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Chromosomal distribution of Heterochromatin Protein 1 (HP1) in *Drosophila:* a cytological map of euchromatic HP1 binding sites

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Abstract

The Heterochromatin Protein 1 (HP1) is a conserved protein which is best known for its strong association with the heterochromatin of *Drosophila melanogaster*. We previously demonstrated that another important property of HP1 is its localization to the telomeres of *Drosophila*, a feature that reflects its critical function as a telomere capping protein. Here we report our analysis of the euchromatic sites to which HP1 localizes. Using an anti-HP1 antibody, we compared immunostaining patterns on polytene chromosomes of the *Ore-R* wild type laboratory strain and four different natural populations. HP1 was found to accumulate at specific euchromatic sites, with a subset of the sites conserved among strains. These sites do not appear to be defined by an enrichment of known repetitive DNAs. Comparisons of HP1 patterns among several *Drosophila* species revealed that association with specific euchromatic regions, heterochromatin and telomeres is a conserved characteristic of HP1. Based on these results, we argue that HP1 serves a broader function than typically postulated. In addition to its role in heterochromatin assembly and telomere stability, we propose that HP1 plays an important role in regulating the expression of many different euchromatic regions.

Introduction

Heterochromatin Protein 1 (HP1) is a phylogenetically strongly conserved chromosomal protein (Jones, Cowell & Singh, 2000) which associates with the heterochromatin and telomeres of *Drosophila melanogaster* (James & Elgin, 1986; James et al., 1989; Fanti et al., 1998a,b). Many features of this protein are already known. Structurally, HP1 has two prominent motifs, the chromodomain (Paro & Hogness, 1991) and chromoshadow domain (Aasland & Stewart, 1995) which are thought to be important for chromatin binding and protein interactions, respectively. It is encoded by the *Su(var)2-5* locus, which acts as a dosage-dependent modifier of position effect variegation (PEV) (Eissenberg et al., 1990). Both the localization of HP1 in heterochromatic regions and its effect on PEV have strongly suggested that this protein plays an essential role in heterochromatin formation. We recently discovered another critical role for HP1, specifically in telomere formation and stability (Fanti et al., 1998a,b). HP1 is a structural component of all *Drosophila* telomeres and when absent, results in extensive telomeric fusions. These observations strongly suggest a role for HP1 in telomere capping.

Previous studies have provided evidence for the presence of HP1 in at least some euchromatic regions (James et al., 1989; Fanti et al., 1998a,b). This localization suggests that HP1 could also play a role in the repression of specific subsets of euchromatic genes (Elgin, 1996). Strong support for this idea has recently been demonstrated by the finding that HP1 is involved in the repression of genes contained in a region that shows intense HP1 staining in polytene chromosomes (Hwang, Eissenberg & Worman, 2001). Localization to euchromatin might also indicate a second possible role for HP1, namely to bind to and regulate sequences that are not canonical genes, but rather

'foreign' sequences such as transposable elements (TE). HP1 might silence TE or other types of repetitive DNAs, which when released from silencing could alter the expression of genes or otherwise perturb the genome. This latter possibility is consistent with two types of data. Firstly, HP1 is present at high concentration in the chromocenter (James et al., 1989) of polytene cells. The chromocenter is comprised predominately of repetitive sequences, many of which are TE or sequences of TE origin (Vaury, Bucheton & Pelisson, 1989; Miklos & Cotsell, 1990). HP1 may have some specificity for regions that are enriched by certain types or families of TE. Secondly, it has been reported that tandem repeats of a mini-*white* transgene show variegated expression that is sensitive to the level of HP1. Significantly, the regions containing these repetitive transgene arrays show a strong accumulation of HP1 in polytene chromosomes (Fanti et al., 1998a).

To explore the significance of the localization of HP1 to euchromatic regions, we performed a detailed cytological analysis of the distribution of HP1 in polytene chromosomes of five different populations of *Drosophila* using an anti-HP1 antibody. Our mapping studies show that in addition to localization in the chromocenter, along the fourth chromosome and at all telomeres, HP1 was located in about 200 euchromatic sites. Comparisons of the immunofluorescence patterns among strains revealed variability in the majority of the HP1 euchromatic sites in presence or absence or in intensity of staining. However, a subset of specific sites are well conserved. Many of the stable, conserved sites do not correspond to sites containing known transposon-like families. Furthermore, we analyzed the HP1 distribution along the euchromatic arms of polytene chromosomes of different *Drosophila* species. We observed that HP1 is located in both the heterochromatin and euchromatin in these species. In addition, we observed that HP1 has maintained its presence in certain homologous regions across the species.

Materials and methods

Drosophila *stocks*

The *Ore-R* stock used in these studies has been maintained in our laboratory for many years. The *Altamura* stock is from flies collected in Altamura near Bari, Italy. The other populations, *Amherst-3* (USA); *Boa Esperance* (Brazil); and *W125* (Russia) were obtained from the Umea (Sweden) stock center. The *Su(var)205* strain was obtained from Wakimoto. The *Su(var)2-5^{02}, Su(var)2-5^{04}, Su(var)2-5^{05}* strains were provided by Reuter. The *P[(neo^{r})HSHP1.83C* stock was provided by Eissenberg. Cultures were maintained at 24°C on standard cornmeal–sucrose–yeast–agar medium. Heat shock experiments were performed according to Eissenberg and Hartnett (1993).

