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The chromosomal distribution of phosphorylated histone H3 differs between plants and animals at meiosis

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Abstract Plant (*Secale cereale*, *Triticum aestivum*) and animal (*Eyprepocnemis plorans*) meiocytes were analyzed by indirect immunostaining with an antibody recognizing histone H3 phosphorylated at serine 10, to study the relationship between H3 phosphorylation and chromosome condensation at meiosis. To investigate whether the dynamics of histone H3 phosphorylation differs between chromosomes with a different mode of segregation, we included in this study mitotic cells and also meiotic cells of individuals forming bivalents plus three different types of univalents (A chromosomes, B chromosomes and X chromosome). During the first meiotic division, the H3 phosphorylation of the entire chromosomes initiates at the transition from leptotene to zygotene in rye and wheat, whereas in *E. plorans* it does so at diplotene. In all species analyzed H3 phosphorylation terminates toward interkinesis. The immunosignals at first meiotic division are identical in bivalents and univalents of A and B chromosomes, irrespective of their equational or reductional segregation at anaphase I. The grasshopper X chromosome, which always segregates reductionally, also shows the same pattern. Remarkable differences were found at second meiotic division between plant and animal material. In *E. plorans* H3 phosphorylation occurred all along the chromosomes, whereas in plants only the pericentromeric regions showed strong immunosignals from prophase II until telophase II. In addition, no immunolabeling was detectable on single chromatids resulting from equational segregation of plant A or B chromosome univalents during the preceding anaphase I. Simultaneous immunostaining with anti-tubulin and anti-phosphorylated H3 antibodies demonstrated that the kinetochores of all chromosomes interact with microtubules, even in the absence of detectable phosphorylated H3 immunosignals. The different pattern of H3 phosphorylation in plant and animal meiocytes suggests that this evolutionarily conserved post-translational chromatin modification might be involved in different roles in both types of organisms. The possibility that in plants H3 phosphorylation is related to sister chromatid cohesion is discussed.

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Introduction

During mitotic and meiotic cell division, the chromatin becomes remarkably condensed to facilitate chromosome segregation. In order to accomplish the structural and functional requirements for alignment, pairing and recombination of homologous chromosomes, the chromosome condensation process at meiosis most likely differs from that at mitosis.

One of the phenomena that correlates with the initiation and maintenance of mitotic chromosome condensation is the phosphorylation of the core histone H3 at Ser10 (Gurtley et al. 1975; Ajiro et al. 1983, 1996), close to its flexible N-terminal end (Paulson and Taylor 1982). While only a few H3 molecules are phosphorylated at interphase, nearly all become phosphorylated during mitosis (Gurtley et al. 1987). The interrelation of H3 phosphorylation and chromatin condensation is not understood. It has been proposed that phosphorylation of the H3 N-terminal tail might reduce its affinity for DNA, facilitating the movement of nucleosomes and the access of condensation factors to the chromatin (Roth and Allis 1992; Hendzel et al. 1997; Sauvé et al. 1999). With an antiserum against phosphorylated H3, Hendzel et al. (1997) demonstrated H3 phosphorylation of mammalian mitotic chromosomes initiating in pericentromeric heterochromatin in late G2 interphase cells, spreading throughout the condensing chromatin, and completing just prior to the formation of prophase chromosomes. Coincidence of H3 phosphorylation and mitotic chromo-

some condensation has meanwhile been reported for a broad spectrum of eukaryotes, such as protozoa (*Tetrahymena thermophila*; Wei et al. 1998), fungi, worms and insects (*Aspergillus nidulans, Caenorhabditis elegans, Drosophila melanogaster;* Wei and Allis 1998), as well as mono- and dicot plants (*Hordeum vulgare, Secale cereale, Vicia faba*; Houben et al. 1999).

Recently, H3 phosphorylation has also been described for meiotic chromosomes of *Tetrahymena* (Wei et al. 1998, 1999) and mice (Cobb et al. 1999a, b). In *Tetrahymena* mutants with unphosphorylatable histone H3, Wei et al. (1999) observed disturbed chromosome segregation during micronuclear mitosis and meiosis. This was interpreted as a consequence of impaired chromosome condensation.

The present study describes the chromosomal distribution of phosphorylated histone H3 during both meiotic divisions in various types of plant and animal bivalents and univalents: (1) bivalent-forming chromosomes with normal meiotic behavior, such as the chromosomes of the normal set (A chromosomes, As) or pairs of supernumerary B chromosomes (Bs); (2) chromosomes forming natural univalents such as the X chromosome or non-associated Bs at metaphase I; (3) univalents in haploids or monosomic addition lines, and (4) artificially induced autosomal univalents. The univalents may show either unipolar (syntelic) or bipolar (amphitelic) orientation at metaphase I and, consequently, reductional or equational segregation at anaphase I. Plant and animal materials are compared and the relationship between histone H3 phosphorylation, meiotic chromosome condensation and sister chromatid cohesion is discussed.

Materials and methods

The following materials were used: (1) Rye (*S. cereale*) 2*n*=14+Bs, from the Korean population Paldang, which contains B chromosomes in 20% of the individuals. (2) Haploid plants of wheat (*Triticum aestivum*, 2*n*=6*x*=42, var. Chinese Spring). (3) A monotelosomic addition line consisting of the 42 chromosomes of wheat plus the long arm of chromosome 5 of rye (5RL). (4) The grasshopper *Eyprepocnemis plorans* (2*n*=22+XX-X0).

All these materials share the feature of forming univalents: two types of natural univalents, such as the X chromosome in the male grasshopper and the rye B chromosomes; univalents produced by lack of homologous pairing, such as those in the haploids or in the monosomic addition line; and artificially induced autosomal univalents as is explained below.

