Cell Tissue Res (2000) 302:189–197
DOI: 10.1007/s004410000270

Immunocytological and FISH analysis of pole cell formation and soma elimination of germ line-limited chromosomes in the chironomid *Acricotopus lucidus*

Wolfgang Staiber

Institute of Genetics
University of Hohenheim
Garbenstr. 30
D-70599 Stuttgart, Germany

Abstract In the chironomid *Acricotopus lucidus*, germ line-soma differentiation becomes evident with the formation of the pole cells and the elimination of the germ line-limited chromosomes (Ks) from the future somatic nuclei of the embryo. Unlike in *Drosophila*, the early nuclear divisions do not proceed synchronously in *A. lucidus*. Usually, only one nucleus, the future pole nucleus, penetrates into the pole plasm always at a telophase stage in the course of a regular mitosis. This happens by chance, depending on the orientation of the mitotic spindles of the early syncytial nuclei. Consequently, the time and the cell cycle at which a nucleus reaches the pole plasm, and pole cells arise, vary between embryos of the same oviposition. When entering into the first germ line mitosis, while polar plasm and syncytial plasm are still not separated, some future somatic nuclei begin to eliminate their Ks. While the soma chromosomes (Ss) undergo normal anaphasic migration to the opposite poles, the K chromatids do not separate and remain in the equatorial plane, as demonstrated by fluorescence in situ hybridization using germ line-specific DNA-probes. The elimination of the Ks do not occur at the same time in all future somatic nuclei. Nondisjunction of Ks was observed in the first mitosis of the pole nucleus, leading to primordial germ cells with different compositions of their K complements. The pattern and timing of elimination mitoses in the embryos indicate that each of the future somatic nuclei seem to regulate the elimination of the Ks autonomously.

Key words Germ line-soma differentiation · Pole cells · Syncytial divisions · Chromosome elimination · Microtubules · *Acricotopus lucidus* (Insecta)

Introduction

In some dipteran families the germ line-soma differentiation during early cleavage divisions is associated with the elimination of specific chromosomes from the future somatic nuclei (for review Tobler 1986; White 1973). Only the pole cells, the germ line progenitors, formed as the first cells on the posterior of the embryo, retain these germ line-limited chromosomes (Ks, derived from "*Keimbahn*"). In *Acricotopus lucidus*, a member of the Orthocladiinae, a subfamily of the Chironomidae, this soma elimination of the Ks is a part of a complex chromosome cycle which the Ks pass through together with the soma chromosomes (Ss) (Bauer and Beermann 1952; Bauer 1970). The factors causing the nuclei in the syncytial compartment of the early embryo to eliminate a specific chromosome complement, and the mechanisms discriminating between Ss and Ks are not known. That the presence of Ks in the germ line is indispensable for the normal development of the germ cells to fertile gametes was demonstrated in Cecidomyiidae (Geyer-Duszynska 1966; Bantock 1970).

In *Drosophila*, pole cell formation is induced by specialized cytoplasm at the posterior polar region of the egg, the germ or pole plasm. This plasm contains polar granules, electron-dense structures, in which factors essential for pole cell formation and so germ cell/line determination are assembled (Okada 1998). Genetic studies have identified so far eight maternal effect genes, and beyond that *oscar, vasa, tudor, nanos*, which play a role in polar granule formation and localization, and also in pole cell formation and migration (Mahowald 1992; St Johnston 1993; Lehmann and Ephrussi 1994).

Some observations of the timing and pattern of the soma elimination of the germ line-limited chromosomes in *Sciara* (called Ls) were reported from a confocal study of the process of programmed elimination of the paternal X during early embryonic development (de Saint Phalle and Sullivan 1996). The sister chromatids of the Ls stick together in a section of their chromosome arms; they therefore cannot move poleward and are eliminated. In chironomids, some details of pole cell formation were reported from a transmission electron microscopy-study of early embryogenesis in *Smittia* (Zissler 1992), but until now only little is known about the mechanism, sequence, and timing of the soma elimination of the Ks. In this study, the processes of soma elimination of the Ks and of pole cell formation were investigated in *A. lucidus* by fluorescence in situ hybridization (FISH) to whole mount early embryos using germ line-specific repetitive DNA probes (Staiber et. al 1997) and by immunostaining of spindle microtubules visualized by the way of confocal laser microscopy.

Materials and methods

Preparation of embryos

All embryos in this study were taken from a laboratory stock of *Acricotopus lucidus* (Diptera, Chironomidae) derived from one egg batch deposited in July 1985 (Staiber 1988). The embryos were dechorionated, fixed, and devitellinized as described by Tautz and Pfeifle (1989) with

modified fixatives. For in situ hybridization embryos were fixed in 10% formaldehyde, 50mM EGTA in PBS, pH 7.0, and for immunostaining of microtubules in freshly prepared 4% para-formaldehyde in microtubule-stabilizing buffer, 100mM PIPES, 1mM MgSO4, 1mM EGTA, pH, 6.8 (Wolf and Bastmeyer 1991). The embryos were stored in 100% methanol at -20°C.

