhimulev, 1993; Koryakov et al., 1996).

The banded structures in chromosome 3 heterochromatin in both *SuUR* SGs and *otu* NCs raise several questions. Do the same DNA sequences polytenize in chromosomes of different cell types? Is this DNA heterochromatic? In this paper, we characterize the polytenized heterochromatic regions in chromosome 3 and correlate these novel heterochromatic features with heterochromatic blocks in mitotic chromosomes revealed by differential staining.

Materials and Methods

Drosophila stocks and DNA clones

Genotypes are given in Table 1. Deficiencies were obtained from Bloomington Stock Center (Bloomington, Indiana). These stocks were used to construct lines with deficiencies and *SuUR* or *otu* mutations as a background. Break points of the deficiencies are shown in Fig. 1. The stock Oregon-R (OR-R) was a wild-type control. All DNA clones used are described in Table 2 and Fig. 1.

Cytology and fluorecsent in situ hybridization (FISH)

NC and SG chromosomes were prepared as described previously (Koryakov et al., 1996). DNA clones were labeled with biotin-16-dUTP (Boehringer Mannheim, Mannheim, Germany) either in PCR or in random-primed polymerase reaction with Klenow fragment.

Squashes of polytene chromosomes were incubated in 2×SSC for 1 hour at 65°C, denaturated in 2×SSC, 0.07 N NaOH for 1.5 minutes, dehydrated in steps of increasing concentrations of cold ethanol (70%, 80%, 100%) for 3-5 minutes each, and air dried. Labeled probe was added to the hybridization mix (50% formamide, 2×SSC, 10% dextran sulphate) to a final volume of 30 μl. Hybridization was performed overnight at 37°C. Unbound probe was removed with three 15 minute washes in 0.2×SSC at 42°C, slides were incubated in blocking solution (2% Blocking reagent (Boehringer Mannheim) for 30 minutes, 4×SSC, 0.1% Triton X-100). Slides were stained with avidin-FITC for 30 minutes at 37°C and washed three times for 5 minutes with 4×SSC, 0.1% Triton X-100. Chromosomes were counterstained with propidium iodide (2 μg/ml in 0.2×SSC) at room temperature for 1-5 minutes, then were treated with Hoechst33542 (2 μg/ml in 0.2×SSC) at room temperature for the same time and rinsed in 0.2×SSC for a few seconds. Finally, 10 μl of antifade solution (2.5 mg/ml, 1,4-diazobicyclo-[2.2.2]-octane in 2×SSC; Sigma) was added before examination by fluorescence microscope. If necessary, the signal was enhanced by treatment with biotinilated anti-avidin antibodies and subsequent staining with avidin-FITC.

Results

Morphology of chromosome 3 pericentric region in SGs and NCs

In SG chromosomes of wild-type larvae, the 3L arm may contain 1-6 unclear bands in the most proximal euchromatic region 80 (Bridges, 1935). The very short 81F region of the 3R arm comprises several thin bands. These euchromatic regions are separated by unstructured diffuse β-heterochromatin (Fig. 2A).

In *SuUR* mutants, a series of distinct bands appear in 80A-

Table 1. *Drosophila* stocks used

Stock	Genotype	Characteristics	Ref.
SuUR	*w; SuUR*	Mutation suppresses under-replication of intercalary and pericentric heterochromatin in salivary gland chromosomes	Belyaeva et al., 1998
otu	*y w sn^3 otu^{11}/FM3*	Mutation causes polytene chromosome formation in oocyte nurse cells	King et al., 1981
Df(3L)1-16	*Df(3L)1-16, kni^{ri-1} p^p/MKRS*	Deficiency of the heterochromatic regions h47-h50d	Marchant and Holm, 1988a; Marchant and Holm, 1988b; Koryakov et al., 2002
Df(3L)2-66	*Df(3L)2-66, kni^{ri-1} p^p/TM3*	Deficiency of the heterochromatic regions h49-h51	Marchant and Holm, 1988a; Marchant and Holm, 1988b; Koryakov et al., 2002
Df(3L)6B-29+Df(3R)6B-29	*Df(3L)6B-29+Df(3R)6B-29, kni^{ri-1} p^p/TM3*	Deficiency of the heterochromatic regions h50-h52, h53p-h58p	Marchant and Holm, 1988a; Marchant and Holm, 1988b; Koryakov et al., 2002
Df(3R)10-65	*Df(3R)10-65, kni^{ri-1} p^p/TM3*	Deficiency of the heterochromatic region h58	Marchant and Holm, 1988a; Marchant and Holm, 1988b; Koryakov et al., 2002
Df(3R)4-75	*Df(3R)4-75, kni^{ri-1} p^p/TM3*	Inversion of the heterochromatic regions h49-h58	Marchant and Holm, 1988a; Marchant and Holm, 1988b; Koryakov et al., 2002