Immunofluorescence and fluorescence in situ *hybridization (FISH)*

The HP1 immunostaining of polytene chromosomes of HP1 mutants, *Ore-R* wild type strain and natural populations was performed according to James et al. (1989) using the C1A9 primary HP1 antibody. All the preparations were incubated with the same primary HP1 antibody solution. Moreover, the same exposure times were used for all the preparations. The secondary antibody was fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse Ig (whole antibody). The C1A9 antibody was diluted 1:50 and the secondary antibodies were diluted 1:20 in PBS with 1% bovine calf serum. Following incubation, the slides were washed three times in PBS, stained with DAPI (4,6-diamidino-2-phenilindole) at 0.01 μg/ml and mounted in anti-fading medium. For each population, salivary glands from 10 larvae were prepared and about 10 good polytene nuclei of each larva were examined. The fluorescence *in situ* hybridization (FISH) was performed according to Pimpinelli et al. (2000). The simultaneous localization of the copia, gypsy, mdg-1, blood, Doc, I, and F transposons along the polytene chromosomes were performed using mixed probes corresponding to their internal fragments as described in Pimpinelli et al. (1995). Two YAC clones, 18F10 and 5B5 which were used to make probes, were obtained from the 'Foundation for Research and Technology – Hellas' (FO.R.T.H., Crete, Greece). These YACs contained DNA sequences located in the region 31 on the cytogenetic map of the left arm of the second chromosome. Chromosome preparations were analyzed using a computer-controlled Nikon E1000 epifluorescence microscope equipped with a cooled CCD camera (Coolsnap). The fluorescent signals, recorded separately as gray scale digital images, were pseudocolored and merged using the Adobe Photoshop program. For HP1 immunostaining analyses, the same CCD camera exposure times were used for each experiment. The fluorescence

intensity quantitation was performed using the Adobe Photoshop program as described by Pimpinelli et al. (1995).

Results

Distribution patterns of HP1 on polytene chromosomes of different natural populations of D. melanogaster

To investigate the chromosomal distribution of HP1, we immunostained polytene chromosomes of *Ore-R* and four other wild type strains of *D. melanogaster* with the C1A9 monoclonal antibody that recognizes this protein. The *Ore-R* strain is a well-established wild type laboratory strain; the other four strains are derived from natural *Drosophila* populations with distinct geographical origins (see Materials and methods). The immunopattern produced with the C1A9 antibody on polytene chromosomes of the *Ore-R* laboratory stock is reported in Figure 1. As has been previously reported, HP1 is abundant on the chromocenter, along the fourth chromosome and at the telomeres (James & Elgin, 1986; James et al., 1989; Fanti et al., 1998a,b) of this strain. Other numerous signals along the euchromatic arms are also visible. Intriguingly, we found that some of the euchromatic signals colocalize with the physiological puffs, including those induced by the ecdysone. As we have previously documented (Fanti et al., 1998a,b), different mutations of *Su(var)2-5*, the locus that encodes HP1, affect the HP1 immunopattern. Specifically, larvae heterozygous for the *Su(var)2-5*^{04} and the *Su(var)2-5*^{05} alleles completely lack HP1 and do not show C1A9 immunosignals on their polytene chromosomes. Hence, these data clearly demonstrate that the immunopattern observed in wild type larvae, including the euchromatic banding pattern, is due to the presence of HP1 and not to cross-reactivity of the C1A9 antibody with other chromodomain containing proteins (see also Figure 1(B)–(D)).

Table 1 reports the immunostaining patterns produced by the C1A9 antibody on polytene chromosomes of the *Ore-R* and four different natural populations. The comparative analysis of these patterns has revealed a series of interesting features regarding the chromosomal distribution of the HP1 protein. First of all, the comparison verifies that HP1 protein is stably and invariably localized on the chromocenter, the fourth chromosome and all the telomeres (James & Elgin, 1986; James et al., 1989; Fanti et al., 1998a,b). In addition to these locations, we also detected HP1 in about 200 euchromatic regions. About 20 of these regions (bold-faced in Table 1), including the intensely staining region 31 on the left arm of the second chromosome, show a stable and invariable positive staining in all populations. Thirty-six other euchromatic regions (bold-faced in Table 1) are also conserved but show a variable fluorescence intensity among the strains. Finally, the vast majority of the euchromatic regions are variable, with a differential presence or absence of the HP1 association depending on the population. In some cases, we noted variability of staining of a subset of these sites among individuals of a single population (denoted by +/− in Table 1).

To test whether the variability in the HP1 staining patterns we observed depended on real differences in the ability of HP1 to bind certain sites, we performed immunostaining experiment on polytenes from hybrids of different populations. Figure 2 shows one example of the HP1 immunostaining of hybrid larvae containing one X from the Boa Esperance stain and one X from Amherst strain. The X chromosome of the first population presents a strong HP1 immunosignal in the 1D region (Figure 2(A)) while in the same region of the X chromosome of the second population lacks an immunopositive band (Figure 2(B)). In the polytene chromosomes of the hybrid larvae, an immunosignal on the 1D region of only one X chromosome is present (Figure 2(C)), verifying that differences in patterns observed are not due to variable staining technique.

HP1 localizes to only a subset of the intercalary heterochromatin regions and does not seem to be related to an enrichment of specific repetitive DNA sequences

Several regions in the euchromatin of *Drosophila* have been defined as intercalary heterochromatin (IH) on the basis of shared criteria in polytene tissue (Kaufmann, 1939; Zhimulev et al., 1982). These criteria are a constricted appearance in polytene chromosomes and underreplication or delayed replication compared to the well-banded, and fully polytenized euchromatic regions. To assess whether regions defined as IH might be preferential sites for HP1 accumulation, we compared the reported IH regions with the HP1 staining regions. We found that HP1 colocalized with only a subset (about 30%) of the IH regions.