Labeling of probes

Probes were generated by PCR amplification of a cloned monomer (AlKe1, 183 bp) and a pentamer (AlKe5-8, 897 bp) of the germ line-specific AlKe-family (Staiber et al. 1997) using the primer pair 5'-AACATAATGTGAAAAATACA-3' and 5'-GACAATAAATTTTATCACAA-3', 200µM dNTPs and 40µM digoxigenin-11-dUTP. Labeled PCR products were cleaned by using spin columns (Qiagen).

For efficient penetration and hybridization to the embryos the size of the PCR products of the AlKe pentamer were reduced by boiling the probe for 40 min. The hybridization mixture consisted of 50% formamide, 4x SSC (pH 7.0), 0.1% Tween 20.

In situ hybridization to whole mount embryos

Fluorescence in situ hybridization (FISH) to whole embryos was performed according to the method of Hiraoka et al. (1993). Additionally, embryos were treated with RNase (1 mg/ml). Hybridization was visualized by a fluorescein conjugated anti-digoxigenin antibody from sheep, Fab fragments (Boehringer Mannheim), 1:10 dilution in 2xSSC/0.1% Tween 20 containing 2 mg/ml bovine serum albumin, 2 h at 37°C. Chromosomes and nuclei were counterstained with 2,5µg/ml propidium iodide in 2xSSC/0.1% Tween 20. Finally, embryos were embedded in Vectashield fluorescence antifade solution (Vector Laboratories).

Anti-tubulin immunofluorescence

Embryos were rehydrated in a methanol/PBS series, rinsed three times in PBT (= PBS/0.1% Tween 20), treated with 1mg/ml RNase in PBT for 4 1/2 h at 37°C, preincubated in 3% normal goat serum in PBT for 1 h at room temperature and incubated in a 1:75 dilution of a mouse monoclonal anti ß-tubulin antibody (Sigma) at 5°C overnight. After thorough washing with PBT, a FITC conjugated goat anti-mouse IgG1 (Serva) diluted 1:50 in PBT, was applied as second antibody for 2h at room temperature.

Microscopy

Digital images were captured with a BioRad MRC 1024 laser confocal imaging system using a Zeiss epifluorescence Axioscope equipped with Planapo 40/1,3 and Planapo 63/1,4 objectives, and were processed with a Corel Draw software package.

Results

Pole cell formation and soma elimination of the germ line limited chromosomes (=Ks) during early embryonic development in *A. lucidus* was studied using confocal laser scanning and differential interference contrast (DIC) microscopy.