Table 2. DNA clones used

DNA clones	Characteristics (vector/size of the clone/localization in chromosome 3)	Source
Dodecasatellite	*pBK6E218*/500 bp/region h53	A. Villasante (Abad et al., 1992)
171 satellite (AATAACATAG)	*pBR322*/125 bp/region h48	A. Lohe (Lohe et al., 1993)
N1156 (AAGAG)	*pBR322*/372 bp/region h57	A. Lohe (Lohe et al., 1993)
AACAC	*pDM3.0*/195 bp/Y chromosome and region h42 in chromosome 2	I. V. Makunin (Makunin et al., 1999)
aDm23-24 (1.688)	*pBR322*/359 bp/h48p, h50d	A. Lohe (Lohe and Brutlag, 1986)

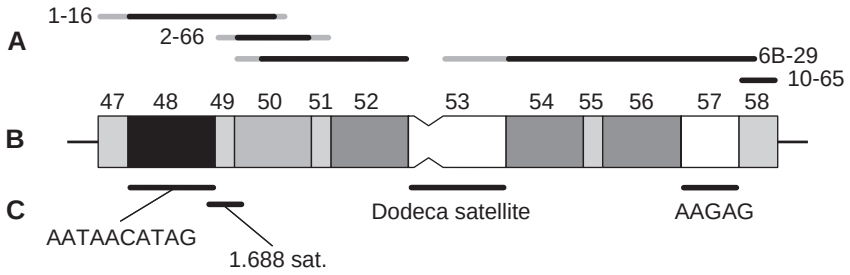

Fig. 1. Location of the deficiency break points (A) and DNA clones (C) in chromosome 3 mitotic heterochromatin (B) (Koryakov et al., 2002). Numbers 47-58 (B) denote differentially stained heterochromatic regions. Gray and black lines indicate the largest and the smallest limits of the deficiencies, respectively.

C, small bands in 81F become a large compact block and new, banded material is visible between chromosome 3 arms. This novel material was called Plato Atlantis, and its banding pattern was divided into six sections: PAA, PAB, PAC, PAD, PAE and PAF. There are constrictions in the PAA and between PAE and PAF regions (Belyaeva et al., 1998; Semeshin et al., 2001) (Fig. 2B).

In *otu* mutant NCs, the proximal 3L euchromatin comprises diffuse bands in section 80, whereas the proximal 3R contains a distinct band in 81F. Novel chromosomal material, visible between sections 80 and 81, was divided into four zones, separated by constrictions, named phD, phE, phF and phG (polytene heterochromatin) by analogy with chromosome 2 (Koryakov et al., 1996) (Fig. 2C). Constrictions indicate that DNA is under-replicated, resulting in frequent chromosomal breakage. Breakage was observed between phE and phF, phF and phG, but not between phD and phE; thus, the latter two regions are always present together in non-rearranged chromosomes.

The pericentric region of chromosome 3 in NCs of flies homozygous for both *otu* and *SuUR* mutations undergoes minimal changes in comparison with *otu* mutants, unlike SG chromosomes. All the structures become larger: if in strain *otu*, the phG section looks like a narrow, unstructured region (Fig. 2C), whereas in strain *otu*; *SuUR*, the phG section is larger and also may contain fine bands (Fig. 2D).

Morphology of heterochromatic zones may vary from chromosome to chromosome. For example, the region phD may have a clear banding pattern with at least three bands (Fig. 2C), or may be very compact, without dividing into bands (Fig. 2E). The region phE consists of puff-like material without a banding pattern (Fig. 2D,G), but occasionally fine friable bands can be seen in this region (Fig. 2F). Usually, the phF block resembles net-like β-heterochromatin (Fig. 2C), but sometimes phF is represented by a block of α-heterochromatin (Fig. 2E).

Localization of chromosomal rearrangements

To find a correlation between definite heterochromatic blocks in mitotic chromosomes and newly polytenized regions, we