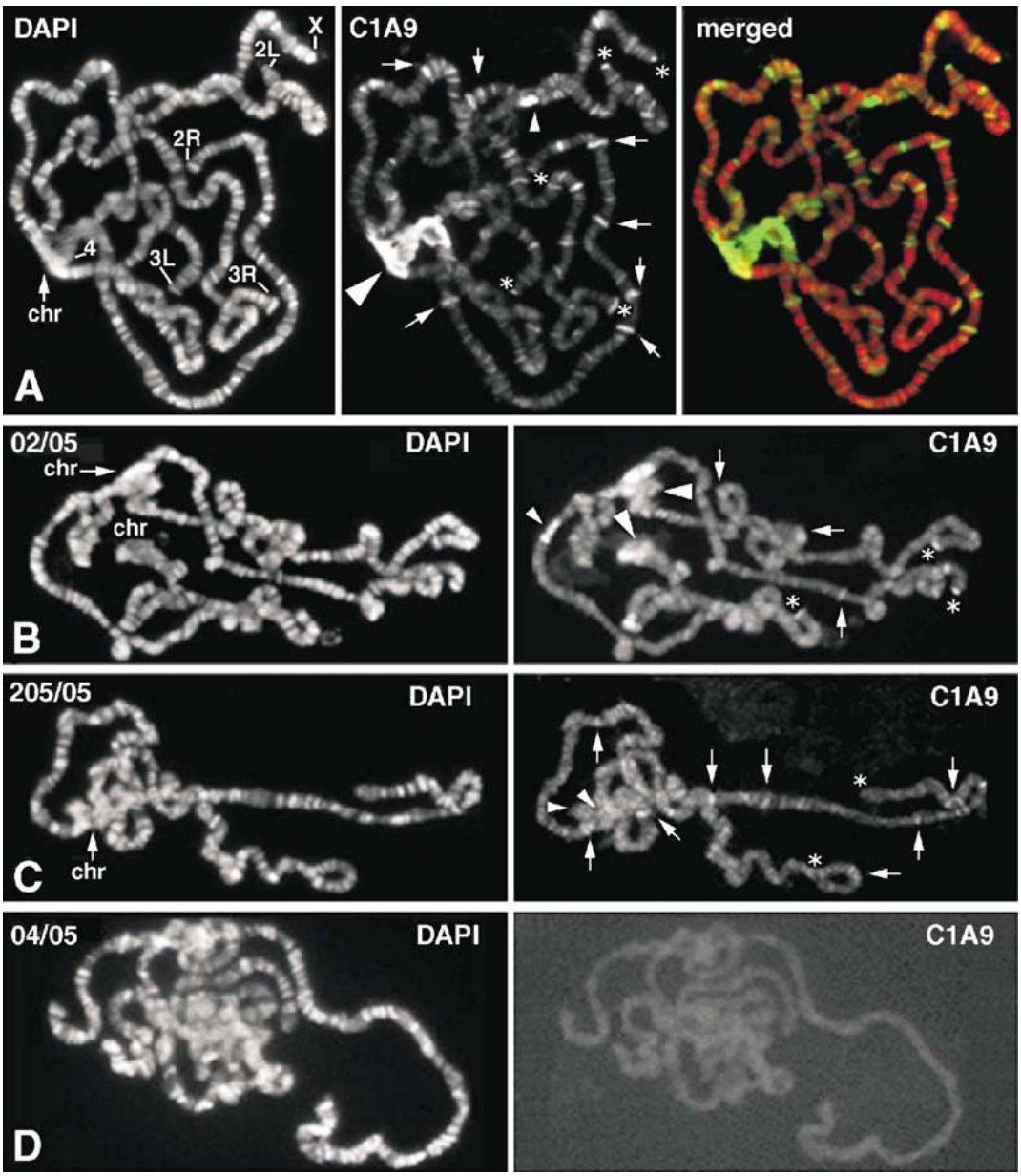

Figure 1. Localization patterns of HP1 in the polytene chromosomes of the *Ore-R* wild type strain and *Su(var)2-5* mutants. (A) The polytene chromosomes of a *Ore-R* larvae were stained with DAPI and immunostained with the C1A9 antibody. The two patterns were then pseudocolored and merged. HP1 is strongly accumulated in the heterochromatic chromocenter (large arrowhead) and is present at the telomeres (asterisks), and in many euchromatic bands (arrows). Note the intense staining throughout the euchromatic region 31 (small arrowhead). (B)–(D) Three different mutant alleles of *Su(var)2-5* were analyzed in heterozygous combination with a protein null allele($Su(var)2\text{-}5^{05}$), so that the effect on immunostaining could be attributed to each mutation tested. (B) Immunopattern on polytene chromosomes of $Su(var)2\text{-}5^{02}/Su(var)2\text{-}5^{05}$ (02/05) mutant larvae. The $Su(var)2\text{-}5^{02}$ allele is a point mutation in the chromodomain and seems to affect mainly the heterochromatic chromocenter binding, as evident by the weaker staining of the chromocenter (large arrowhead) compared to the intensity seen in wild type larvae. In contrast, the signals along the euchromatic arms (arrows), the 31 region (small arrowhead) and telomeres (asterisks) were not affected by the mutation. (C) Immunopattern on polytene chromosomes of $Su(var)205/Su(var)2\text{-}5^{05}$ (205/05) mutant larvae. The *Su(var)205* mutation produces a protein with an insertion of eight amino acids in the carboxy-terminal region. Note that the chromocenter (arrowheads) and the telomeres (asterisks) appear unstained and only few euchromatic immunosignals are present (arrows). (D) Immunopattern on polytene chromosomes of $Su(var)2\text{-}5^{04}/Su(var)2\text{-}5^{05}$ (04/05) mutant larvae. The $Su(var)2\text{-}5^{04}$ mutation is equivalent to a null mutation in that it produces a polypeptide lacking the domain required for nuclear targeting. The chromosomes appears completely unstained, providing evidence that the C1A9 immunopattern depends exclusively on the presence of HP1. X: X chromosome; 2L and 2R are the left and right arm of chromosome 2, respectively; 3L and 3R are the left and right arm of chromosome 3; 4: chromosome 4; chr: chromocenter.