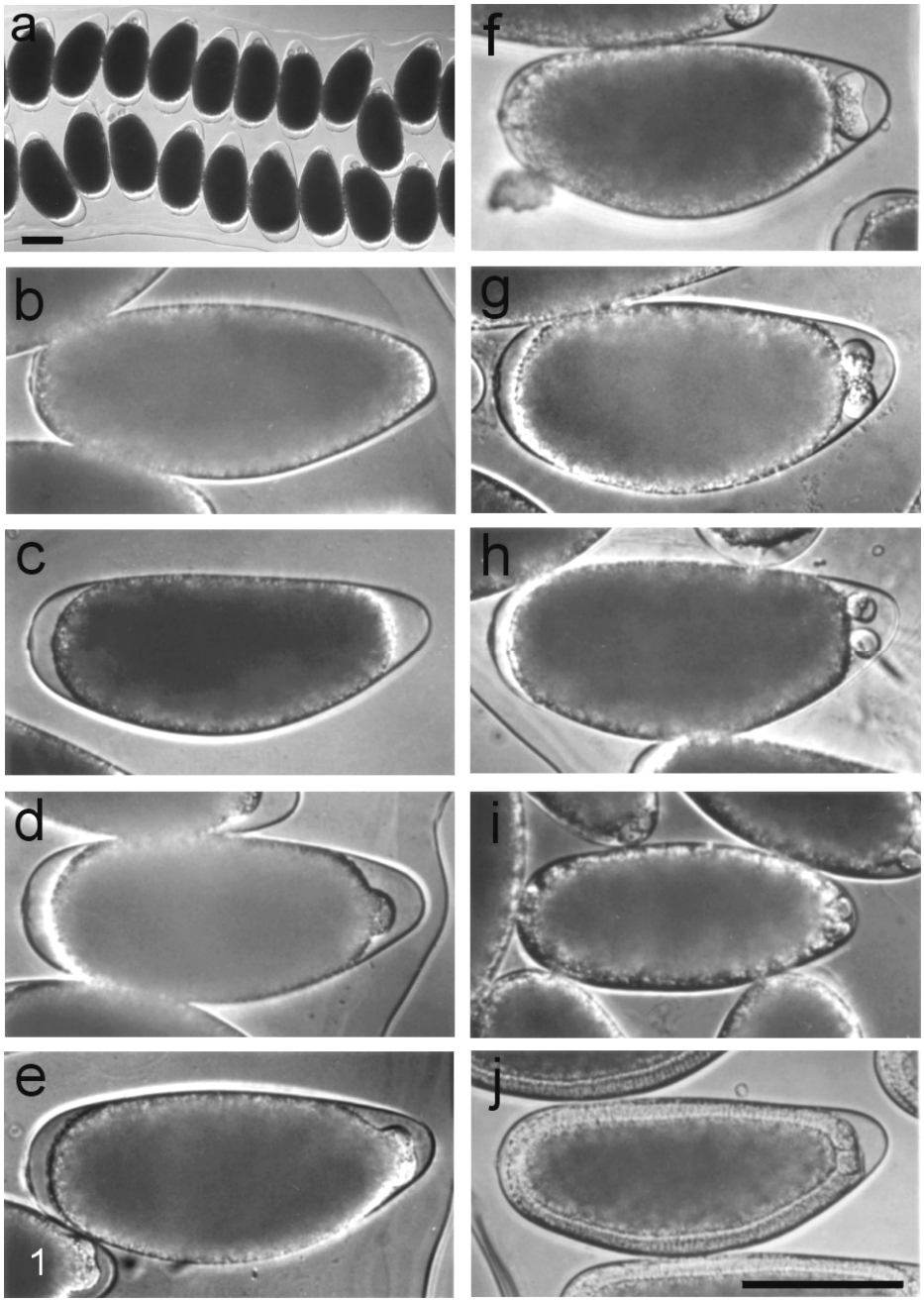

Fig. 1a-j Pole cell formation and early embryonic development in *A. lucidus*. DIC images of living embryos. **a** Section of an egg tube, 1 3/4 h AED (20-22°C), showing two rows of embryos in different stages of pole cell formation. **b** Newly-laid egg. **c** Embryo, 1/2 h AED. Gaps appear between vitelline membrane and embryo poles. **d-g** Polar bud and pole cell formation in embryos, 1 1/2-2 h AED. **h** Embryo, 3 h AED. **i** Syncytial blastoderm, 7 h AED. **j** Cellular blastoderm, 8-9 h AED. *Left*, anterior pole. *Bars* **a**, **j** 100 µm.

In *A. lucidus*, shortly after copulation, the female deposits into water 100-300 eggs in a transparent gelatineous tube-like envelope (Fig. 1a). The clear eggshell allows one to observe pole cell formation and the following embryogenesis directly without any pretreatment of the

eggs (Fig. 1b-j). Newly laid eggs are completely filled with yolk mass (Fig. 1b). The first signs of development are when gaps appear between vitelline membrane and embryo poles about 1/2 h after egg deposition (AED) at room temperature (20-22°C) (Fig. 1c). In a normally developing embryo, the beginning of pole cell formation (about 1,5-2 h AED) is marked by a slight surface bulge at the posterior end of the embryo which signals the arrival of a nucleus in the germ plasm. Following the formation of a clear polar bud (Fig. 1d) and the first appearance of slight constrictions (Fig. 1e), the first mitosis of the polar nucleus occurs (Fig. 1f, g) and is completed by the formation of two pole cells (Fig. 1h). The pole cells separate from the syncytial part of the embryo and from each other in one step at the same time. During the syncytial blastoderm with the formation of somatic buds (Fig. 1i) and the cellular blastoderm (Fig. 1j), the two primordial germ cells can be clearly distinguished from the somatic cells by their larger size. It is important to note that pole cell formation does not occur synchronously in the different embryos of the same egg deposition. This is seen in the section of an oviposition (1 3/4h AED) in Fig. 1a, where some embryos had already completed pole cell formation while others have still not even formed polar buds.

The visualization of spindle microtubules in whole mount embryos via immunofluorescence, using an anti ß-tubulin antibody combined with propidium iodide fluorescence staining of the chromatin, allows the clear identification of all mitotic stages, especially of telophase nuclei and their corresponding counterparts within the syncytium (Fig. 2a). Using only propidium iodide staining, it is not possible to distinguish interphase from telophase nuclei. So in all embryos examined, which showed a slight bulb at the posterior, indicating the arrival of a nucleus in the pole plasm, the nucleus was identified as a telophase nucleus (Fig. 2a).

A series of confocal images of the arrival of a nucleus in the pole plasm, and of successive stages of pole cell formation and first germ line mitosis is to be seen in Fig. 2b-i. The slight surface bulge at the posterior of the embryo (Fig. 2b, section of Fig. 2a) increases in size, forming a clear polar bud during transition from prophase (Fig. 2c) to metaphase (Fig. 2d) of the pole nucleus. Prophasic nucleus and metaphase plate, including the developing spindle, lie close to the posterior pole membrane of the embryo. In most cases, the spindle poles are arranged in right angle to the anterioposterior axis of the embryo (Fig. 2d). Occasionally, the spindle poles may also be orientated in the direction of the longitudinal axis (Fig. 2e). This was found in one of 45 pole cell metaphases and anaphases analysed by laser scanning microscopy. The chromosomes then segregate and start their anaphase migration to the opposite poles (not shown). With their arrival at the spindle poles and entry into telophase, the constrictions between the arising pole cells and the embryo, as well as between both pole cells, increase (Fig. 2f). The pole cells separate from the syncytial part of the embryo and from each other in one step (Fig. 2g).