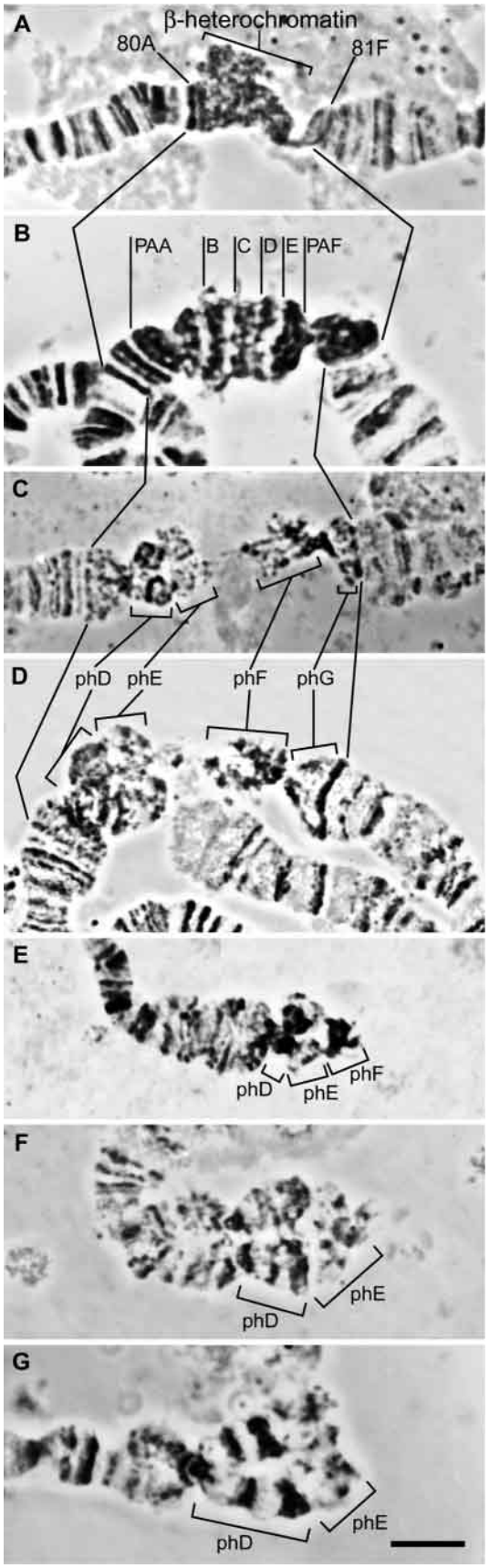

Fig. 2. Morphology of the chromosome 3 heterochromatin in polytene chromosomes from SGs of Oregon-R (A) and *SuUR* mutants (B), from NCs of *otu* mutants (C,E) and *otu*; *SuUR* mutants (D,F,G). Homologous regions are connected by lines. Bar, 5 μm.

analyzed several chromosomal rearrangements with known break points within mitotic heterochromatin. Heterozygous deficiencies *Df(3L)1-16*, *Df(3L)2-66*, *Df(3L)6B-29* + *Df(3R)6B-29* and *Df(3R)10-65*, which remove regions h47-h51, h49-h50d, h50-h58p and h58, respectively, were used with the homozygous mutation *SuUR* as a background (see map of the deficiencies in Fig. 1A). In addition to these deficiencies, we studied the rearrangement *Df(3R)4-75*, which contains a long paracentric inversion (Koryakov et al., 2002).

Investigation of the rearrangements in heterochromatin is a very complex problem for several reasons. First, both in SGs and in NCs, banded structures do not arise in every chromosome, and often instead of these structures one can see a mass of unstructured heterochromatin. Second, even the presence of a banding pattern does not mean full polytenization; by contrast, it remains partial, which results in multiple ectopic contacts. Third, the structures under investigation are small in size, and frequently it is very difficult to map precisely a deficiency, removing only 2-3 small heterochromatic blocks. Taking into account all of these complexities, we made the conclusions about rearrangements based on analysis of many chromosomes of every stock.

Mapping the deficiency *Df(3L)1-16* in SG chromosomes shows that it removes material from the proximal part of Plato Atlantis from the region PAA to PAF. Results (Fig. 3A) demonstrate that all the bands of Plato Atlantis are present in the normal homolog, whereas the region of compact bands of the section PAA joins PAF in the deleted homolog. Analysis of the same rearrangement in NC chromosomes demonstrates that it removes the most part of phD, except the proximal portion, as indicated by an arrow in Fig. 3B.

Chromosome 3 in the SGs of larvae with the deficiency *Df(3L)2-66* lacks material from the proximal part of PAC to PAF (Fig. 3C), whereas both phD and phE regions are visible in the normal homolog of NC chromosomes. At the same time in the rearranged homolog, only the most distal part of phE is present along with phD (Fig. 3D). The blocks phF and phG are not affected by the rearrangement (not shown).

The deficiency *Df(3L)6B-29* + *Df(3R)6B-29* removes the longest parts of chromosome 3 heterochromatin. In the SG chromosomes, one of the break points is in the distal part of PAA, whereas the proximal part of this region, indicated by an arrow, is visible only in one homolog. The second break point was mapped in the euchromatic region 82B of chromosome 3R. In the rearranged homolog, region 82B ectopically connects to the region 101E of chromosome 4, which is followed by diffused material marked by a white arrow and the region PAA (Fig. 3E). In NCs, chromosome 3L with the deficiency lacks phD and phE (Fig. 3F), and chromosome 3R lacks material up to the region 82B (Fig. 3G). As found in SGs, the rearranged homolog in NCs has a small amount of friable material between break points (marked by an arrow in Fig. 3F). This material probably corresponds to h53 or at least a part of this region, containing the centromere, which could not be deleted.

