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The *Drosophila* GAGA transcription factor is associated with specific regions of heterochromatin throughout the cell cycle

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In virtually all eukaryotes the centromeric regions of chromosomes are composed of heterochromatin, a specialized form of chromatin that is rich in repetitive DNA sequences and is transcriptionally relatively silent. The *Drosophila* GAGA transcription factor binds to GA/CT-rich sequences in many *Drosophila* promoters, where it activates transcription, apparently by locally altering chromatin structure and allowing other transcription factors access to the DNA. Here we report the paradoxical finding that GAGA factor is associated with specific regions of heterochromatin at all stages of the cell cycle. A subset of the highly repetitive DNA sequences that make up the bulk of heterochromatin in *D.melanogaster* are GA/CT-rich and we find a striking correlation between the distribution of GAGA factor and this class of repeat. We propose that GAGA factor binds directly to these repeats and may thereby play a role in modifying heterochromatin structure in these regions. Our observations demonstrate for the first time that a transcriptional regulator can associate with specific DNA sequences in a fully condensed mitotic chromosome. This may help explain how the distinctive character of a committed or differentiated cell can be maintained during cell proliferation.

Key words: cell cycle/*Drosophila*/GAGA/heterochromatin/transcription factors

Introduction

The term heterochromatin was first used by Heitz (1928) to describe the regions of chromosomes that remain condensed throughout the cell division cycle. These regions, which are often clustered at the centromeres and telomeres, have a number of properties that distinguish them from euchromatin: they have a low density of genes, a high density of middle and highly repetitive DNA, and they replicate late during the S phase of the cell cycle (reviewed in John, 1988). Although heterochromatin is present in virtually all eukaryotes, its functions remain controversial. Some suggest that it is essential for processes such as recombination and chromosome pairing, while others suggest that it has no essential function and is simply maintained at certain chromosomal locations through a variety of genetic mechanisms (John, 1988).

What causes specific regions of chromatin to become condensed into heterochromatin? The repetitive DNA sequences that make up the bulk of heterochromatin have evolved rapidly and tend not to be conserved between species (John, 1988). Recent results in *Drosophila*, however, suggest that any repeated sequence that is long enough to fold back on itself and pair somatically will tend to form heterochromatin (Dorer and Henikoff, 1994). How this somatic pairing might trigger heterochromatin formation is not known. A number of proteins that specifically associate with centromeric heterochromatin have been isolated. In mammals, for example, the CENP-A and CENP-B centromeric proteins (Earnshaw and Rothfield, 1985) are thought to interact directly with heterochromatic DNA: CENP-A behaves biochemically like a histone and has limited homology to histone H3 (Palmer *et al.*, 1991), while CENP-B is a member of the helix–loop–helix family of DNA binding proteins and appears to bind to a specific DNA sequence in human heterochromatin (Sullivan and Glass, 1991). The function of these proteins is unknown.

In *Drosophila*, position effect variegation (PEV) has been used as an assay to define genes that can influence heterochromatin structure (Grigliatti, 1991). PEV occurs when a euchromatic gene is placed (usually by a chromosomal rearrangement) close to a heterochromatic region; transcription from the euchromatic gene is often repressed in a stochastic, but clonally heritable, fashion, leading to a variegated expression of the gene. The variegation seems to be caused, at least in part, by the spreading of heterochromatin into the neighbouring euchromatin, which then represses transcription from the euchromatic gene. Mutations that modify PEV are thought to affect proteins that influence such spreading and are therefore likely to be involved in regulating chromatin structure (Henikoff, 1992; Reuter and Spierer, 1992; Karpen, 1994). This appears to be the case for the heterochromatin protein 1 (HP-1), which is located in heterochromatin and is encoded by the suppressor of variegation *Su(var)*[2-5] (James and Elgin, 1986; Eissenberg *et al.*, 1990). Sequence analyses of three other genes that modify PEV when mutated suggest that the genes encode DNA binding proteins (Reuter *et al.*, 1990; Garzino *et al.*, 1992; Dorn *et al.*, 1993).