Living spermatocyte cultures and univalent induction in *E. plorans*

Living spermatocyte cultures were made following the technique described by Nicklas et al. (1982) from testicular follicles obtained by biopsy from young adult males of the grasshopper *E. plorans*. In the testicular follicles of grasshoppers, cells are arranged in cysts of rather synchronous cells, in various stages of meiosis and spermatogenesis. Cysts from premeiotic mitosis to mature sperm can be found. In this species, the X chromosome appears as a univalent in male meiosis and shows regular behavior, migrating undivided to one pole at anaphase I and separating chromatids at anaphase II.

Autosomal univalents were induced by heat treatment of young adult males at 44°C for 4 days, followed by recovery in normal laboratory conditions (Rebollo and Arana 1995). The meiotic behavior of autosomal univalents is highly irregular (Rebollo and Arana 1995, 1997; Rebollo et al. 1998), showing a great variety of segregation abnormalities.

Plant chromosome preparation

Anthers were fixed for 30 min in freshly prepared 4% (w/v) paraformaldehyde solution containing phosphate-buffered saline (PBS, pH 7.3), washed for 45 min in PBS and digested at 37°C for 25 min in a mixture of 2.5% pectinase, 2.5% cellulase Onozuka R-10 and 2.5% pectolyase Y-23 (w/v) dissolved in PBS. Anthers were then washed for 15 min in PBS and squashed between a glass slide and coverslip in PBS. After freezing in liquid nitrogen, the coverslips were removed and the slides were transferred immediately into PBS.

For routine controls and quantitative estimations of configuration frequencies, anther squashes were made in 1% acetocarmine.

Mitotic chromosomes of rye were prepared from root tips according to Houben et al. (1999).

Plant immunostaining techniques

To avoid nonspecific antibody binding, slides were incubated for 30 min in 8% BSA (w/v), 0.1% Triton X100 in PBS at room temperature prior to two washes in PBS for 5 min each, and incubated with the primary antibody in a humidified chamber. The primary antibodies of a polyclonal rabbit antiserum that specifically recognized histone H3 phosphorylated at Ser 10 (Upstate Biotechnology, USA) were diluted 1:500 in PBS, 3% BSA. After 12 h incubation at 4°C and washing for 15 min in PBS, the slides were incubated in rhodamine-conjugated anti-rabbit IgG (Dianova) diluted 1:200 in PBS, 3% BSA for 3 h at room temperature. After final washes in PBS, the preparations were mounted in antifade containing 4′,6-diamidino-2-phenylindole (DAPI) as counterstain.

For simultaneous immunostaining with anti-α-tubulin, a microtubule stabilizing buffer (50 mM PIPES, 5 mM $MgSO_4$, 5 mM EGTA, pH 6.9) was used instead of PBS. The anti-α-tubulin antibody (N356, Amersham) diluted 1:50 was detected after incubation with fluorescein isothiocyanate (FITC)-conjugated anti-mouse antibody (Dianova) diluted 1:100. Fluorescent signals were observed using a microscope equipped with epifluorescence optics. The images, recorded with a cooled CCD camera, were pseudo-colored and merged with the program Adobe Photoshop 4.0.

Animal immunostaining techniques

Immunostaining was carried out on cultured spermatocytes previously tracked in vivo. Cells were labeled following the technique employed by Nicklas et al. (1995) for kinetochore staining with 3F3/2 antibody (Gorbsky and Ricketts 1993), without microcystin LR in the lysis/fixative buffer. A monoclonal anti-α-tubulin antibody (Boehringer) and the anti-phosphorylated H3 serum were used as primary antibodies at 1:50 and 1:100 dilutions in PBS, 1% BSA, respectively. FITC-conjugated anti-mouse (Boehringer) for tubulin, and rhodamine-conjugated anti-rabbit (Dianova) for phosphorylated H3 were used as secondary antibodies at 1:25 and 1:50 dilutions, respectively. Preparations were counterstained with DAPI before mounting in Vectashield. Cells were examined in an Olympus BX-60 epifluorescence microscope, photographed on 400 ISO color negative film, and the negatives scanned at 1340 dpi. The images were optimized for best contrast and brightness with Adobe Photoshop 4.0.

Table 1 Chromosomal distribution of phosphorylated histone H3 at mitosis and meiosis. (P, pericentromeric distribution of the immunolabeling; +, immunolabeling visible along the entire chromosome; –, immunolabeling not detectable; R, reductional segregation; E, equational segregation; L, leptotene; Z, zygotene; P, pachytene; D-Dk, diplotene-diakinesis; MI, metaphase I; AI, anaphase I; I, interkinesis; PII, prophase II; MII, metaphase II; AII, anaphase II; TII/In, telophase II/interphase transition)

Material	Type of chromosome	Mitosis	First meiotic division								Second meiotic division			
					L	Z	P	D–DK	MI	AI	I	PII	MII	AII	TII/In
Rye	As	P	Bivalents	R	–	+	+	+	+	+	–	P	P	P	–
	Bs	P	Bivalents	R	–	+	+	+	+	+	–	P	P	P	–
Univalents	R	–	+	+	+	+	+	–	P	P	P	–			
				E	–	+	+	+	+	+	–	–	–	–	–
Haploid wheat	As	P	Bivalents	R	–	+	+	+	+	+	–	P	P	P	–
			Univalents	R	–	+	+	+	+	+	–	P	P	P	–
				E	–	+	+	+	+	+	–	–	–	–	–
Wheat-5RL addition line	Mono 5RL	P	Univalent	R	–	+	+	+	+	+	–	P	P	P	–
				E	–	+	+	+	+	+	–	–	–	–	–
Eyprepocnemis plorans	As	+	Bivalents	R	–	–	–	+	+	+	–	+	+	+	–
			Univalents	R	–	–	–	+	+	+	–	+	+	+	–
				E	–	–	–	+	+	+	–	+	+	+	–
	X	+	Univalent	R	–	–	–	+	+	+	–	+	+	+	–

Results

A summary of the immunolabeling patterns obtained with antibodies against phosphorylated H3 during the different stages of meiosis in plant and animal meiocytes is presented in Table 1.