Mapping of the rearrangements *Df(3R)10-65* and *Df(3R)4-*

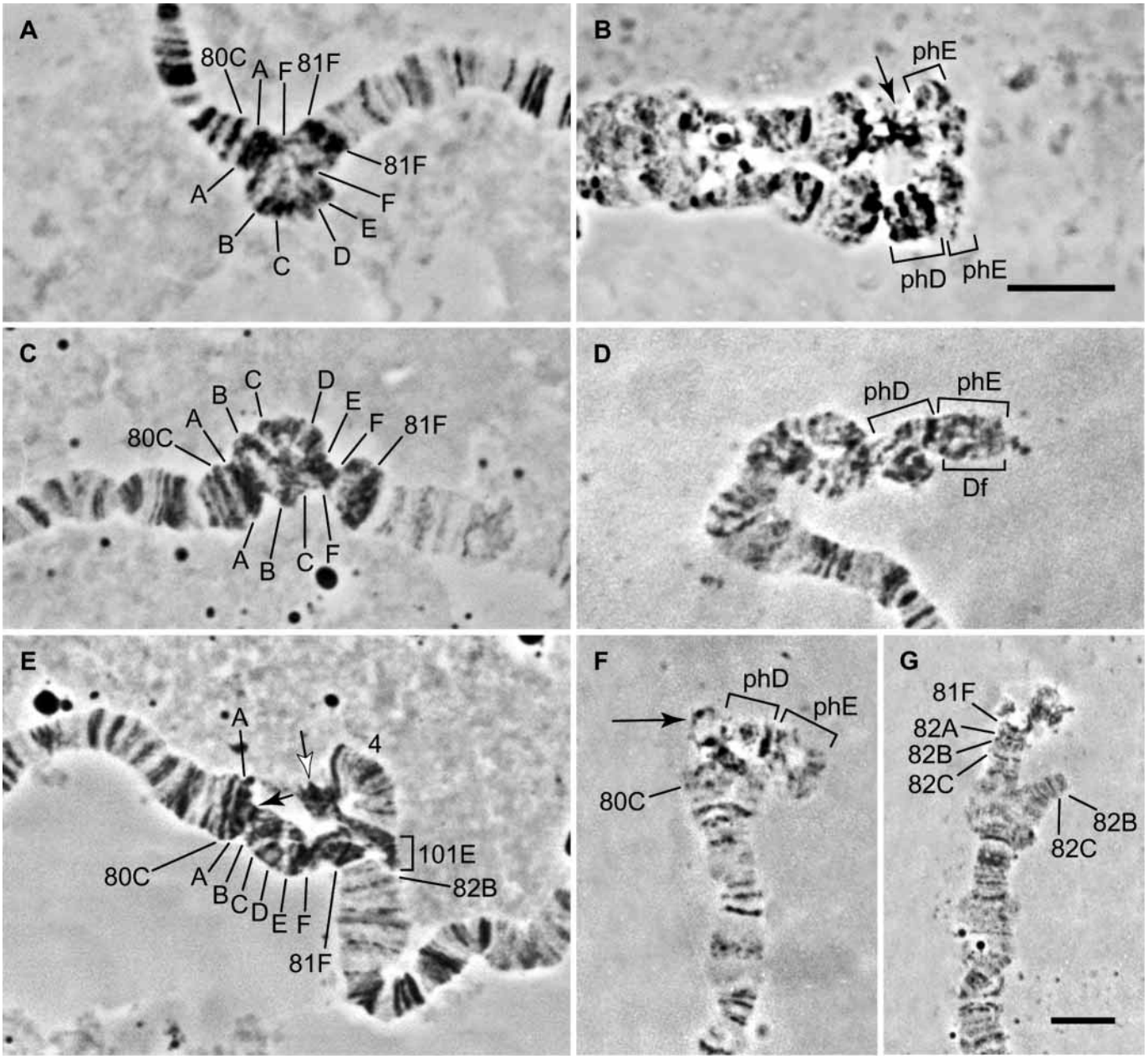

Fig. 3. Heterozygous heterochromatic deficiencies in SG chromosomes of *SuUR* (A,C,E) and NCs of *otu* mutants (B,D,F,G): *Df(3L)1-16* (A,B), *Df(3L)2-66* (C,D), *Df(3L)6B-29* + *Df(3R)6B-29* (E,F,G). The material removed by the deficiency is marked by a bracket in the normal homolog (D). See text for explanation of the arrows. Bar, 5 μm.

75 showed that they are more complex than it was known before. According to genetic (Marchant and Holm, 1988a; Marchant and Holm, 1988b) and cytological data in mitotic chromosomes (Koryakov et al., 2002), the rearrangement *Df(3R)10-65* is a simple deficiency; however, analysis of NC polytene chromosomes reveals that this stock contains a long pericentric inversion. Break points of the inversion are located in constrictions 80C-phD and phG-81F. We could not determine what part of the chromosome is deleted, probably due to its small size (data not shown).

It was shown genetically that *Df(3R)4-75* is a deficiency of part of the 3R arm (Marchant and Holm, 1988a; Marchant and Holm, 1988b), whereas analysis of the mitotic chromosome demonstrated that this stock contains a heterochromatic inversion (Koryakov et al., 2002). Mapping of the rearrangement in SG chromosomes with *SuUR* mutation showed that, in addition to heterochromatic, there is at least one euchromatic inversion with break points in 79E and 83F (data not shown). The presence in so short a chromosome length of at least two inversions and numerous ectopic contacts does not allow us to perform more-detailed mapping.