Table 1. HP1 distribution along the euchromatin of polytene chromosomes from the *Ore-R* laboratory strain and four different natural population[a]

X chromosome	Populations					2nd chromosome	Populations					3rd chromosome	Populations				
	Or R	(A)	(B)	(C)	(D)		Or R	(A)	(B)	(C)	(D)		Or R	(A)	(B)	(C)	(D)
1 A 1,2	++	++	++	++	++	**21 A 1,2**	+	+	+	+++	+++	**61 A 1–3**	+++	+	+	+	+
1 B 11	+	−	+	−	−	**21 B 4,5**	+	+	+	+++	+++	61 C 1-4	+	−	+	−	+−
1 D 1,2	+	++	+	−	+−	22 A 6	+	−	+	+	+−	61 D 3,4	+	+	−	−	+
2 B	+	−	+	+	+	22 D 1-5	+	−	+	+−	+	61 E 3	+	+	+	−	−
2 D 1,2	++	+	+	+−	+	23 C 1,2	+	++	−	−	+	61 F 5-8	−	+	−	−	−
2 E 1,2	−	−	+	−	−	23 D 1,2	−	−	−	+−	−	62 B 9	−	+	+	−	−
2 F 4-6	−	−	+	−	+	23 E	+	−	−	+	−	**62 C 3,4**	+++	+	+	+++	+
3 D 1,2	+	++	−	−	+	24 A 3-5	+	+−	−	+	+−	62 E	+	−	+	−	−
3 E 3,4	++	+	+	+	+	24 D 2,3	+	+	−	+	+−	62 F 1,2	++	−	+	+	+
4 D 3-7	+	+	+++	+	+	**24 F 3-8**	+	+	+	+	+	63 C	−	−	−	+	−
4 E 3	+	+	−	−	−	25 B	+	+	?	+	−	63 D 3	−	+	−	−	−
4 F 9,10	+	+	−	−	−	**25 D 1-5**	++	++	++	++	++	63 E 5-9	−	+	−	−	−
5 A 7	+	+	+	−	−	26 A 2,3	+−	−	−	+−	+−	63 F 3-7	+	++	−	+−	+−
5 C 3-10	−	−	+	?	−	26 B 10,11	+−	−	+	−	−	64 B 14-17	+	−	−	+	−
5 E	−	−	−	+	+	27 E 1,2	+	++	−	−	+	65 D 4-6	+	−	−	+	+
6 A 3,4	+−	−	−	+	−	28 A 3-6	+	+	−	−	?	66 D 13-15	+	+	−	+	−
6 D 7,8	+	+	+	+	+	**28 B 1,2**	+	+	+	+	++	66 F 5	+	−	−	−	−
6 E 9-11	++	++	+	+	+	28 F 5	+−	−	+−	−	−	67 B	+	?	−	+	−
7 A 3-5	++	+	+	+	+	29 F 1,2	+−	−	−	+	−	67 C 6-11	+	+−	−	−	−
7 B 3,4	++	+	+	+−	+	**30 A 3-6**	+	++	+−	+	+	67 D 7	+−	+	+−	−	−
7 D	+	−	−	+	+	30 B 3-12	+	+−	+−	+	+	67 E 5-7	+−	+	−	+	+
8 C 7,8	+	+	+	+	+	30 F	+	+−	+	−	−	67 F 4	−	+	+−	−	+
8 E 5-12	+	+	+	+	+	**31B-31E**	+++	+++	+++	+++	+++	68 C 1,2	+	−	−	−	−
8 F 10-9 A1	−	−	+	−	+	**32 C 3-5**	+	+	+	+	+	69 D 3-4	+	?	−	?	+
9 A 4	+	−	−	−	−	**33 B 1,2**	+	+	+	+	+	69 F 3-7	+	+	−	+	+
9 D 1,2	+−	+	+	−	−	33 C	+	?	−	−	−	**70 F 1,2**	++	+	++	++	+
9 E 1,2	+	+	+	−	?	**33 E 3-10**	+	+	+	+	+	**71 D 1,2**	++	+	++	+	+
10 A 3-11	++	++	++	++	++	**34 A 5,6**	++	++	+	+	+++	72 D 7-9	+	+	−	+	+
10 C 1,2	++	++	++	++	++	34 B 6,7	+	+	−	−	−	73 A 5	−	+−	−	+	+
10 F 10,11	+	+	++	?	−	35 B	+	−	+	+	+	73 E 3-6	++	?	−	+++	−
11 B 17,18	+	+	+	+	+	36 A 6,7	+	+	+	−	+	74 D 4	++	+−	−	+−	+

Table 1. (*continued*)