In plants the phosphorylation pattern of chromosomal histone H3 alters during meiosis

The immunolabeling patterns obtained with antibodies against phosphorylated H3 during the different stages of meiosis were similar for the chromosomes of the monocot species *S. cereale* and *T. aestivum*. Immunostaining of interphase cells was hardly observed. The first diffuse immunosignals were detectable during the transition from leptotene to zygotene (Fig. 1A, B). Strong immunosignals appeared to be dispersed randomly throughout the nuclei from zygotene to diplotene (Fig. 1C, D). With the further compaction of chromosomes during diakinesis, immunosignals were scattered over the chromosomes (Fig. 1E). At metaphase I, congressed rye and wheat bivalents (Figs. 1F, 2A), rye Bs (not shown) and wheat univalents (Fig. 2A, B) were entirely and strongly immunolabeled. During diakinesis and metaphase I, the histone H3 of the centromeric regions appeared slightly more strongly phosphorylated than the rest of the chromosomes (Fig. 1E, F, arrowed). At metaphase I, all bivalents were syntelically oriented, and the chromosomes migrated reductionally to the poles at anaphase I (Fig. 1G), still homogeneously labeled.

At telophase I (Fig. 1H), the phosphorylation of histone H3 gradually disappeared. At interkinesis, the short stage between the first and second meiotic divisions, in which no DNA replication occurs, the chromatin displayed only faint immunostaining (Fig. 1I). At the light microscopy level, it seems that the degree of chromatin condensation at interkinesis is higher than at mitotic interphase (Fig. 1O).

The H3 phosphorylation pattern at second meiotic division was similar to that observed in mitotic cells (Houben et al. 1999), where mainly the pericentromeric regions are immunolabeled at metaphase and anaphase (Fig. 1P, Q). At early prophase II, the recondensation of the chromosomes coincided with renewed phosphorylation of histone H3. The phosphorylation initiated in discrete areas and pronounced immunofluorescent spots became visible, while other areas were less intense and more diffusely labeled (Figs. 1J, 3A, upper cell). At late prophase II and metaphase II, the centromeric regions were phosphorylated, whereas the chromatid arms were very weakly labeled (Figs. 1K; 3A, lower cell, B, C).

The status of phosphorylation remained unchanged after the separation of the sister chromatids at anaphase II (Fig. 1L). At telophase II (Fig. 1M), the phosphorylation of histone H3 disappeared gradually with the decondensation of the chromosomes (Fig. 1N).

Histone H3 phosphorylation patterns differ between formerly reductionally and equationally divided plant univalents during the second meiotic division

At metaphase I, most chromosomes in the haploid wheat (Fig. 2A, B), and 66% of rye B chromosomes, form univalents. A high proportion of wheat univalents, 50% of B univalents and 75.2% of rye univalent 5RL of the wheat-rye addition line were syntelically oriented and migrated reductionally to the poles at anaphase I, still homogeneously labeled. The amphitelically oriented univalents divided equationally at anaphase I, their sister chromatids separating to different poles (Fig. 2C, one chromatid arrowed). Phosphorylation of H3 at first mei-