Summarizing all the data, we can conclude that newly appeared heterochromatic structures are formed by pericentric heterochromatin rather than by proximal euchromatin. Also, left and right arms make an unequal contribution to formation of heterochromatic structures. We determined that, in SG

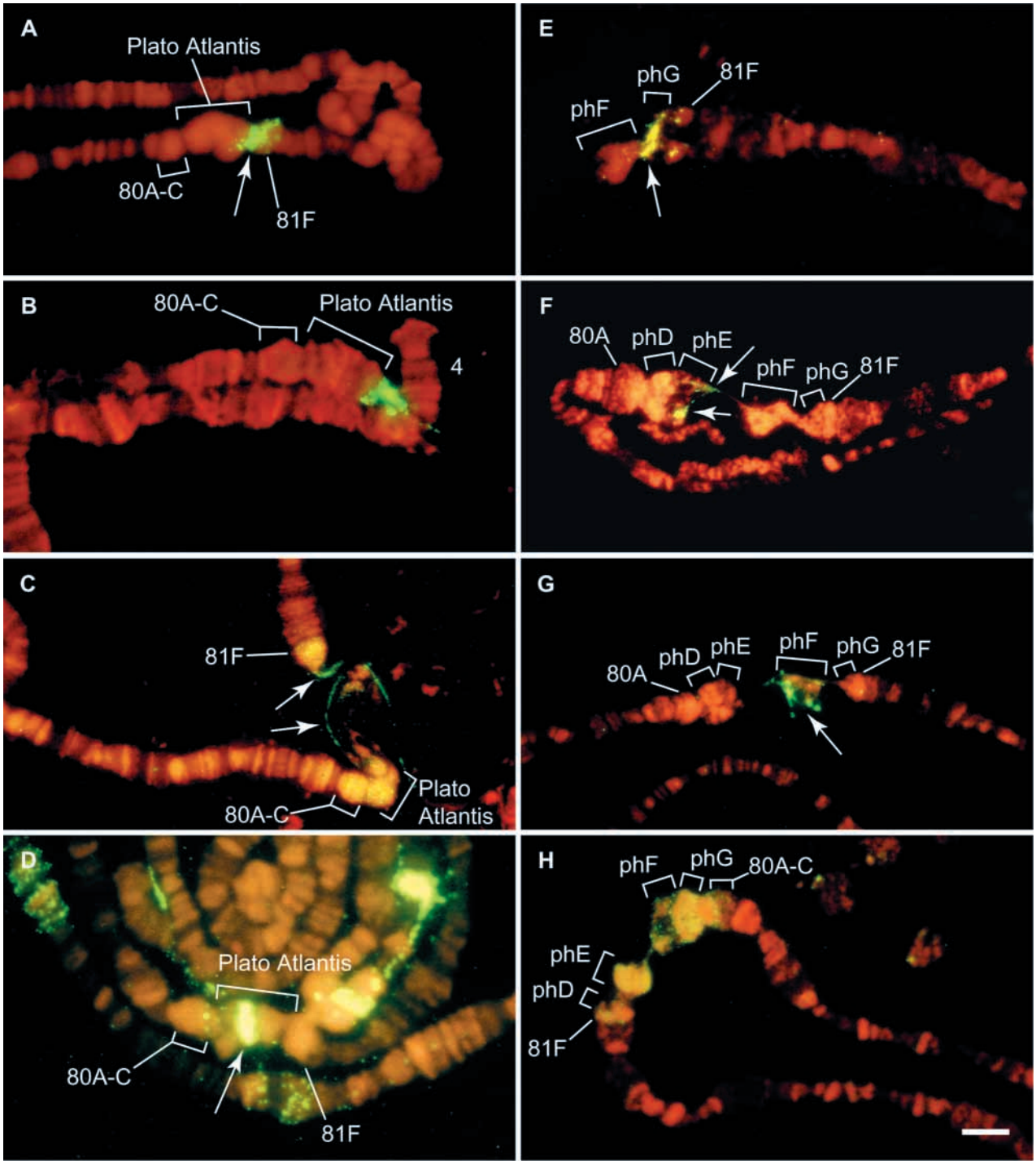

Fig. 4. FISH of the satellite clones AAGAG (A,E), AATAACATAG (B,F), dodecasatellite (C,G), 1.688 (D) and AACAC (H) on the SG (A,B,C,D) and NC (E,F,G,H) polytene chromosomes. Arrows indicate localization of the label. Bar, 5 μm.

chromosomes of the *SuUR* mutants, heterochromatic structures are formed by the blocks h47-h50d; by contrast, in NC chromosomes, in addition to these blocks, h50p, h53 and h57 are also present.

FISH of DNA clones from heterochromatin

Another approach for heterochromatin mapping in polytene chromosomes is fluorescent in situ hybridization (FISH). We hybridized clones of satellite DNA, defining distal portion of left arm heterochromatin (block h48, satellite AATAACATAG), centromeric area (block h53, dodecasatellite) and distal portion of right arm heterochromatin (block h57, satellite AAGAG) (Lohe et al., 1993; Abad et al., 2000) (Fig. 1C), the third chromosome 1.688 satellite (Koryakov et al., 1999; Abad et al., 2000), and AACAC, which

was not shown for chromosome 3 (Makunin et al., 1999). All these clones were hybridized to SG polytene chromosomes of OR-R strain as a control.

In SGs of the wild-type strain, all these clones demonstrate a signal of hybridization as one or several spots in the chromocenter. In the case of the dodecasatellite, in addition to spots we revealed diffused labeling around the chromocenter (data not shown).