The *Drosophila* GAGA transcription factor was initially identified as a transcriptional activator that binds to GA/CT-rich elements found in the promoter regions of many *Drosophila* genes (Biggin and Tjian, 1988; Soeller *et al.*, 1993). More recently it has become apparent that the factor activates transcription indirectly by opening chromatin structure and allowing other factors access to the DNA (Kerrigan *et al.*, 1991; Lu *et al.*, 1993; Tsukiyama *et al.*, 1994). Here we report the unexpected finding that GAGA

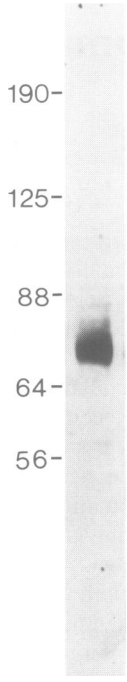

Fig. 1. A Western blot of a 0–3 h *Drosophila* embryo extract using affinity-purified anti-Z13 antibodies. The antibodies recognize a prominent band of ~67 kDa and a few fainter, higher molecular weight bands.

factor is associated with regions of heterochromatin throughout the cell cycle, probably via a direct interaction with a GA/CT-rich subset of the highly repetitive DNA sequences found in *D.melanogaster* heterochromatin. We speculate that GAGA factor may normally play a role in modifying heterochromatin structure, a possibility that is supported by the recent findings of Farkas *et al.* (1994) that mutations in the GAGA factor gene enhance PEV.

Our findings also demonstrate that GAGA factor can associate with specific DNA sequences even when the DNA is highly compacted in the mitotic chromosome. This may help explain how certain proteins can impose a transcriptionally active state on chromatin that can be transmitted to the two daughter cells when a cell divides.

Results

Antibodies raised against the Z13 protein stain the centromeric region of Drosophila chromosomes at all stages of the cell cycle

We isolated the phage Z13 during a screen of a λ expression library with a rabbit antiserum raised against an 85 kDa *Drosophila* centrosomal protein. We subcloned the 1.8 kb cDNA from this phage into the pGEX3 expression vector and isolated the protein encoded by the cDNA as a fusion with glutathione *S*-transferase (GST−Z13). We used the fusion protein to immunize a rabbit and then to affinity-purify anti-Z13 antibodies from the rabbit serum. In Western blotting experiments the affinity-purified anti-Z13 antibodies recognized a major band of ~67 kDa and a series of minor higher molecular weight bands in *Drosophila* embryo extracts (Figure 1), suggesting that the Z13 cDNA did not encode the 85 kDa centrosomal protein. Moreover, in indirect immunofluorescence studies on fixed embryos, the affinity-purified antibodies did not stain centrosomes, but instead stained nuclei in an interesting pattern (Figure 2).

In early embryos, the purified antibodies stained bright dots in the interphase nucleus. Most of the dots were clustered at the apical surface of the nucleus (Figure 2A and B), where the centromeric region of each chromosome is located (Hiraoka *et al.*, 1990). As the nuclei entered mitosis, the dots remained associated with the condensing chromatin (Figure 2C), eventually clustering at the metaphase plate (Figure 2D). At anaphase (Figure 2E) and telophase (Figure 2F) most of the dots were clustered in the centromeric regions, which lead the chromosomes as they move toward the spindle poles. This was most easily seen when the images of the chromatin and the antibody staining were superimposed (Figure 3A). Thus, the Z13 protein seems to be associated with the centromeric regions of chromosomes throughout the cell cycle.

The Z13 cDNA encodes the GAGA transcription factor

We sequenced both strands of the 1.8 kb Z13 cDNA and found that it was >99% identical to the published sequence of a cDNA encoding GAGA factor (Soeller *et al.*, 1993) (not shown). Several lines of evidence suggest that the staining pattern observed with the Z13 antibodies accurately reflects the distribution of GAGA factor. (i) Purified GAGA factor consists of a major polypeptide of ~67 kDa and a series of minor, higher molecular weight proteins, the largest being ~90 kDa (Biggin and Tjian, 1988; Soeller *et al.*, 1993). This is the same pattern that is recognized by the Z13 antibodies in Western blotting experiments with *Drosophila* embryo extracts (see Figure 1). (ii) When we used the ~67 kDa region of these blots (that contained the major GAGA factor polypeptide) to affinity-purify antibodies from the original Z13 antiserum, the purified antibodies gave the same staining pattern as antibodies purified with the Z13 fusion protein (not shown). (iii) A rabbit antiserum raised against purified *Drosophila* GAGA factor (kindly provided by C.Wu) stained early embryos in a pattern that was very similar to that seen with the purified Z13 antibodies (J.W.Raff, unpublished observations; C.Wu, personal communication). A similar pattern was also seen with an antiserum from a second rabbit immunized with the Z13 fusion protein (not shown).