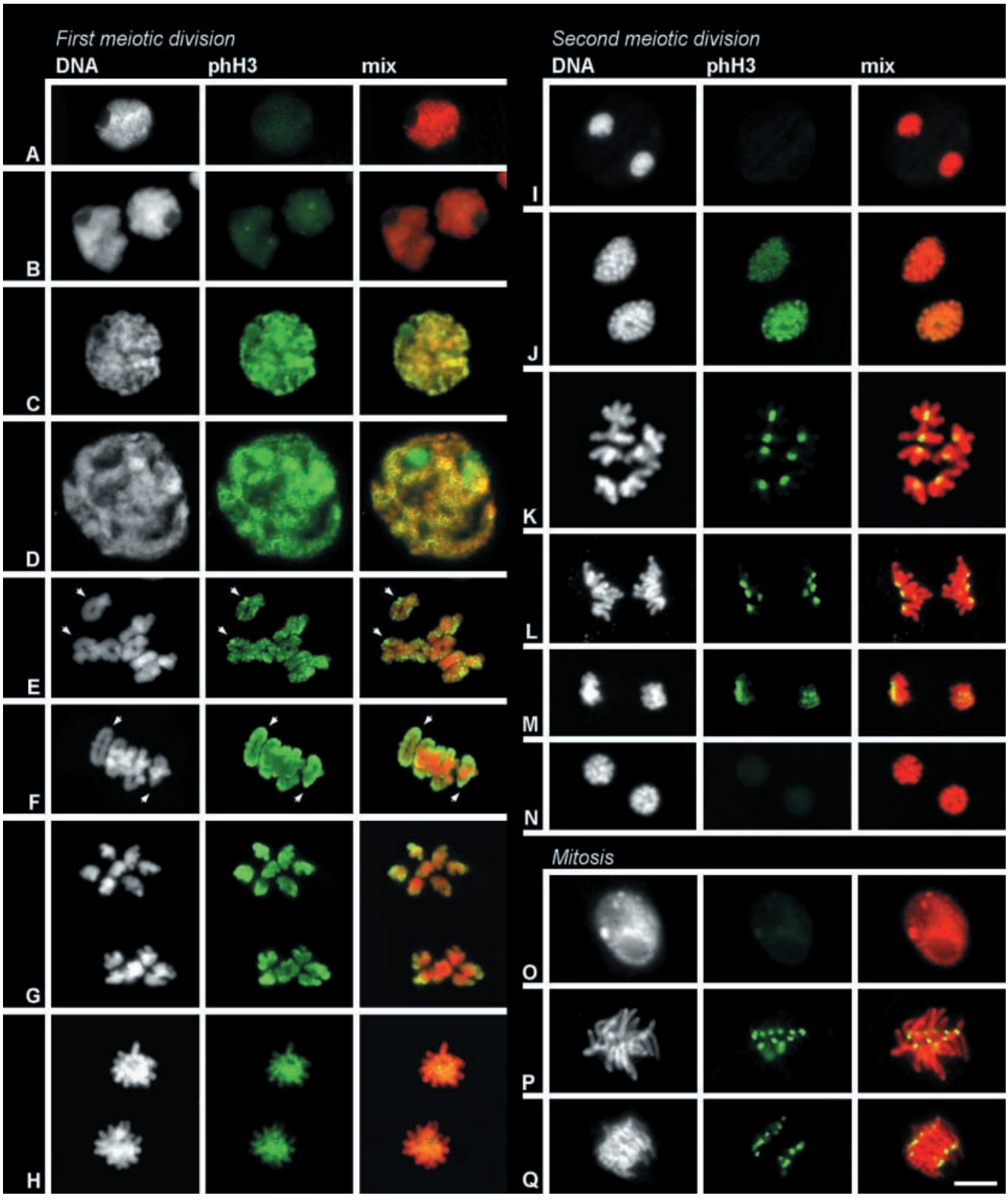
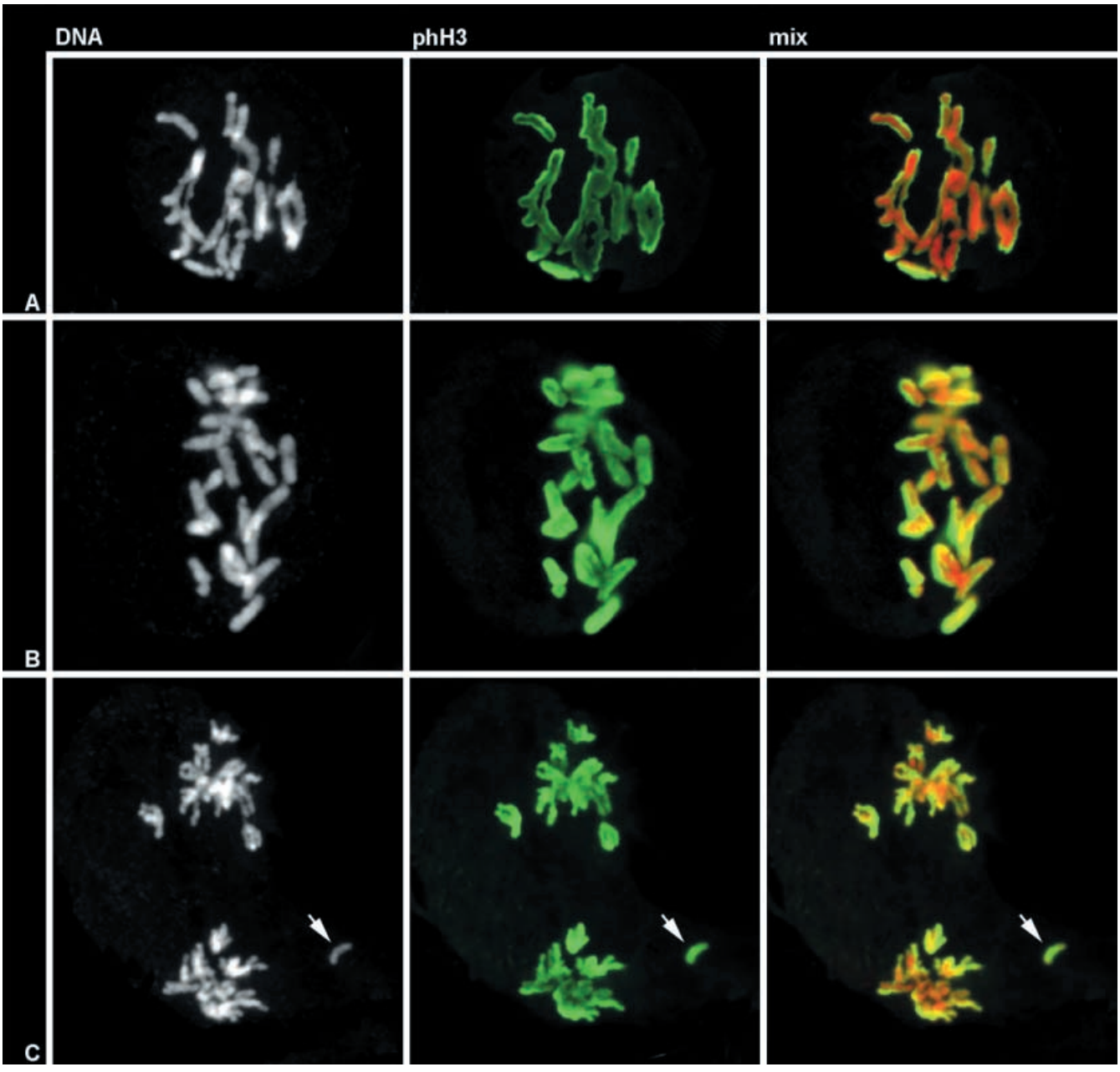


Fig. 1 Immunolocalization of phosphorylated histone H3 during meiosis (**A–N**) and mitosis (**O–Q**) of *Secale cereale* without B chromosomes. The different meiotic and mitotic stages were distinguished according to the morphology of the chromosomes. First meiotic division: **A** preleptotene-leptotene, **B** leptotene, **C** zygotene-pachytene, **D** diplotene, **E** diakinesis, **F** metaphase I, **G** anaphase I, **H** telophase I, **I** interkinesis. Second meiotic division: **J** prophase II, **K** metaphase II, **L** anaphase II, **M** telophase II, **N** interphase (tetrads). Mitosis: **O** interphase, **P** metaphase, **Q** anaphase. *DNA column* DAPI staining; *phH3 column* phosphorylated H3 labeling; in the *mix column*, the images of the DNA stained (*red*) and immunolabeled (*green*) cells are merged. *Bar* represents 5 µm (**Q**)