X chromosome	Populations					2nd chromosome	Populations					3rd chromosome	Populations				
	Or R	(A)	(B)	(C)	(D)		Or R	(A)	(B)	(C)	(D)		Or R	(A)	(B)	(C)	(D)
11 D 3-11	+	+	+	−	+	36 F	+	+	+	−	−	75 B 1,2	+	+	−	+−	+
11 F 7-9	+	−	+	+	−	**38 B 3-6**	+	+−	++	+	+++	75 F 1,2	+−	−	−	+−	−
12 A 1,2	+−	+	−	−	−	38 C 1,2	+	−	−	+	+−	**76 D 3,4**	+	+	+	+	+
14 A 1,2	+++	+++	+++	+++	+++	39 A 1,2	+	+−	−	−	+	76 E 1,2	+	+−	+	+−	+
14 C 6,7	++	++	++	++	++	39 C 1,2	+	−	+	−	−	77 E 5-8	+	+−	−	+−	−
14 F 4	+	+	−	−	−	40 A 1-4	+	−	++	++	+	78 B 1,2	+	−	−	−	−
15 C 1,2	+−	+	−	−	−	41	+	+	−	?	−	79 E 3	+	−	−	−	−
16 A 3,4	+	+	−	−	−	42 A 3	++	−	−	−	−	82 F	++	−	+	++	+
16 B 12	+	+	+	+	−	42 A 12	++	−	−	−	−	83 C 5-9	+	−	+	+	−
16 C 9,10	+	+	−	−	+−	42 B	+	−	+	?	−	83 F 1,2	+	−	+−	+	+
16 E 1,2	+	+	+	+	−	42 E	+	−	−	−	−	84 B 3-6	++	−	++	++	+
17 F 1,2	+	−	+	?	−	43 C	+	−	+	−	−	**84 F 14-16**	+	+	+−	++	+−
18 F 4,5	+	−	+	?	+	**43 E**	++	+	++	++	−	**85 F 1-8**	++	+	+	+	+
20 A	+	+	−	+	−	45 B 1,2	+	+−	+	+	−	86 E 12-18	+	+	−	−	+
						45 F	+	−	−	−	−	87 B	+	−	−	+	+
						46 C	+	+−	+	+	−	87 D	+−	−	+−	−	+−
						46 F	+	−	−	−	−	87 F 4-10	+	+	−	++	+−
						47 A 14-16	+	−	+−	+	−	88 A 6-8	+	+−	−	++	+
						47 D 5-8	+	−	+	−	−	**88 C**	++	+	+	++	++
						47 E 3-6	−	+−	−	−	+	**88 D**	++	+	+	+	+
						48 B 1-5	+	+−	−	−	+	88 E	+−	+	−	+	−
						48 C	+	+−	+	+	+	**88 F 7-9**	++	++	+	++	+
						49 F 1-8	+	−	+	+	+	89 A	+−	−	−	+	−
						50 C 6-10	+	++	+	+	+++	**89 B**	+	+	+	+	+
						50 D 4,5	+	+−	+	−	++	**90 BC**	++	+	+	+	+−
						50 E 1,2	++	+	−	+	+++	**90 D 3-6**	++	++	++	++	++
						51 F	+	−	−	−	−	91 A 1-3	−	+−	−	+	+−
						52 A	+	−	−	−	−	**91 D 3-5**	++	++	+	+	+
						52 C 3,4	+++	++	+++	++	+	**92 A**	++	++	+	+++	++
						53 D 3-9	++	+	+	+	+	92 B 4-11	+	+	−	−	−
						54 A 1,2	+	+	+−	+	−	92 C 3-6	+	+	−	−	+−

55 A	+	−	+	−	−	93 A 6,7	−	+−	−	?	+−
55 E 1,2	+	+	−	−	+−	93 B	−	−	−	−	+
56 D	+	−	−	−	−	93 C	+−	−	−	−	+
56 E	+++	+++	+++	+++	+++	**93 D 6,7**	++	++	++	++	++
57 B 3	+−	−	+	−	−	94 A 12-16	+	−	+	+	+
57 C 5-9	+	+	−	−	+	**94 C 1-5**	++	++	+	++	+
57 F 3-11	+	+	+	+	+	94 E	+	−	−	−	+
58 E	++	++	++	++	++	95 E 1,2	+−	−	−	+−	−
59 D 5-11	+	+	+	+	+	95 F	+−	−	−	−	+
60 B 12,13	+++	+++	+++	+++	+++	96 E 1,2	+	−	+−	−	+
60 F	++	+	+	+	+	96 F 12-14	+	+−	+−	−	+
						98 D 6,7	+	+	+	++	+
						98 E 5,6	+	+	−	+	+
						98 F 1,2	+	−	+	+	−
						99 B 3	+	−	−	+	+−
						99 B 6-11	+	+	−	+	+
						99 C 1-6	+	−	+−	−	−
						99 D 5-9	−	−	−	+	−
						99 F	+	−	+−	+	−
						100 B 6-9	+	−	−	+	−
						100 F	+	+	+	+	+

[a] The populations are: (A) = Boa Esperance (Brazil; Minas Gerais); (B) = Altamura (Italy); (C) = Amherst-3 (USA); (D) W125 (Russia).

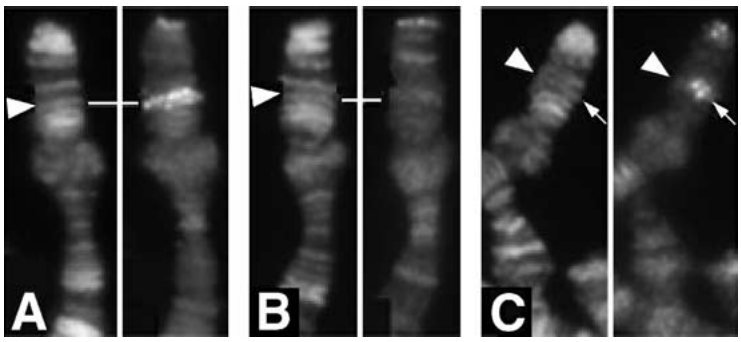

Figure 2 The differences between HP1 signals observed among the different populations reflect real differences in HP1 binding. (A)–(C) compares the staining patterns in the distal part of the X chromosome from larvae of two different strains and in a hybrid individual carrying both X chromosomes. In each figure the left image shows the DAPI staining and the right image shows the staining with the C1A9 antibody. (A) Boa Esperance; (B) Amherst and (C) the Boa Esperance/Amherst hybrid. Note that in the sub-telomeric region 1D of the X chromosome (arrowheads), the larva from the Boa Esperance population has a strong HP1 immunosignal. This same region lacks a signal in the larva from the Amherst population. In Boa Esperance/Amherst hybrid, the 1D region of one chromosome shows the HP1 signal (arrow) while the other homolog shows no signal (arrowhead).