GAGA factor is associated with heterochromatin both in older embryos and in cell lines in culture

To test whether GAGA factor was only localized to centromeric regions of chromosomes during the early syncytial nuclear cycles (where there are no G1 or G2 phases of the cell cycle), we used the affinity-purified Z13 antibodies to stain both older embryos and a *Drosophila* cell line in culture. In older embryos the Z13 antibodies stained interphase nuclei brightly. Some of the staining was diffusely spread throughout the nucleus and some was distributed in a punctate pattern (Figure 3B). At least some of the punctate staining probably reflected centromere labelling, as at anaphase the dots could again be seen to be associated with the centromeric regions of chromosomes (arrow in Figure 3B). The centromere

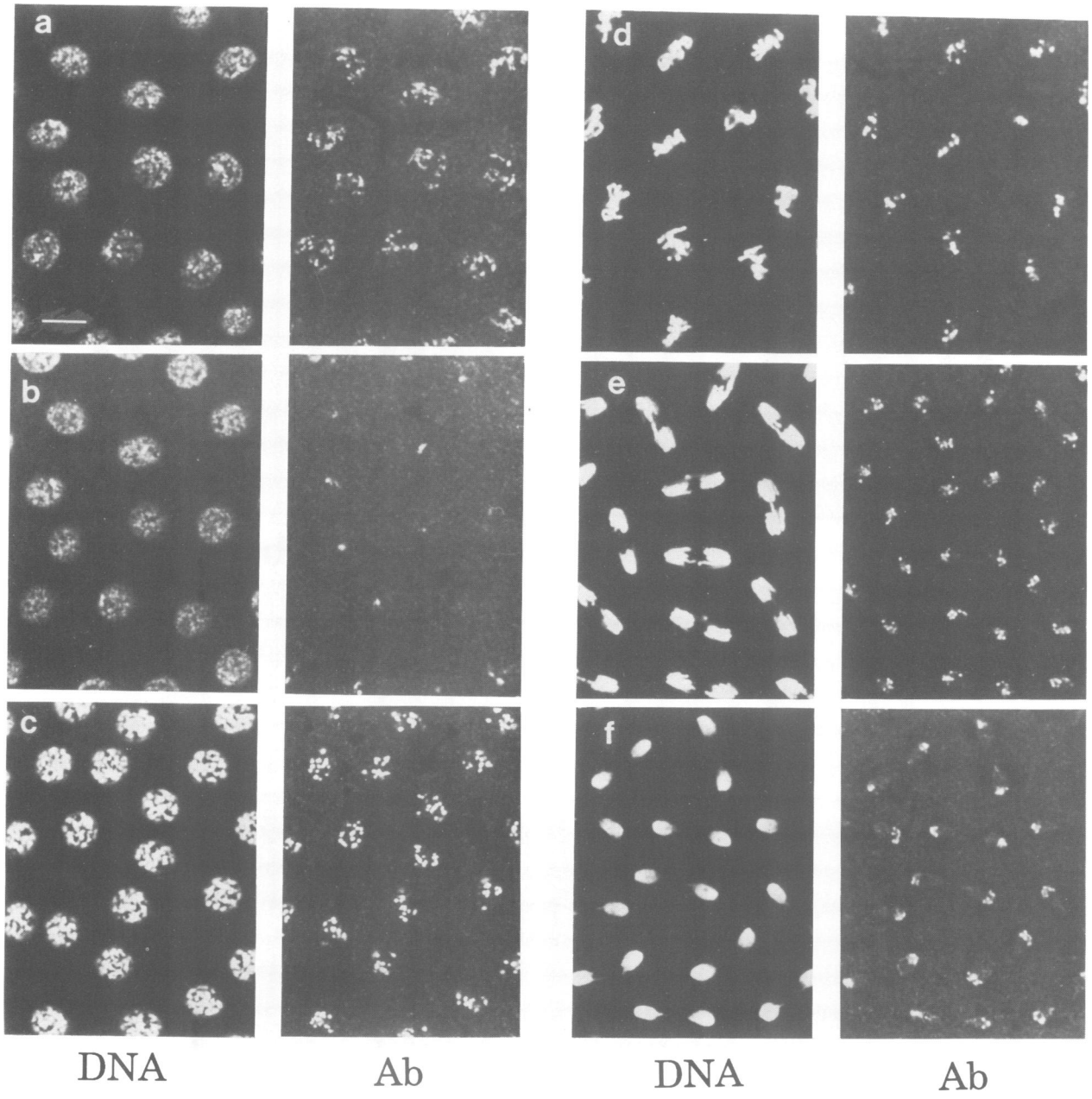

Fig 2. Comparison of the distributions of the Z13 protein and DNA in nuclear cycle 11 *Drosophila* embryos. (**a** and **b**) The same field from an embryo with the nuclei in interphase is shown at two different focal planes; surface view (a) and bottom view (b). The Z13 antibodies (right panels) stain a large number of elongated dots that are largely clustered at the apical surface of each interphase nucleus. (**c**) A field from an embryo with the nuclei in prophase. (**d**) A field from an embryo with the nuclei in metaphase showing the Z13-staining dots clustered at the metaphase plate. (**e**) A field from an embryo with the nuclei in anaphase showing that most of the Z13-staining dots are associated with the centromeric regions of the chromosomes which lead the chromosomes toward the spindle poles. (**f**) A field from an embryo with the nuclei in telophase, showing the majority of the Z13-staining dots clustered at the centromeric regions of the decondensing chromosomes. Scale bar 8 μm

staining in older embryos was not nearly as striking as that observed in the pre-cellularization embryos (Figure 3A). This was probably because the older nuclei are transcriptionally active and the overall levels of GAGA factor are much higher, giving a higher level of staining throughout the nucleus during interphase and throughout the cytoplasm during mitosis. We obtained similar results when the Z13 antibodies were used to stain a *Drosophila* cell line in culture (Figure 4).