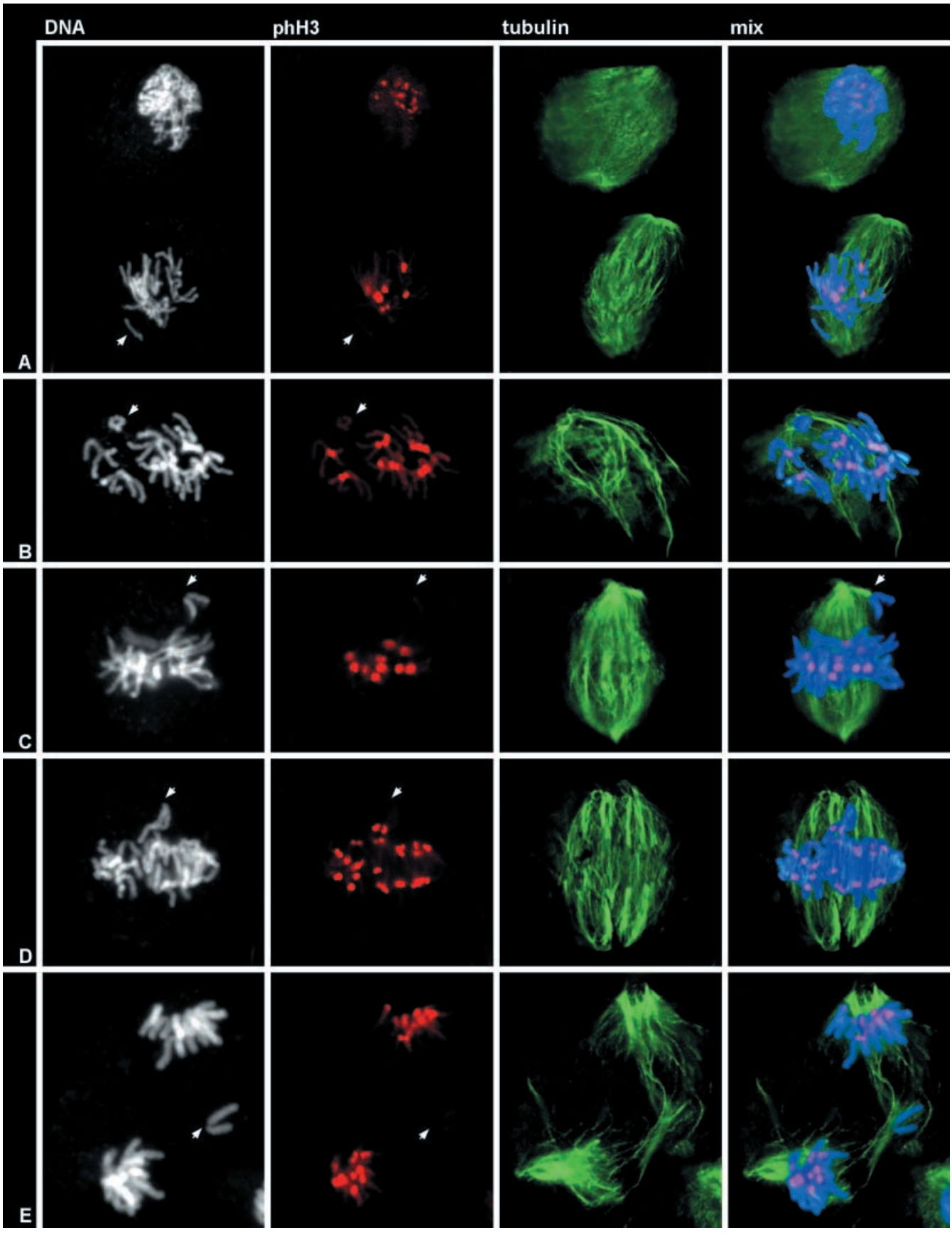
otic division was identical for equationally and reductionally dividing chromosomes.

To ascertain the fate of the equationally divided univalents, the frequency of cells with equationally dividing rye B univalents at anaphase I, the frequency of cells with uncoupled B chromatids at metaphase II and the frequency of cells with laggards at anaphase II were scored in 100 cells of ten rye anthers stained with acetocarmine. The frequencies coincided (33%), indicating that single chromatids at metaphase II and chromatid laggards at anaphase II are products of equational division of univalents at first meiotic division.

The frequency of cells with uncoupled chromatids at metaphase II was 25% for rye A chromosomes in the addition line, and more than 95% for haploid wheat. As in the case of rye Bs, single chromatids are easily detected

Fig. 2 Immunolocalization of phosphorylated histone H3 on meiocytes of haploid *Triticum aestivum* at metaphase I (**A**, **B**), and anaphase I (**C**). In **A** bivalents as well as univalents are shown, whereas in **B** there are only univalents. Note the prematurely separated sister chromatid *arrowed* in **C**. *DNA column* DAPI staining; *phH3 column* phosphorylated H3 labeling; *mix column* merged DNA staining (*red*) and immunolabeling (*green*) images

Fig. 3A–E Simultaneous immunostaining of phosphorylated histone H3 and α-tubulin on meiocytes of haploid *Triticum aestivum* during the second meiotic division. **A** Prophase II, **B, C** metaphase II, **D** early anaphase II, **E** late anaphase II. Single chromatids are *arrowed*. **C** The interaction between tubulin fibers and the kinetochore of a single chromatid is illustrated. *DNA column* DAPI staining; *phH3 column* phosphorylated H3 labeling; *tubulin column* α-tubulin labeling; *mix column* merged DNA (*blue*), phH3 (*red*) and tubulin (*green*) images ▶