Another possibility is that the euchromatic regions to which HP1 localizes may be characterized by certain type of repetitive DNA sequence or by repeat DNA in general. This idea is an extension of the notion that the heterochromatic localization of HP1 might depend on an enrichment of repetitive DNAs. Therefore, we asked if HP1 co-localized with known repetitive sequences in the euchromatin. First, we compared the HP1 euchromatic localization pattern to the distribution of DrD sequences. Miklos et al. (1988) showed that DrD is a repetitive DNA sequence that is present in the chromocenter and throughout the fourth chromosome in a pattern that appears grossly to resemble that of HP1. We compared the DrD pattern to that HP1 euchromatic sites detailed in Table 1 and found only a scarce overlap of sites. We extended this analysis to other known repetitive DNAs by performing C1A9 immunostaining and sequential FISH on polytene chromosomes. For efficiency, we surveyed numerous transposon-like families using mixed probes for the FISH assay (see Materials and methods). The mixed probe contained a variety of TE, specifically the internal fragments of the copia, gypsy, mdg-1, blood, Doc, I and F transposons, which previous studies showed are enriched in certain regions of the heterochromatin (e.g. Pimpinelli et al., 1995). The comparisons of the HP1 immunopattern and FISH patterns revealed very few sites of overlap in the euchromatin (Figure 3(A)). Although only a limited number of transposon-like sequences was analyzed in this experiment, we believe it is significant that HP1 immunosignals were hardly ever detected in those regions that were highly enriched in TEs as indicated by intense FISH signals. This observation indicates that there is not a strict correlation between the HP1 euchromatic binding and the presence of transposon-like middle repetitive DNA.

Finally, we further investigated the nature of the intense staining of HP1 along region 31. This region, specifically region 31B–31E contains several megabases of DNA and is characteristically stained throughout its length with the C1A9 antibody (Figure 3(B)). We obtained two YAC clones derived from region 31 and used these clones as probes for FISH. The rationale for this experiment was based on the prediction that if the HP1 localization depends on some specific repeated sequence, then *in situ* hybridization of large clones derived from any part of region 31 should have given a pattern coextensive with the HP1 pattern. As shown in Figure 3(C) and (D), we observed a different result. Each YAC probe produced a single signal in a specific part of region 31. These results strongly suggest that the YAC clones do not share specific repetitive sequence that otherwise might have accounted for the dispersed staining of HP1 throughout region 31. Although these experiments do not lead to a definitive conclusion about the basis of HP1 recognition in euchromatin, we think that taken together, they suggest that the euchromatic binding of HP1 does not depend upon a enrichment of repetitive DNAs.

HP1 shows conserved patterns of localization among Drosophila *species*

Several studies have previously shown that HP1 is phylogenetically strongly conserved, not only in its