We also used the Z13 antibodies to stain the polytene chromosomes of salivary gland nuclei, where the centromeric regions are greatly under-represented (Rudkin, 1969; Gall *et al.*, 1971) and all of the centromeres are clustered in a relatively small central domain, the chromocentre (Lefevre, 1976). As reported previously with other anti-GAGA factor antibodies (Tsukiyama *et al.*, 1994), the purified Z13 antibodies stained a large number of bands spread throughout the euchromatic arms of the polytene chromosomes (not shown), presumably reflecting GAGA factor binding to GA/CT-rich elements in the euchromatin. GAGA factor was largely excluded from the chromocentre, although on close inspection it could be seen to be present in a small number of discreet bands within the chromocentre (not shown). As the precise organization of

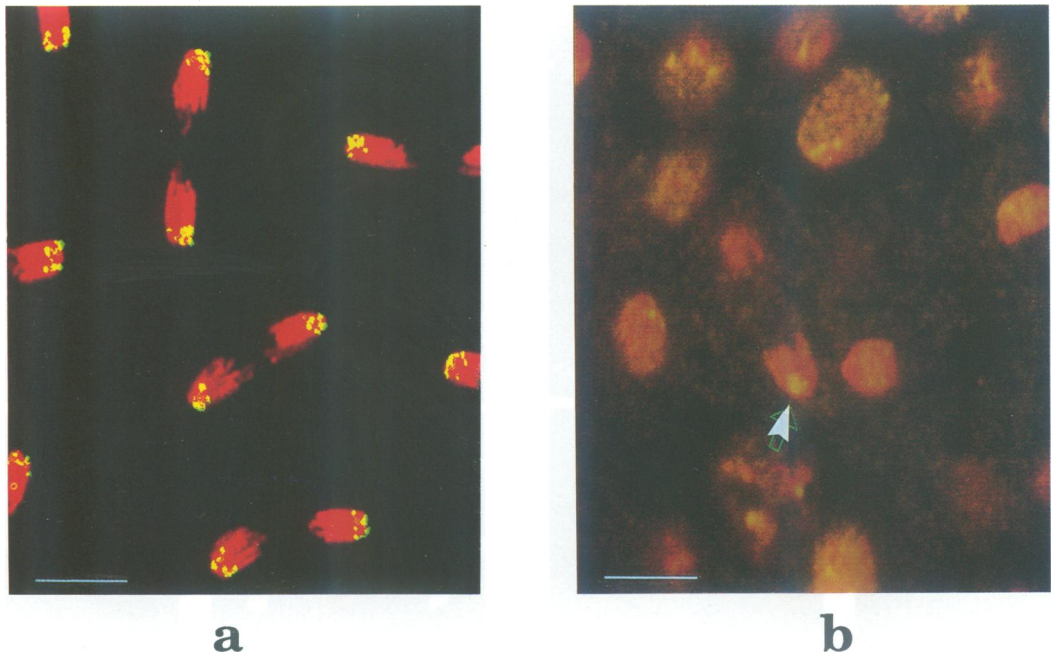

Fig. 3. The Z13 protein (GAGA factor) is associated with the centromeric regions of chromosomes. Confocal micrographs showing the distribution of GAGA factor (green) superimposed on chromatin (red) (regions of overlap appear yellow) in a field from an embryo with the nuclei in anaphase of nuclear cycle 11 (**a**) or in a field from a 3–4 h embryo in which the nuclei have cellularized, lost mitotic synchrony and adopted a more typical cell cycle pattern (**b**). In both cases the GAGA factor can be seen to be associated with the centromeric regions of anaphase chromosomes (arrow in b). The association is much less striking in the older embryo (b), presumably because there is a much higher level of GAGA factor in cells that are transcriptionally active and much of the extra GAGA factor is not associated with the centromeric regions. [The increased abundance of GAGA factor in the older embryo (b) is not accurately reflected in this micrograph, because the level of GAGA factor staining (green) has been electronically reduced using the confocal microscope computer so that the centromeric staining of GAGA factor in the anaphase cell is emphasized.] Scale bar 5 μm.