DNA
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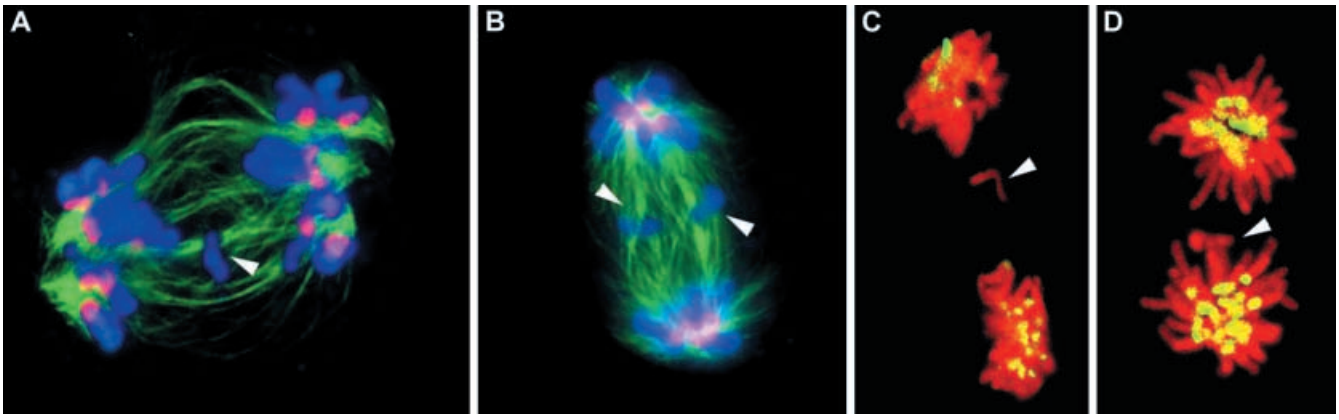

Fig. 4A–D Histone H3 of condensed prematurely separated A or B chromatids is not phosphorylated at the centromeric region. **A, B** Anaphase II cells of rye showing lagging B chromatids (*arrowed*); DNA *blue*, phosphorylated H3 labeling *red*, α-tubulin labeling *green*. **C, D** Anaphase II chromosomes (*red*) of a wheat 5RL monosomic addition line after immunostaining of phosphorylated H3 (*yellow*). The lagging rye chromatids are *arrowed*

by their faulty congression at the metaphase II plate (Fig. 3C) or by their impaired segregation at anaphase II, where they often appear as laggards (Figs. 3E; 4). They were condensed in a similar manner to the sister chromatids of reductionally divided chromosomes (Fig. 3, 4). However, the immunostaining revealed that, within the same metaphase II cell, only chromosomes consisting of two sister chromatids had strong signals at their pericentromeric regions, whereas single chromatids showed no immunosignal (Fig. 3A, B, C, arrowed). At anaphase II, these single A and B chromatids also revealed unphosphorylated H3 (Figs. 3D, E; 4, arrowed).

To analyze whether the different H3 phosphorylation status of the pericentromeric chromatin affects the ability of the kinetochore to attach to spindle microtubules, triple-staining with anti-phosphorylated H3, anti-tubulin antibodies and DAPI was performed (Figs. 3; 4A, B). Anti-tubulin staining also confirmed the affiliation of single chromatids lacking phosphorylated H3 to the cells analyzed. The triple-staining experiment demonstrated that the kinetochores interact with microtubules, irrespective of their level of H3 phosphorylation. Figures 3C, E and 4A, B show single A and B chromatids without phosphorylated H3 at the pericentromeric chromatin, in interaction with apparently normal chromosomal fibers. The centromeric regions of such single chromatids appear to have a normal degree of condensation.

Histone H3 phosphorylation patterns in *E. plorans* at meiosis and mitosis

In *E. plorans*, the meiotic H3 phosphorylation pattern obtained (Fig. 5) coincided with that previously reported by Cobb et al. (1999a, b) for *Mus musculus*. The onset of phosphorylation was detected at diplotene (Fig. 5C, C′, D, D′, a) and the immunosignals were distributed all over the condensed chromosomes. At metaphase I, both the autosomal bivalents and the X univalent were strongly immunolabeled (Fig. 5D, D′, b, X arrowed).

At anaphase I the condensed chromosomes showed strong immunolabeling, which gradually disappeared during telophase I, and was not observed at interkinesis (Fig. 5D, D′, c, d, e). The immunosignals reappeared at prophase II, and were distributed over the entire length of the condensed chromosomes during the second meiotic division.

This behavior was the same for all types of chromosomes studied, i.e., the autosomal bivalents, the X chromosome, and the induced autosomal univalents, irrespective of their reductional or equational segregation at anaphase I (Fig. 5E, E′, F, F′, G, G′, G″). Also, in the case of *E. plorans*, there is evidence that the single chromatids at metaphase II or lagging chromosomes at anaphase II are the result of equationally dividing univalents at first division (Rebollo and Arana 1997).

The mitotic H3 phosphorylation pattern was observed in mitosis in those testicular follicles where spermatogonial cells were dividing. The immunosignals appeared not at the initiation of chromosome condensation, but at mid-prophase (Fig. 5A, A′). It was not possible to determine whether the H3 phosphorylation initiates on the pericentromeric regions or scattered along the chromosomes. At metaphase and anaphase, the whole chromosome showed strong immunolabeling, which disappeared during telophase (Fig. 5B, B′).