Satellites AAGAG (Fig. 4A) and AATAACATAG (Fig. 4B) in SG chromosomes of the *SuUR* mutants label region PAF. The dodecasatellite displays two types of signals: a diffuse weak signal distributed uniformly all around Plato Atlantis, 80A-C and 81F; and a bright site in PAF (Fig. 4C). Also, we noted that, if satellites AAGAG and AATAACATAG after first enhancement of FISH produce a bright signal of hybridization, then the dodecasatellite gives a very weak signal. Probably, this results from a higher polytenization level of AAGAG and AATAACATAG in comparison with the dodecasatellite.

FISH in SG chromosomes reveals the 1.688 satellite in the central part of Plato Atlantis, in region PAC (Fig. 4D). For the satellite AACAC in SG chromosomes after enhancement of brightness, we found a hybridization signal not only in pericentric heterochromatin of chromosome 3, but also in numerous euchromatic sites both in OR-R and in *SuUR* strains (data not shown).

Satellite AAGAG in NC chromosomes hybridizes with the region phG (Fig. 4E). We observed an intensive fluorescence signal even without brightness enhancement both in chromosomes of *otu*; *SuUR* and *otu* mutants. Satellite AATAACATAG labels the proximal portion of phE (Fig. 4F). In this case, the signal was visualized only after a second FISH enhancement. In addition, we noted significant differences in the rate of signals in chromosomes of *otu* and *otu; SuUR* strains: a very weak signal in *otu*, and a bright signal in *otu; SuUR*. The label of dodecasatellite is situated in the distal part of phF (Fig. 4G), and signal was clearly seen even after one enhancement. Sequence AACAC in NC chromosomes, like in SG chromosomes, hybridizes diffusely all around heterochromatin (Fig. 4H).

Results of hybridizations described above demonstrate that Plato Atlantis and structures in NC chromosomes are not identical. If, in Plato Atlantis, the satellites AAGAG, AATAACATAG and dodecasatellite are found in one narrow zone (PAF) then, in NC chromosomes, they mapped in three clearly distinguished regions (phE, phF and phG).

Discussion

What kind of DNA polytenizes in heterochromatin?

Pericentric heterochromatin is almost completely under-replicated in polytene chromosomes of *Drosophila* (Rudkin, 1965; Berendes and Keyl, 1967; Mulder et al., 1968). Unexpectedly, in *otu* mutant NC chromosomes, blocks of banded material were present in the heterochromatin region (Mal'ceva and Zhimulev, 1993). In SG chromosomes of *SuUR* mutants, a reproducible banding pattern appears in the pericentric region of chromosome 3 in place of small lumps of unstructured heterochromatin (Belyaeva et al., 1998; Semeshin et al., 2001). Even in these mutants, the bulk of the heterochromatin remains unpolytenized, so we have addressed the question: what kind of sequences pass additional replication cycles?

The possibility that the novel banded structures are not heterochromatic, but originate from proximal euchromatin, was investigated by deletion mapping. The location of satellites from different parts of heterochromatin to the same region in SGs (Fig. 5A) or close regions in NCs (Fig. 5C) supports an origin of these structures from the most proximal euchromatin of the 3L arm. However, deletion mapping conflicts with this conclusion (Fig. 5D-F). Satellite AATAACATAG, found in the region h48 (Lohe et al., 1993), should be located in regions PAA-PAD of SG chromosomes and in phD of NC chromosomes. This contradiction can be resolved if there is a minor site of this satellite in addition to the major one. According to this hypothesis, the minor site, situated somewhat proximally to h48, polytenizes in SG and NC chromosomes, and this site is seen in FISH experiments, while the major site remains under-replicated.

Availability of several sites, both major and minor, was already shown for the 1.688 satellite. At first, this sequence was found in the X chromosome as a unique large block in heterochromatin (Lohe et al., 1993), and as very short repeats (2-3 copies) in euchromatin (DiBartolomeis et al., 1992). Later, homologous sequences were discovered in chromosomes 2 and 3 (Koryakov et al., 1999; Abad et al., 2000). For this reason, it is possible that sites of dodecasatellite and AAGAG, detected in polytene chromosomes, also correspond to minor sites, which are invisible in mitotics, rather than to major sites in regions h53 and h57. However, data of FISH for satellites AAGAG and dodeca at least do not conflict to rearrangement mapping data.

The situation with the satellite AACAC is somewhat different. In mitotic chromosomes, this sequence was located in chromosomes 2 and Y. In SG polytenes of OR-R and *SuUR* strains, it was found in two bright sites and across the chromocenter as a fine net (Makunin et al., 1999). In our study, this satellite was identified in chromosome 2 after brightness enhancement (not shown) and in numerous sites in hetero- and euchromatin. Probably there are several minor sites of AACAC all around heterochromatin and in euchromatin as well. Also, unspecific hybridization to the sequence AC/TG is possible. This dinucleotide repeat is found in the *Drosophila* genome approximately once per every 30-100 kb (Cirera et al., 1994). Unspecific hybridization may result from errors of Taq polymerase during PCR labeling.

The data obtained mean that, in both types of heterochromatic regions with banding pattern (NCs of *otu* mutants and SGs of *SuUR* mutants), there are no long tracts of satellite DNAs. There are only small sites of 1.688 satellite and, probably, short fragments of AACAC or at least AC/TG. Large blocks of satellites locate either in the narrowest region of Plato Atlantis (PAF), or in friable β-heterochromatic portions of NC chromosomes (Fig. 5A-C).