A highly repeated germ line-specific DNA sequence family, named AlKe family (Staiber et al. 1997), specifically located in the centromeric regions of the Ks can be used as probe in FISH to identify the Ks in early cleavage divisions, in elimination mitoses, and in pole cell mitoses.

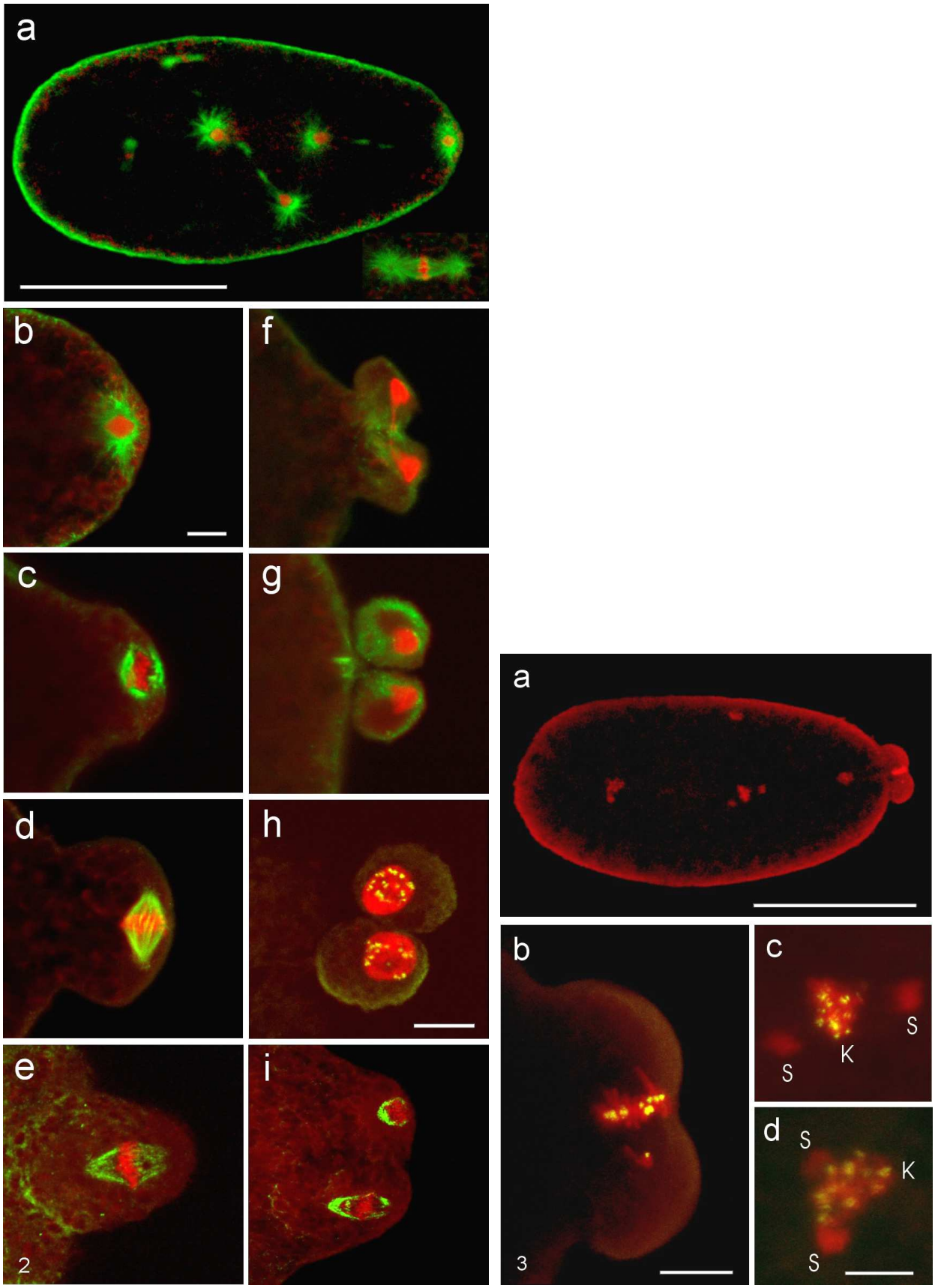

Fig. 2a-i Confocal images of pole cell formation in whole mount embryos. **a-g, i** Anti ß-tubulin immunofluorescence of spindle microtubules. **h** FISH detection of the centromeric regions of the Ks using germ line-specific AlKe DNA sequences as probe. **a** Arrival of a telophase nucleus in the posterior pole plasm. Two telophases and five metaphases were found in this embryo. Insert presents one of the metaphases. **b** Posterior section of the embryo in **a** with the future germ line nucleus. **c-e** Polar bud

formation showing a prophase nucleus **(c)** and two metaphase nuclei with spindle pole to spindle pole orientations in right angle to **(d)** and in direction of **(e)** the anterioposterior axis of the embryo. **f** Telophase. **g** Cytokinesis. **h** Clear AlKe FISH signals demonstrate that Ks are present in both primordial germ cells. **i** After having reached the posterior pole plasm at the same time, two nuclei start mitosis, leading to the formation of four pole cells. Nuclei are counterstained with propidium iodide. *Bars* **a** 100 µm; **b** 10µm.