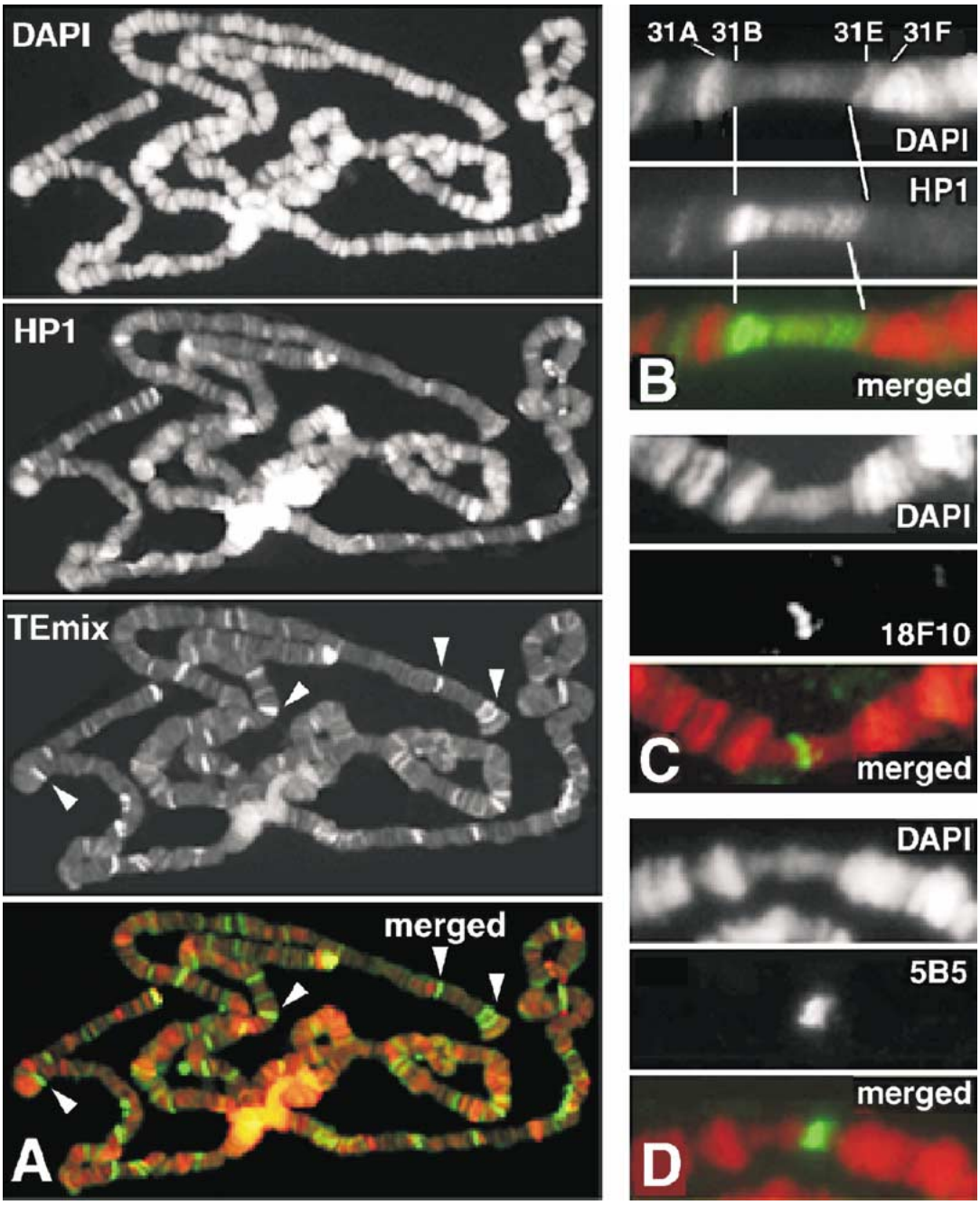

Figure 3 The euchromatic sites of HP1 localization do not correlate with the localization of several known TE or repetitive DNA sequences. (A) A comparison of the sites of HP1 localization and several known TEs in the *Ore-R* strain. The same polytene chromosome squash is shown in the four panels. From top to bottom are: the DAPI staining pattern, the C1A9 antibody immunopattern, the FISH pattern of a mix of probes corresponding to several TEs (see Materials and methods), and the merged image showing both the immuno- and the FISH patterns (the HP1 signals are red, the FISH signals are green, the regions of overlap are yellow). Note that there are numerous regions enriched in TE-like sequences (arrowheads in the TEs pattern, and arrowheads and green signals in the merged patterns) that lack HP1 immunosignal. (B)–(D) Immunopattern and FISH localization of two YAC clones containing DNA from region 31 on the polytene chromosomes of an *Ore-R* wild type larva. (B) Region 31 is shown at high magnification, stained with DAPI (above) and immunostained with the C1A9 antibody (middle). The two patterns were pseudocolored and merged (below) with DAPI in red and HP1 in green. Note as the HP1 stains bands distributed throughout region 31, specifically in 31B–31E. (C) and (D) show the FISH using the 18F10 and 5B5 YAC clones, respectively. These two different YACs are derived from region 31. Note that for each clone, only one a single signal is located in region 31 and that the two signals are at different sites.