There is an interesting correlation between the polytenized heterochromatic regions and the location of essential 'heterochromatic' genes. There are 16 complementation groups in chromosome 2 heterochromatin (Hilliker and Holm, 1975; Hilliker, 1976; Schupbach and Wieschaus, 1989; Eberl et al., 1993), which can be divided into three groups in the right arm (in distal, proximal and middle parts) and one group in the distal part of the left arm (Dimitri, 1991; Dimitri et al., 2003).

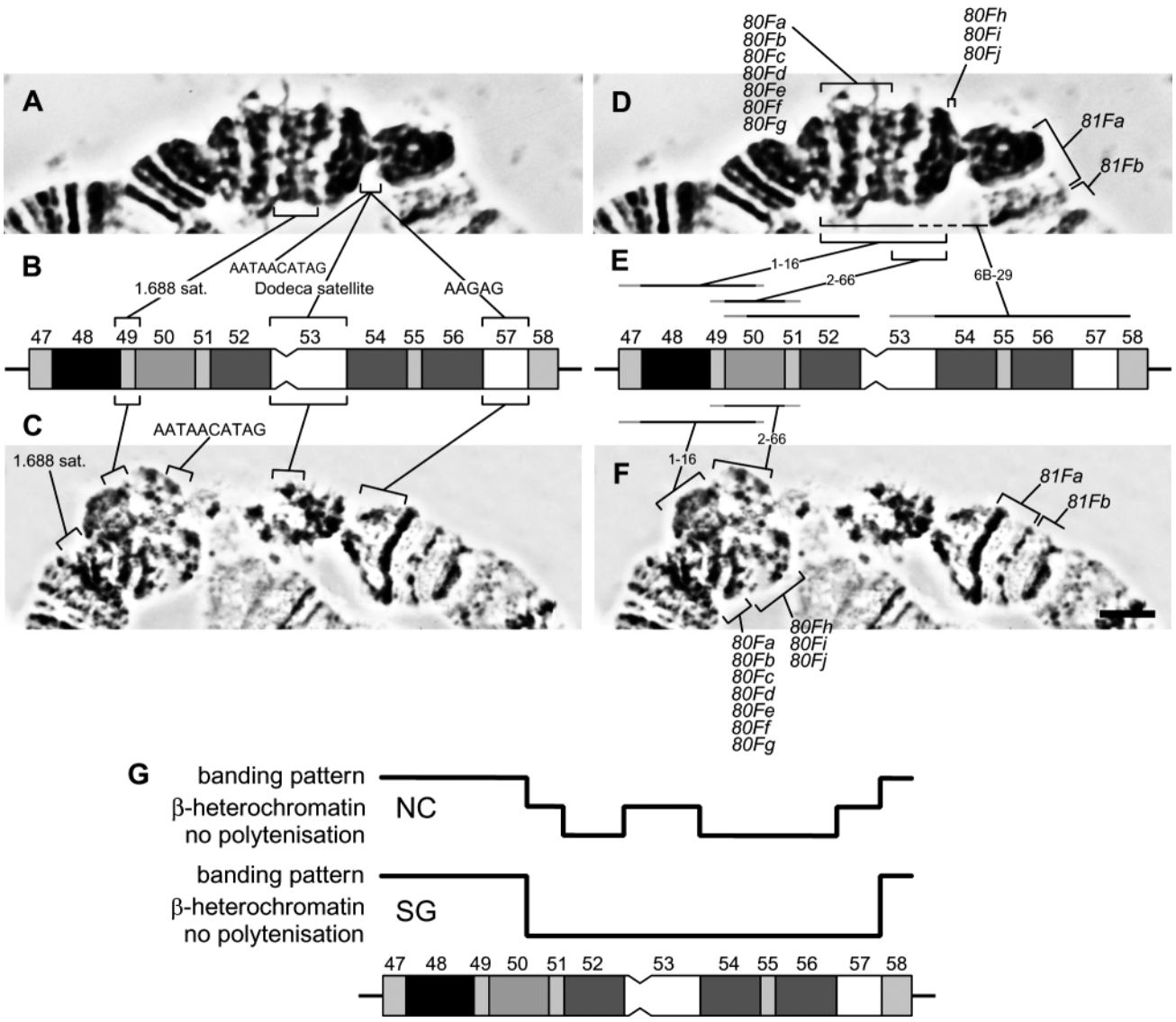

Fig. 5. Correspondence between heterochromatin in SG chromosomes of *SuUR* mutants (A,D), mitotic chromosomes (B,E) and NC chromosomes of *otu-SuUR* nutants (C,F). Location of satellite sequences (A-C) and rearrangements and genes (D-F). Panel G demonstrates regions of mitotic heterochromatin polytenizing both in SG and NC chromosomes. Bar, 3 μm.