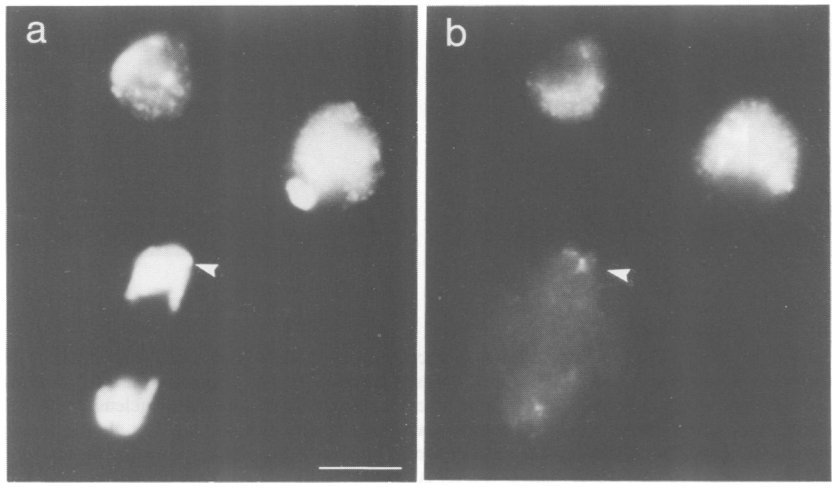

Fig. 4. The Z13 protein (GAGA factor) is detected in the centromeric regions of chromosomes in cells in culture. *Drosophila* Schneider cells were grown in culture, fixed and stained with DAPI to reveal DNA (**a**) and with the Z13 antibodies (**b**). At anaphase, GAGA factor could be seen to be concentrated in the centromeric regions of the condensed chromosomes (arrowheads). In these rounded mitotic cells only a few of the centromeric dots could be brought into one focal plane at the same time. Scale bar 5 μm.

heterochromatic DNA in these salivary gland nuclei is unknown (Lefevre, 1976), the significance of this result is uncertain. We believe that GAGA factor is associated with some of the highly repetitive DNA sequences found in the most proximally located heterochromatin (see below). These sequences are thought to be the most under-represented in the salivary gland nuclei, perhaps explaining the low abundance of GAGA factors at the chromocentre. Alternatively, the heterochromatin in these specialized nuclei may be packaged in a form that excludes GAGA factor.

GAGA factor co-localizes with the GA/CT-rich repeats found in heterochromatin

As GAGA factor has been shown to bind directly to GA/CT-rich DNA sequences (Biggin and Tjian, 1988; Soeller *et al.*, 1993), it seems likely that its association with centromeric regions of chromosomes reflects a direct