Discussion

Histone H3 phosphorylation at Ser10, associated with chromatin condensation in mitosis and meiosis, has been described in species ranging from *Tetrahymena* (Wei et al. 1998) to mammals (Hendzel et al. 1997; Cobb et al. 1999a, b), indicating that H3 phosphorylation during nuclear division is a highly conserved process among eukaryotes. However, the immunolabeling patterns obtained with antibodies against phosphorylated H3 in the present work show that there are remarkable differences in the chromosomal distribution of phosphorylated H3

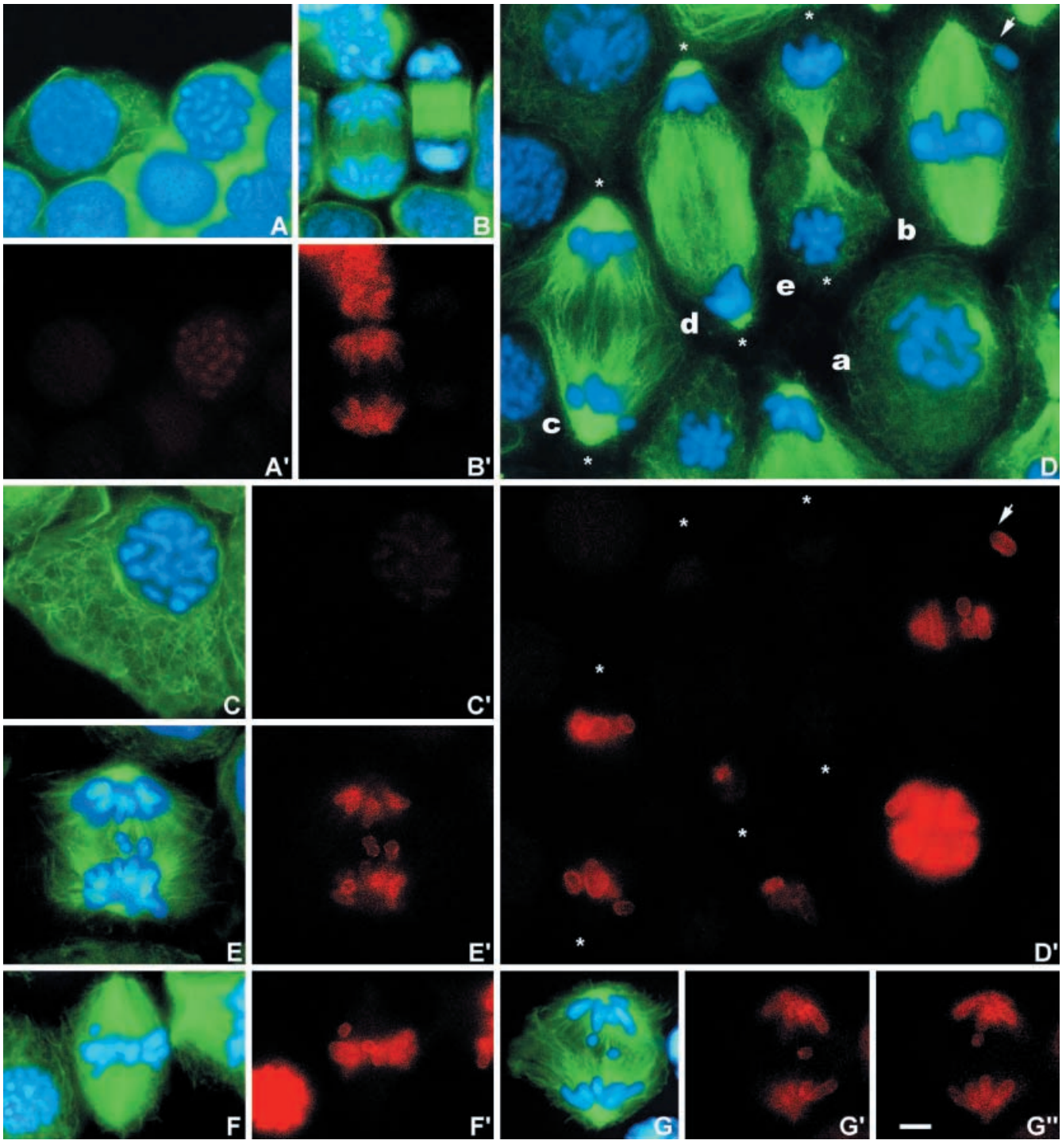

Fig. 5A–G Immunostaining of phosphorylated H3 and α-tubulin during mitosis and meiosis of *Eyprepocnemis plorans*. **A–G** DAPI and α-tubulin staining; **A′–G′** phosphorylated H3 labeling. **A, A′** early (*left*) and late (*right*) mitotic prophase; **B, B′** mitotic anaphase (*left*) and telophase (*right*); **C, C′** diplotene; **D, D′** *a* diakinesis, *b* metaphase I (X *arrowed*), *c* anaphase I, *d* telophase I, *e* cytokinesis; *asterisks* indicate the poles; **E, E′** anaphase I with two lagging univalents amphitelically oriented; **F, F′** metaphase II with a single chromatid out of the plate; **G, G′, G″** anaphase II with two lagging chromatids (**G′** and **G″** are two focal planes of the same cell). **E–G** Cells from heat-treated individuals. *Bar* represents 5 μm (**G″**)

between animal and plant cells, and between mitosis and the first and second meiotic divisions.

Unlike mitosis, where the phosphorylation of H3 starts distinctly in the pericentromeric chromatin of mammals (Hendzel et al. 1997) and plants (Houben et al. 1999), at first meiotic division the phosphorylation of H3 initiates, and remains uniform, along the chromosomes. In plants, the first immunosignals were observed when the initiation of chromosome condensation began during the transition from leptotene to zygotene. At this stage, chromosomes undergo a global structural reorganization,

the chromatids separate slightly and the chromosome volume increases (Dawe et al. 1994). By contrast, in *E. plorans*, the onset of phosphorylation was detected at diplotene, as it was in *M. musculus* (Cobb et al. 1999a, b).