Fig. 3a-d Correlation between entrance into the first mitosis of the germ line nucleus and the first occurrence of K elimination mitoses in *A. lucidus*. FISH with an AlKe probe. **a** Embryo in the fourth mitotic cycle containing five nuclei. Only the propidium iodide fluorescence channel is presented. Three of the four future somatic nuclei are eliminating their Ks (those lying along the anterioposterior axis), while the pole nucleus is in metaphasic stage. Germ plasm and somatic plasm are still not separated. **b** Section of the embryo in **a** showing the Ss and the AlKe fluorescence-labeled Ks aligned in the metaphase plane. There is nondisjunction and premature movement of one K to the lower spindle pole. **c,d** Anaphases of elimination mitoses in prospective somatic nuclei. Sections of **a**. Only the S chromatids have separated and move to the spindle poles, while the Ks remain in the equatorial plane. Nuclei are counterstained with propidium iodide (*S* soma chromosomes; *K* germ line-limited "*Keimbahn*" chromosomes). *Bars* **a** 100µm; **b-d** 10µm.

Using these K-specific AlKe sequences for FISH on whole mount embryos, it can be demonstrated that the pole cells contain Ks, as is to be seen in Fig. 2h.

Early syncytial mitoses before pole cell formation do not proceed synchronously in *A. lucidus*. In the embryo in Fig. 2a seven metaphases (insert in Fig. 2a) and two telophases can be observed at the same time.

Generally, two pole cells originate at the posterior tip of the embryos, but two nuclei can also reach the pole plasm at the same time, forming two polar buds (Fig. 2i) from which then four pole cells arise. In five different randomly choosen egg tubes of *A. lucidus* (2-3 h AED) the portion of embryos with four pole cells varied from 0 of a total of 162 embryos, to 4 of a total of 178 embryos, 4 of a total of 234 embryos, 6 of a total of 296 embryos, and 7 of a total of 259 embryos.

During the first syncytial cleavage divisions, the telophase nuclei can move away from each other by spindle elongation within the embryo to up to 100 µm. This is something less than half of the length of an embryo. On the average, the distance between anterior and posterior poles of an embryo amount to about 220 µm.

As mentioned before, the future pole nucleus penetrates into the pole plasm at the telophase stage in the course of a regular mitosis. This happens by chance depending on the orientation of the spindles of the early syncytial mitoses. Consequently the time, and the cell cycle, at which a nucleus reaches the pole plasm, a polar bud is formed, and pole cells arise varies by about 1/2h between embryos of the same oviposition.

The earliest observed penetration of a telophase nucleus into the pole plasm was during the third mitotic cycle. In such an embryo, two of three nuclei were located in the interior of the yolk mass. These two nuclei passed through mitoses with pole to pole orientation of their spindles along the anterioposterior axis. Thereby, one of the four resulting telophase nuclei reached the germ plasm during spindle elongation. The third, but nondividing, nucleus was located dorsally near the surface of the embryo. The next step in development of such an embryo, now containing

five nuclei, is illustrated in Fig. 3a. In this embryo the Ks were selectively stained in their centromeric regions by FISH with an AlKe probe (fluorescein signals in Fig. 3b-d). In Fig. 3a only the propidium iodide fluorescence is shown. In the now fourth mitotic cycle, at the time when the germ line nucleus enters into the first mitosis (Fig. 3b), the soma elimination of the Ks occurred in the three nuclei arranged along the longitudinal axis (Fig. 3c, d). In such an elimination mitosis, the Ss segregate equally and migrate to the opposite poles, while the Ks remain in the equatorial zone. No elimination of Ks was observed at that time in the nucleus located near the embryo surface.

It is important to note that pole cells/pole plasm had still not separated from the syncytial plasm at the time when some of the future somatic nuclei were eliminating their Ks. But a telophase nucleus can also reach the pole plasm for the first time during the fourth or fifth mitotic cycle. This depends on the random orientation of the mitotic spindle poles. The soma elimination then starts in the following (the fifth or the sixth) mitotic cycle. Thus pole cell formation and the start of the soma elimination of the Ks can occur at different times, i.e. asynchronously between embryos of the same oviposition.

In the metaphase of the first germ line mitosis shown in Fig. 3b, one single chromosome, identified by the AlKe signal as a K, moved prematurely and undivided to the lower pole. This nondisjunction of a K, observed only once in the present study, would result in two primordial germ cells with different compositions of their K-complements.