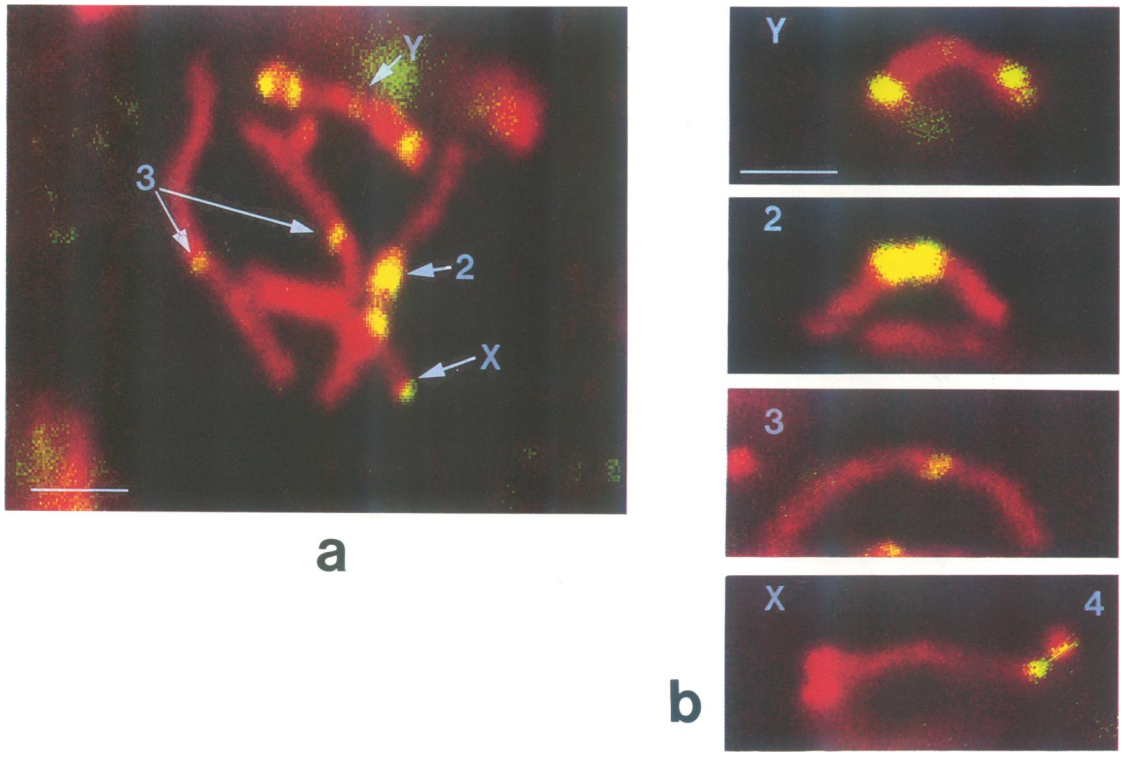

Fig. 5. GAGA factor is located in specific regions of heterochromatin on each mitotic chromosome. (**a**) A field from an embryo treated with the microtubule depolymerizing drug colchicine. The individual metaphase chromosomes have spread out so that each chromosome can be identified. The two third chromosomes (the largest chromosomes), the monocentric X chromosome, the Y chromosome and a single second chromosome are visible in this focal plane, as indicated. (**b**) A composite figure taken from different fields shows the GAGA factor distribution on each chromosome. The bottom panel shows the X chromosome on the left and the dot-like fourth chromosome on the right. The GAGA factor has a characteristic distribution on each chromosome. At this level of resolution, the distribution is the same as that reported for the AAGAG and AAGAGAG repeats on mitotic chromosomes (Lohe *et al.*, 1993). Scale bar 2 μm.

interaction with DNA. In *D.melanogaster* two of the most common arrays of tandem repeats found in heterochromatin are AAGAG and AAGAGAG, which have been estimated to comprise 5.6 and 1.5% of the total genomic DNA respectively (Brutlag, 1980; Lohe and Roberts, 1988). From the known binding properties of GAGA factor to various promoter elements (Biggin and Tjian, 1988; Soeller *et al.*, 1993), it seems likely that the factor could bind directly to both of these repeats.

These GA/CT-rich repeats are distributed in heterochromatin in a characteristic pattern that is unique to this class of repeat: they are present in the centromeric regions of all the chromosomes, although in widely varying amounts, and toward the tips of the Y chromosome, which is largely heterochromatic (Lohe *et al.*, 1993). To test whether the distribution of GAGA factor on individual chromosomes coincides with the distribution of these repeats, we treated embryos with colchicine to induce a metaphase arrest and then stained them with anti-Z13 antibodies. GAGA factor has a reproducible and highly characteristic distribution on each metaphase chromosome (Figure 5A and B): a small amount is located at the tip of the X chromosome, a small amount on one side of the primary constriction of the third chromosome, a large amount spread throughout the centromeric region of the second chromosome, a small amount close to the centromere and larger amounts toward the tips of the Y chromosome, and a small amount close to the centre of the fourth chromosome. At this level of resolution, the chromosomal distribution of GAGA factor is the same as the reported distribution of the AAGAG and AAGAGAG repeats on mitotic chromosomes, strongly suggesting that GAGA factor is associated with heterochromatin through a direct interaction with these repeats.

To further test this hypothesis, we used the Z13 antibodies to stain embryos from the distantly related species *D.virilis*. Chromosomes from this species are rich in heterochromatin, but apparently lack GA/CT-rich repeat sequences (Gall and Atherton, 1974). As expected, the antibodies stained interphase nuclei, but did not stain the centromeric regions of mitotic chromosomes in these embryos (not shown).

Discussion

We find that the *D.melanogaster* GAGA transcription factor is associated with specific regions of heterochromatin. These regions coincide with the previously reported distribution of a class of highly repetitive DNA sequences that are GA/CT-rich (Lohe *et al.*, 1993). These repeat sequences have a higher GA/CT content than many of the known promoter elements that have been shown to bind GAGA factor, making it highly likely that GAGA factor is associated with heterochromatin through a direct interaction with these repeats. To our knowledge, GAGA factor is the first protein known to affect chromatin structure that is associated with any of the highly repetitive DNA sequences found in heterochromatin.