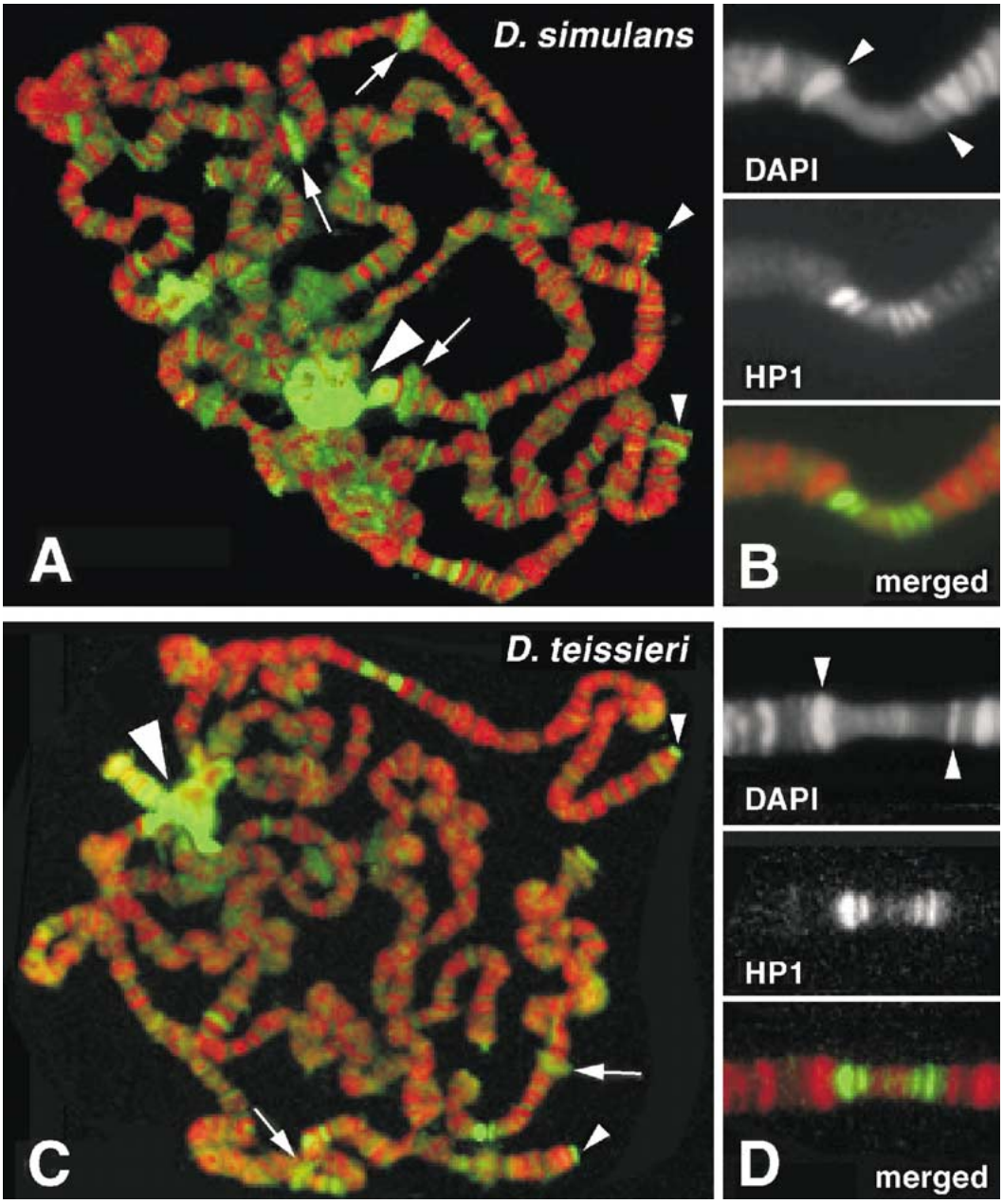

Figure 4 HP1 shows similar chromosomal distributions in other *Drosophila* species. HP1 immunopattern for *D. simulans* (A) and *D. teissieri* (C) reveals shared characteristics such as: intense staining on the heterochromatic chromocenter (large arrowheads), staining at the telomeres (small arrowheads) and staining at multiple euchromatic locations. Interestingly, the euchromatic sites include the physiologically regulated puffs (arrows). Moreover, HP1 staining in *D. simulans* (B) and *D. teissiere* (D) is intense on the region corresponding to region 31 of *D. melanogaster.*

biochemical features but also in its heterochromatic localization (see Singh et al., 1991; Jones et al., 2000, for review). To asses whether the euchromatic sites of HP1 are also present in other *Drosophila* species, we performed immunostainings experiments with the C1A9 antibody on polytene chromosomes of *D. simulans*, *D. sechellia*, *D. mauritiana*, *D. teissieri* and *D. virilis*. As expected, we observed HP1 localization in all of these species. In addition, we found that HP1 is present at all telomeres and at several euchromatic

sites. Intriguingly, we found that HP1 localization on a subset of the euchromatic region, such as region 31, is a conserved feature of the protein (see Figure 4 for examples).

Discussion

We have performed a detailed cytological characterization of the distribution of the HP1 protein in the polytene chromosomes in several different strains of *D. melanogaster* and several different species of *Drosophila*. In particular, we focus here on the localization of HP1 in the euchromatin to better understand the significance of the staining patterns and its underlying basis. We found that of the several hundred euchromatic sites of HP1 localization in *D. melanogaster*, several dozen sites are stably maintained in different populations. Others show variability in their signal intensity or are lacking in certain strains. The presence of HP1 along the euchromatin of other *Drosophila* species suggests that this is a conserved feature of the protein.

It has recently been found that different HP1 isoforms exist in mammals, including humans. These isoforms have evolved different binding affinity for heterochromatin or euchromatin (Wregget et al., 1994; Horsley et al., 1996; Furuta et al., 1997; Minc et al., 1999, 2001; Minc, Courvalin & Buendia, 2000). More recently, two HP1-related proteins, designated HP1b and HP1c, have been identified in *D. melanogaster*. By the analysis of their cytological immuno patterns, it has been suggested that original HP1 is located only in heterochromatin, while HP1b is located both in heterochromatin and euchromatin, and HP1c is located only euchromatin (Smothers & Henikoff, 2001). These conclusions contrast with our previous and present data that unequivocally show a euchromatic distribution of HP1 using C1A9 antibody. The euchromatic immunosignals described in this report are surely due to HP1, and not to other proteins, as has been shown previously (Fanti et al., 1998a,b) by the absence of C1A9 signals in the HP1 null genotypes.

Given that current models of HP1 postulate a role in heterochromatin formation, the fact that HP1 is also present at hundreds of sites in the euchromatin raises the issue of the significance and basis for its euchromatic localization. One simple answer would be to postulate a role of HP1 in the formation of IH. However, our comparative analyses do not support this possibility. We found that sites of HP1 accumulation and intercalary heterochromatic regions do not overlap extensively. We also argue that HP1 localization in euchromatin is likely not due to a recognition of sites enriched in repetitive DNAs. We found a scarce overlap between HP1 and several known families of transposable element-like DNA sequences. Furthermore, FISH analysis using two large genomic clones of region 31 did not indicate the existence of repetitive sequences along this region of abundant HP1 binding. We think that it is more likely that HP1 recognizes different euchromatic regions via different binding modalities, probably due to interactions of different sets of protein factors. It has been shown that HP1 may in fact interact with different proteins to form different chromatin complexes (Pak et al., 1997; Huang et al., 1998; Delattre et al., 2000; Bannister et al., 2001; Lachner et al., 2001; Nielsen et al., 2001; Song et al., 2001; Scholzen et al., 2002). It is reasonable to imagine that some of the different HP1 chromatin complexes could be related to different functional states of gene activities. Although the majority of the data so far available have suggested a main role of HP1 in heterochromatin formation and telomere stability, other recent data have also suggested its possible role in gene repression. It has been recently shown, that HP1 is involved in silencing euchromatic genes that are located along the chromosomal region 31 (Hwang, Eissenberg & Worman, 2001). In addition, it has been recently shown that in mammals, HP1 interacts with the retinoblastoma (Rb) protein to repress euchromatic genes (Nielsen et al., 2001). Although these data have reinforced the suggestion for a repressive role of HP1 in gene expression, we here observed, however, that several euchromatic regions, where HP1 is located, correspond to physiological puffs. In addition, we also observed that HP1 accumulates in heat shock induced puffs (data not shown). Since puffs represent the cytological phenotype of gene activity, this is particularly suggestive of a possible involvement of HP1 also in the activation of gene expression. The association of HP1 to the actively transcribed loci is particularly striking and challenges the canonical view that assigns an exclusive role of HP1 in heterochromatin formation and gene silencing.

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