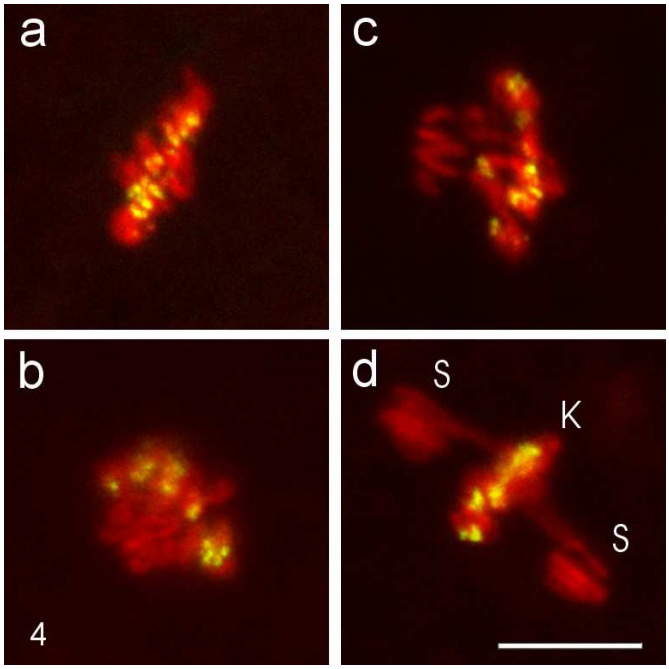

Fig. 4a-d FISH using a germ line-specific AlKe DNA probe to syncytial future somatic nuclei. **a** Side view and **b** top view of a metaphase plate. **b** Ss and Ks lie separated from each other within the plate. **c** Early anaphase of an elimination mitosis. The Ss start the movement to the opposite spindle poles. **d** Late anaphase of an elimination mitosis. Only chromosomes exhibiting AlKe signals, thus identified as Ks, remain back at the equatorial plate. All stages derived from the same embryo. *Bar* 10µm.

Four mitotic stages from the syncytial somatic compartment of an embryo, in which the centromeric regions of the Ks are visualized by FISH, are presented in Fig. 4. In Fig. 4a, Ks and

Ss are aligned in the equatorial plane (side view). The distribution of AlKe hybridization signals in the top view of the metaphase plate in Fig. 4b indicates that Ks and Ss are arranged in separated groups. In the early anaphase of an elimination mitosis in Fig. 4c, only the S chromatids separate and migrate to the opposite poles, while the Ks stay behind in the equatorial plate. Only one K seems to be pulled in direction to the left spindle pole. In the mid anaphase in Fig. 4d, all chromosomes exhibiting AlKe signals are eliminated and begin to clump together.