As GAGA factor is an activator of transcription (Biggin and Tjian, 1988; Lu *et al.*, 1993; Soeller *et al.*, 1993; Tsukiyama *et al.*, 1994) and heterochromatin is largely transcriptionally inactive, the presence of GAGA factor in heterochromatin might seem surprising. There is increasing evidence, however, that GAGA factor activates transcription indirectly, by locally altering chromatin structure. GAGA factor binding creates DNase I hypersensitive sites in DNA both *in vivo* and *in vitro* and this opening of the DNA appears to allow other transcriptional activators access to the DNA (Lu *et al.*, 1993; Tsukiyama *et al.*, 1994). Moreover, GAGA factor binding to GA/CT-rich promoter regions *in vitro* appears to disrupt the nucleosomal arrangement in these regions, while promoting an orderly positioning of nucleosomes on adjacent DNA (Tsukiyama *et al.*, 1994); this property could explain how GAGA factor activates transcription, as the precise positioning of nucleosomes within promoter regions can affect transcriptional activity (Grunstein, 1990; Felsenfeld, 1992). Thus GAGA factor seems to act more as a chromatin modifying factor than a direct transcriptional activator.

What might be the function of GAGA factor in heterochromatin? It is possible that it serves no function there and simply binds to the GA/CT-rich sequences in heterochromatin without physiological consequence. We think this unlikely for two reasons. First, as just described, the binding of GAGA factor to DNA has important effects on chromatin structure. Second, mutations in the GAGA factor gene have recently been shown to act as enhancers of PEV (Farkas *et al.*, 1994). Therefore, reducing the level of active GAGA factor seems to enhance heterochromatin formation (as assayed by its effects on PEV), suggesting that GAGA factor can inhibit the spreading of heterochromatin into adjacent euchromatic regions. Thus, the presence of GAGA factor in heterochromatin is unlikely to have a completely neutral effect.

One possibility is that GAGA factor may act to open heterochromatic DNA and thereby allow the expression of some of the essential genes that are located within *D.melanogaster* heterochromatin (Hilliker *et al.*, 1980; Gatti and Pimpinelli, 1992); the regions of heterochromatin that bind GAGA factor could act as boundaries that prevent the spreading of heterochromatin into transcriptionally active regions. Alternatively, GAGA factor may play a more general role in locally modifying heterochromatin structure, perhaps promoting an altered nucleosomal arrangement or allowing some other heterochromatin-specific factors access to the DNA. As neither the GA/CT-rich repeats nor GAGA factor are abundant in the heterochromatin of *D.virilis*, it seems clear that GAGA factor itself cannot be essential for the formation or function of all heterochromatin. However, GAGA factor belongs to a large family of proteins that are related over their N-terminal 120 amino acids (Dorn *et al.*, 1993; D.Read and T.Kornberg, personal communication). At least two members of this family are transcription factors (Harrison and Travers, 1990; DiBello *et al.*, 1991) and another is encoded by a gene that enhances PEV when mutated (Dorn *et al.*, 1993). It is possible that other members of this family might perform functions similar to GAGA factor in the heterochromatin of other *Drosophila* species that lack GA/CT rich repeats and in those regions of *D.melanogaster* heterochromatin where GAGA factor is not bound.

A detailed analysis of GAGA factor mutants should help establish what role, if any, GAGA factor has in heterochromatin.

Whatever the role of GAGA factor in heterochromatin, our findings have important implications for understanding a fundamental property of committed or differentiated cells—their ability to maintain their committed or differentiated state through repeated rounds of cell division. In *Drosophila*, for example, homeotic selector genes in the *bithorax* and *antennapedia* complexes specify the anterioposterior character of the segments of the fly (Lawrence, 1992). These genes must be continuously activated or repressed in cells throughout most stages of their development for the cells to retain a memory of their segmental position and behave accordingly (Bienz, 1992; Lawrence, 1992). Two classes of protein play an important role in maintaining the activated or repressed state of these genes, at least during the later stages of development: the *polycomb* group (PcG), which suppresses their transcription, and the *trithorax* group (TrxG), which activates their transcription. It has been proposed that these proteins bind to a region of chromatin and determine whether it is transcriptionally active or inactive; this transcriptional state is then passed on to the two daughter cells when a cell divides (Paro, 1990, 1993; Bienz, 1992). The GAGA factor mutations were originally studied because of their *trithorax*-like phenotype (Farkas *et al.*, 1994), suggesting that GAGA factor, like the other TrxG proteins, is a component of a system that can impose a transcriptionally active state on chromatin in a clonally heritable fashion.

Perhaps the simplest way of ensuring that the transcriptional state of a region of chromatin is inherited by each daughter cell after cell division is for the relevant chromosomal proteins to remain associated with the DNA throughout the cell division cycle. It has not been possible, however, to test whether such a simple mechanism operates for the TrxG or PcG proteins (or for any other transcriptional regulator). The association of GAGA factor with large contiguous tracts of heterochromatic DNA has allowed us to directly observe its interaction with specific DNA sequences throughout the cell cycle in *Drosophila* embryos. As the heterochromatic DNA is decondensed during interphase in the early nuclear cycles of the *D.melanogaster* embryo (Sonnenblick, 1950), our results demonstrate that GAGA factor can interact with DNA through all phases of the cell cycle. This provides the first direct evidence that it is mechanistically possible for a transcriptional regulator to associate with specific DNA sequences during mitosis. If GAGA factor can remain associated with condensed DNA, it seems likely that the TrxG and PcG proteins, and perhaps other classes of transcriptional regulators, might also remain associated with DNA during the process of cell division.