In NC chromosomes, namely gene-containing regions form large diffuse heterochromatic blocks. For example, the major part of the left arm does not contain known genes, and no noticeable structures are found in NC chromosomes, but right arm heterochromatin consists of at least two large blocks (Koryakov et al., 1996). A similar situation was found in chromosome 3. It was shown that all the genes of the left arm are grouped in regions h47-h51, and of the right arm in region h58 (Koryakov et al., 2002). Mapping of deletions indicates that almost all Plato Atlantis are formed by the sections of chromosome 3 removed by the deficiencies *Df(3L)1-16* and *Df(3L)2-66*, which contain all known genes for 3L arm heterochromatin (Marchant and Holm, 1988a; Marchant and Holm, 1988b) (Fig. 5D,E). The part of chromosome 3 heterochromatin in NCs, which can produce the banding pattern (phD and phE), is formed by these regions as well (Fig. 5E,F). Right arm heterochromatin contains only two known genes locating in the most distal part (Marchant and Holm, 1988a; Marchant and Holm, 1988b; Koryakov et al., 2002). In Plato Atlantis, 3R heterochromatin is not presented almost completely and, in NCs, it consists of a pair of typical β-heterochromatic diffuse blocks (phF and phG) (Fig. 5D-F).

X chromosome heterochromatin does not contain unique genes (reviewed by Gatti and Pimpinelli, 1992), no new blocks appear in polytene chromosomes, and new bands in division 20 in *SuUR* mutants are not related with heterochromatin (Koryakov et al., 1999; Kolesnikova et al., 2001). It seems evident that predominantly gene-containing regions of heterochromatin undergo polytenization or banding pattern formation in polytene chromosomes of SGs of *SuUR* mutants and NCs of *otu* mutants. This conclusion is in good correlation with data about polytenization of heterochromatic genes (Devlin et al., 1990; Berghella and Dimitri, 1996).

Differences in heterochromatin polytenization in somatic and germline cells

In spite of many common features, there is a very important difference between chromosomes of SGs and NCs. Polytene chromosomes of normal morphology do not develop in NCs and appear only in some mutants such as *otu* (King et al., 1981). This gene influences egg chamber formation, the system of actin microfilaments and the transfer of substances from NCs to the growing oocyte (Storto and King, 1988). In some way, the absence of normal product of this gene causes polytene chromosome formation, but it does not increase the polytenization level of heterochromatin. In SGs, the situation is opposite, polytene chromosomes develop normally, but heterochromatin is not polytenized, and partial polytenization can be caused only by the mutation *SuUR*. In addition to this, in SG chromosomes this mutation makes global changes in pericentric heterochromatin, but has little effect in NCs.

Plato Atlantis and blocks in NC chromosomes are different with respect to several other traits. First, a single banded structure was found between the arms of chromosome 3 in SGs whereas, in NC chromosomes, four structures appear, which have both euchromatic and heterochromatic morphology. Second, three satellite DNAs locate at the same site in SG chromosomes, whereas they are in three clearly distinguishable blocks in NCs. Third, regions affected by deficiencies *Df(3L)1-16* and *Df(3L)2-66* in NC and SG chromosomes are different.

According to genetic data, these deficiencies do not overlap (Marchant and Holm, 1988a; Marchant and Holm, 1988b), but they partially overlap by cytological data (Koryakov et al., 2002) (Fig. 5E). Based on rearrangement mapping, we can determine where heterochromatic genes are located in Plato Atlantis. Deficiencies *Df(3L)1-16* and *Df(3L)2-66* do not overlap in sections PAA-PAC, which means that seven distal genes (*l(3)80Fa* to *l(3)80Fg*) are in this interval. In PAD-PAE, these deficiencies overlap, and proximal break points coincide with each other. It is followed by the conclusion that three proximal genes (*l(3)80Fh* to *l(3)80Fj*) locate somewhere in the narrowest portion of PAE, which could not be distinguished by cytological methods in SGs. The position of satellite AAGAG and mapping of *Df(3L)6B-29 + Df(3R)6B-29* allows us to conclude that the gene *l(3)81Fa* is situated in 81F-82B, and the gene *l(3)81Fb* is situated distally to 82B (Fig. 5D).

In NC chromosomes, deficiencies *Df(3L)1-16* and *Df(3L)2-66* are clearly discerned, and the overlapping zone seems to be very small and locates between blocks phD and phE. This makes it possible to put genes *l(3)80Fa* to *l(3)80Fg* into phD, and genes l*(3)80Fh* to *l(3)80Fj* into phE. Like in SGs, one of the right arm genes (*l(3)81Fa*) is situated in 81F-82B, and another one (*l(3)81Fb*) locates distally to 82B (Fig. 5F).

Summarizing data of the morphological analysis and detailed comparison of rearrangements, we construct a scheme comparing two types of polytene and mitotic chromosomes (Fig. 5G). We conditionally marked out three levels of polytenization: absence, middle level with diffuse β-heterochromatin formation and high level with banding pattern formation. It is seen that the same regions of mitotic heterochromatin behave differently in NC and SG chromosomes. In both cases, the regions PAA-PAE and phD are homologous and formed by the blocks h47-h50d, whereas other blocks (h50p, h53 and h57) are partially polytenized in NCs, but absent in SGs. It is clear that the genetic systems controlling replication of DNA and especially heterochromatin in somatic and germline cells may be different.

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