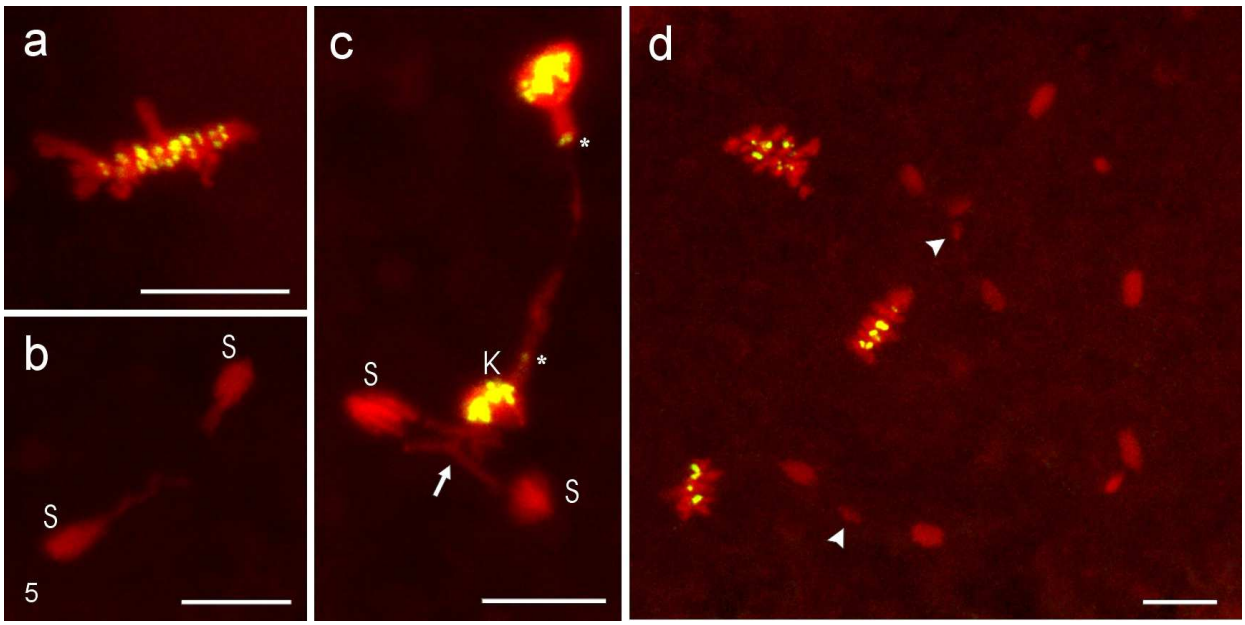

Fig. 5a-d Nonsynchronous elimination of the Ks within embryos of *A. lucidus*. Ks are not eliminated from the future somatic nuclei at the same time. FISH with AlKe probes. The mitotic phases in **a-c** derived from the same embryo that has completed pole cell formation. **a** Side view of a metaphase plate containing Ks. **b** Telophase of a nucleus that has eliminated its Ks in one of the previous mitoses. **c** Two phases connected by a chromatin bridge indicating that they are daughter nuclei of a previous mitosis. The clear AlKe fluorescence signals (*stars*) on both ends of the bridge indicate that the latter is probably the result of a chromosome rearrangement involving two Ks. Both nuclei are in different stages of the cell cycle. The upper nucleus is in interphase, while the lower is in anaphase of an elimination mitosis. The sister chromatids of two Ks showing no AlKe signals (*arrow*), and therefore most probably K9s, are stretched towards the opposite poles. **d** Section of the somatic compartment of an embryo showing some nuclei in metaphase stage that still possess their Ks (AlKe signals) and some telophases that are just eliminating one K (no AlKe signal, so K9, a*rrowheads*). The telophases have lost most of their Ks in one of the previous mitoses. This asynchrony indicates that the nuclei regulate the elimination of the Ks autonomously. *Bars* 10µm.

The mitotic phases shown in Fig. 5a-c derived from one embryo in which pole cell formation has already been completed. There are nuclei/phases which still possess their Ks (the metaphase in Fig. 5a and the upper nucleus in Fig. 5c), as well as nuclei that have already eliminated all their Ks (the anaphase nuclei in Fig. 5b) and phases in which the soma elimination is just proceeding (lower phase in Fig. 5c). In Fig. 5c, there is a chromatin bridge of about 20 µm in length between the two phases with clear AlKe fluorescence signals on both ends. This indicates that the bridge resulted from a rearrangement between two Ks and that both nuclei are daughter nuclei of a previous mitosis. The lower nucleus in Fig. 5c is in late anaphase of an elimination mitosis, while the upper nucleus is in interphase, still possessing its Ks. In the elimination

mitosis the sister chromatids of two chromosomes, which remain in the equatorial zone but exhibit no AlKe signals, are stretched towards the in opposite the poles. Most probably these chromosomes are K9s. K9 is the only K of the nine different K types which contain no AlKe sequences in its centromeric region (Staiber et al. 1997).

That the elimination of the Ks does not proceed synchronously in the different somatic nuclei, and that sometimes the elimination of all the Ks does not occur during only one mitosis, is impressively demonstrated in Fig. 5d. The confocal image of a section of an embryo shows three metaphases possessing their Ks, clearly seen on the bright FISH AlKe signals, and the two telophases exhibiting no fluorescence signals but just eliminating one small K (arrowheads in Fig. 5d). Most probably the small K is a K9. In the case of the telophase nuclei, the Ks must therefore have been eliminated in one of the previous mitoses.