Materials and methods

Molecular biology methods

All molecular biology techniques were performed using standard protocols, as described in Sambrook *et al.* (1989), except where stated.

Cloning of the Z13 cDNA

A λ Zap (Stratagene) cDNA expression library made from 0–4 h old embryonic mRNA (kindly provided by Bruce Hay) was screened as

described previously (Kellogg and Alberts, 1992) with a rabbit antiserum raised against an 85 kDa centrosomal protein. The 1.8 kb cDNA was recovered in the pSK vector by phagemid rescue as per the manufacturer's instructions (Stratagene). As described in the Results, this cDNA did not encode the 85 kDa protein. We assume that the antiserum used to screen the cDNA library contained antibodies that recognized the GAGA factor protein. On Western blots, weak staining of an ~67 kDa protein could be seen with this serum (J.W.Raff, unpublished observations).

Sequencing of the Z13 cDNA

Both strands of the 1.8 kb cDNA were sequenced using the Sequenase 2.0 kit as per the manufacturer's instructions. Some sequencing was also performed by the central sequencing facility at UCSF. The sequence was virtually identical to the published sequence of the GAGA factor cDNA (Soeller *et al.*, 1993). The only difference in the coding sequence was a frameshift caused by the addition of an extra A nucleotide in a stretch of four A nucleotides at position 1154 in the published sequence. This frameshift was presumably introduced as a cloning artefact, as downstream from the extra A the sequence was identical to the remainder of the GAGA factor cDNA. In addition, we performed *in situ* hybridizations with the Z13 cDNA to polytene salivary gland nuclei. The probe hybridized to a single site at 70EF, the site of the previously isolated GAGA cDNA (Soeller *et al.*, 1993).

The frameshift in the Z13 cDNA introduced a stop codon after a further 17 amino acids. Thus, the Z13 antibodies were raised against the N-terminal 321 amino acids of GAGA factor plus 17 random amino acids. This does not seem to have effected the GAGA factor specificity of the anti-Z13 antibodies (see Results).

Production and purification of anti-Z13 antibodies

The 1.8 kb cDNA was subcloned into the pGEX3 expression vector (Smith and Johnson, 1988). This vector was transformed into DH5α cells, which were then used to produce the GST−Z13 fusion protein. The fusion protein was purified on a glutathione column, as described previously (Raff *et al.*, 1993), and was used to immunize two rabbits. All immunizations and bleeds were performed by the Berkeley Antibody Company (Richmond, CA). Anti-Z13 antibodies were purified from one of these rabbits by first depleting the antiserum of anti-GST antibodies by repeated passage over a GST affinity column until no further antibodies bound. The residual antibodies were then passed over a GST−Z13 affinity column and the bound antibodies were eluted from the column and stored, as described previously (Raff *et al.*, 1993).

Antibody staining of embryos and tissue culture cells

Embryos were fixed with either methanol (Figure 2) or formaldehyde (Figure 3) and stained with purified anti-Z13 antibodies used at 0.5–1 μg/ml as described previously (Kellogg and Alberts, 1992). The chromatin was detected with a mouse monoclonal anti-histone antibody used at a 1/100 dilution. The anti-Z13 antibodies were visualized with fluorescein-coupled goat anti-rabbit IgG antibodies (Cappel, diluted 1/500) and the anti-histone antibodies with rhodamine-coupled goat anti-mouse IgG antibodies (Cappel, diluted 1/500). Embryos were examined on a Bio-Rad M600 confocal microscope. Schneider cells were grown in D-22 insect medium supplemented with 10% fetal calf serum. The cells were fixed in 3.0% formaldehyde, 0.1% glutaraldehyde in phosphate-buffered saline (PBS), followed by several 5 min washes in PBS plus 0.1% Triton-X100. The fixed cells were then stained with the Z13 antibodies at 0.5–1 μg/ml and immunostaining was detected with fluoroscein-coupled goat anti-rabbit IgG antibodies (Cappel, diluted 1/500). DNA was stained with DAPI and the cells were examined on a Nikon microphot microscope and photographed with Kodak TMAX-100 film.

Western blotting

Western blotting was performed as previously described (Raff *et al.*, 1993) using the purified anti-Z13 antibodies at 1–2 μg/ml.

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