From metaphase I to interkinesis, the H3 phosphorylation patterns were similar in *E. plorans* and plant meiocytes. In both cases, the condensed chromosomes showed strong immunolabeling that gradually disappeared during telophase I. At the light microscopy level, it seems that the degree of chromatin condensation at interkinesis is higher than at mitotic interphase for the species analyzed. In most animals, telophase I and interkinesis are absent, and the anaphase chromosomes pass directly to late prophase II. The contraction of chromosomes is retained; in fact, it persists until the whole meiotic cycle is completed (Swanson et al. 1981). The fact that histone H3 becomes dephosphorylated at interkinesis and phosphorylated again during prophase II, indicates that this post-translational modification is reversible and can occur independently of the DNA replication process.

The spatial and temporal course of H3 phosphorylation during the second meiotic division resembles that of mitosis of the same species. In rye and wheat the immunolabeling was restricted to pericentromeric positions, whereas in *E. plorans*, the chromosomes or chromatids were labeled throughout their length.

Surprisingly, the single chromatids of equationally segregated A or B chromosome univalents, of both grass species analyzed, revealed no detectable phosphorylated histone H3 during the entire second meiotic division. In *E. plorans*, however, this phenomenon was not observed, and the single chromatids, resulting from heat-induced univalents, showed the same immunolabeling as the normally dividing chromosomes.

It should be noted that normal plant chromosome arms were condensed at second meiotic division in the absence of detectable levels of phosphorylated H3. Furthermore, those single chromatids lacking immunosignals even at the pericentromeric region, were equally condensed. These observations prompt the question of whether or not phosphorylation of histone H3 is an absolute requirement for the initiation and/or maintenance of chromosome condensation. Van Hooser et al. (1998) have already observed that hypotonic treatment of mitotic mammalian cells can cause H3 dephosphorylation without chromosome decondensation, and suggested that H3 phosphorylation is not required for the maintenance of high levels of chromosome condensation. Also, in *Tetrahymena* mutants with unphosphorylatable H3, the condensation of chromosomes during mitosis and meiosis did not fail completely (Wei et al. 1999). At mitosis, such cells exhibited difficulty in passing out of anaphase, and some chromosomes could not segregate properly and lagged between the two daughter nuclei (Wei et al. 1999). Also, the condensation status of early prophase chromosomes of mice spermatocytes was unaffected after artificial induction of precocious histone H3 phosphorylation by okadaic acid treatment. Further histone H3 phosphorylation was not inhibited, even though chromosome condensation was artificially inhibited, demonstrating that the phosphorylation of histone H3 can be uncoupled from meiotic chromosome condensation (Cobb et al. 1999a, b). Thus, histone H3 phosphorylation is not sufficient for the condensation of mitotic and meiotic chromosomes. Perhaps the phosphorylation at Ser10 of histone H3 is not necessary at all for the condensation of meiotic plant chromosomes, and other chromosomal components are more important in this process.

Alternatively, the phosphorylation of H3 might be necessary for condensation, but only a few phosphorylated H3 molecules, not detectable by epifluorescence microscopy, would be sufficient to initiate the condensation of chromosomes during the second meiotic division. In any case, the different longitudinal phosphorylation of the chromosomes remains unexplained. Also, it cannot be ruled out that in plant chromosomes the pericentromeric regions and chromosome arms use different condensation mechanisms.

Since, in our experiments, H3 phosphorylated and nonphosphorylated chromatids showed a normal degree of condensation during the second meiotic division, it is tempting to speculate that the phosphorylation of H3 may play different roles affecting chromatin structure in plants and animals. One possible assumption would be that the pattern of phosphorylated histone H3 found in plant cells is closer to that of sister chromatid cohesion than to chromosome condensation in both meiotic divisions. At first meiotic division, the condensed sister chromatids are entirely H3 phosphorylated and closely associated along their entire length (Fig. 1C–F). Cohesion along the chromatid arms serves to keep the bivalents intact by counteracting the spindle forces that act at the kinetochores. This association along the chromosome arms disappears at anaphase I, allowing the homologs to segregate to opposite poles (Bickel and Orr-Weaver 1996).

At second meiotic division, as in mitosis, the phosphorylation of histone H3 occurs only at the pericentromeric regions (Fig. 1K–M), where sister chromatids cohere until the onset of anaphase II. The situation is different for chromatids already separated by equational segregation of univalents during the first division. Since no chromosome reduplication occurs at interkinesis, such chromatids lack a sister to cohere with and therefore need not, or cannot, get H3 phosphorylated at the pericentromeric chromatin. Since the kinetochores of such chromatids interact with microtubules, it can also be concluded that the phosphorylation of H3 of the pericentromeric chromatin is not essential for the successful interaction between microtubules and kinetochores.

However, the correlation between sister chromatid cohesion and phosphorylation of H3 is not perfect because the processes do not begin or end simultaneously. Firstly, it is generally assumed that sister chromatid cohesion is already established during S-phase (Uhlmann and Nasmyth 1998) and is maintained during interkinesis (Bickel and Orr-Weaver 1996). Secondly, sister chromatids eventually separate at the arm region at anaphase I, and along the entire chromatid length during anaphase of

mitosis and second meiotic division, without observable dephosphorylation of histone H3 until telophase.

It is possible that the phosphorylation of histone H3 has a more complex role, including both condensation and sister chromatid cohesion. A mechanistic link between cohesion and condensation has been proposed recently (Biggins and Murray 1999). Guacci et al. (1997) have demonstrated that the product of the *MCD1* gene physically links sister chromatids, and also recruits condensation proteins to condense the chromosomes of budding yeast. The evolutionarily conserved SMC family, with the related but distinct condensin and cohesin protein complexes, is apparently also involved in sister chromatid cohesion and chromosome condensation (Hirano 1999), as is topoisomerase II (Rose et al. 1990; Anderson and Roberge 1996; Cobb et al. 1999b).

Taken together, our results indicate that the changes in chromosome structure during the cell mitotic and meiotic cycles are not universal. Further research will be needed to reach a deeper understanding of the mechanisms governing these complex changes.

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