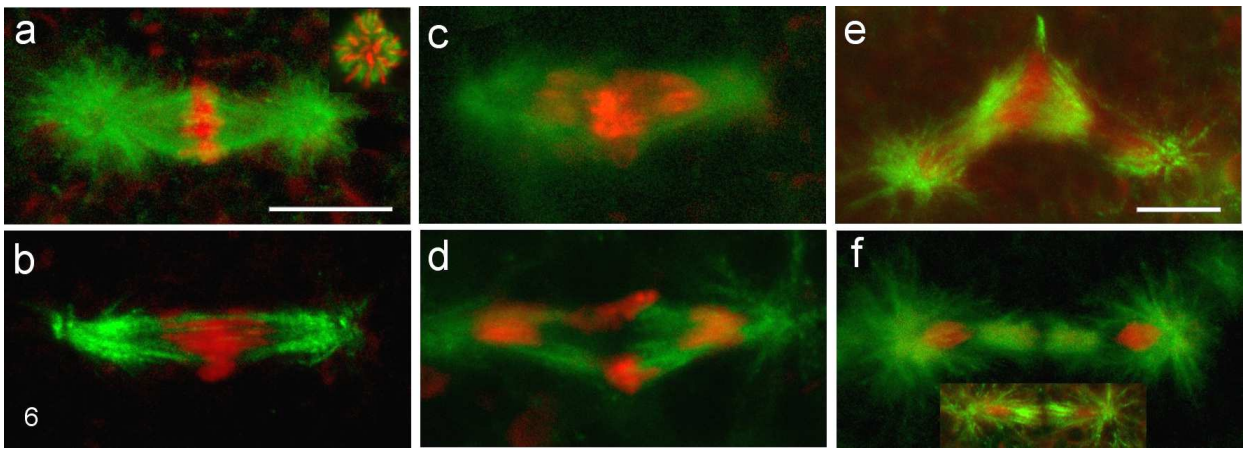

Fig. 6a-f Microtubule arrangements in regular mitoses and in K elimination mitoses visualized by anti ß-tubulin immunofluorescence. **a** Metaphase of a regular early mitosis containing Ss and Ks derived from the embryo in Fig. 2a. Astral microtubules are well established around the centrosomes on both spindle poles. **b** Early, **c** mid and **d** late anaphases of elimination mitoses. **e** Telophase of an elimination mitosis. High densities of microtubules are present on both sides of the equatorial zone adjacent to the eliminated Ks. **f** Similar microtubule arrays in telophases of syncytial nuclei, either containing Ks or having lost the Ks (*insert*). *Bars* 10µm.

The changes of spindle microtubule arrangements in early syncytial mitoses and during K elimination mitoses are presented in Fig. 6. In the metaphase of an early syncytial mitosis (Fig. 6a), Ks and Ss are completely aligned in the equatorial zone. Forces of kinetochore microtubules pull the centromeres of the sister chromatids somewhat in direction to the opposite spindle poles. Aster-like microtubule arrays are clearly visible around the centers of both spindle poles containing the centrosomes. In the top view of a metaphase plate in the insert in Fig. 5a, bundles of microtubules pass through the spaces between the chromosomes. In the early anaphase of an elimination mitosis in Fig. 6b, only the Ss separate and beginn their migration to the opposite poles. The Ks remain back in the equatorial plate, as clearly seen in the mid anaphase in Fig. 6c. In the upper part of the equatorial plane of the late anaphase in Fig. 6d, both sister chromatids of an eliminated K lie side by side, and no pole orientation of the centromeres is visible. Frequently, during late anaphase and telophase, both half spindles curve forming a bow (Fig. 6e).

In telophase, dense bundles of pole-oriented microtubules are concentrated on both sides of the equatorial plane adjacent to the eliminated K chromatin (Fig. 6e). But this microtubule arrangement on both sides of the equatorial zone is not specific for elimination mitoses. The same repelling microtubule arrays occur in telophases of early embryonic mitoses still containing their Ks (Fig. 6f), as well as in telophases of syncytial mitoses of future somatic nuclei that have already eliminated their Ks (Fig. 6f, insert). For some time the eliminated K complements are visible as clumps of chromatin within the yolk (not shown).

As a result of the K elimination mitoses in the somatic compartment of the embryo, the daughter nuclei now contain only the six Ss, while the germ line transmits in addition the Ks to the next generation.

Discussion

The elimination of the special chromosome complement of the Ks from the future somatic nuclei during early syncytial divisions in *A. lucidus* is correlated in time with the formation of the pole cells, the primordial germ cells, which transmit the Ks to the next generation. Using immuno-staining of spindle microtubules and confocal imaging it was demonstrated that in *A. lucidus* the pole nucleus penetrates into the posterior germ plasm at the telophase stage in the course of a normal mitosis. This can occur in the third, fourth or fifth mitotic cycle, depending on the random orientation of the spindle poles in the first syncytial divisions. This observation differs from observations in other chironomids and in *Drosophila*, from which a distinct cycle-dependent migration of one nucleus (*Smittia*, after the 2nd mitotic division), or of some nuclei (*Drosophila*, during cycle 9) into the polar plasm, probably mediated by cytoskeletal elements is reported (Zissler 1992; Baker et al. 1993; Foe et al. 1993; St Johnston 1993). The asynchrony in pole cell formation between the different embryos of the same egg deposit, determined in vivo using DIC microscopy, complements the above observations of differences in timing of the nuclear arrival in polar plasm.

With the entrance of the polar nucleus into prophase/prometaphase stage of the first mitosis, some but not all of the future somatic nuclei start to eliminate their Ks. This takes place during the fourth to the six mitotic cycle. From earlier investigations of the chromosome cycle in *A. lucidus*, Thudium (1974) and Bauer and Beermann (1952) reported that the soma elimination of the Ks occurs in the fifth or the sixth cleavage division, respectively.

The cause leading some syncytial nuclei to switch from normal mitosis with equal segregation of all chromosomes to an elimination mitosis differentiating between two chromosome complements is unknown. The arrival of a nucleus in the pole plasm, the entrance into mitosis of the polar nucleus and/or the beginning of the separation of the germ plasm from the syncytial plasm may provide the condition for the start of eliminating the Ks in some of the future somatic nuclei.

In *Drosophila* the actin cytoskeleton is needed for stable association of *nanos*, *oskar*, *germ cell-less* and cyclin B mRNAs and Oskar and Vasa proteins, at the posterior pole in the early embryo (Lantz et al. 1999). Possibly, changes in arrangement of the posterior cytoskeleton and/or changes in concentration of one or of some of the above mentioned factors during formation of the pole cells initiate the beginning of the soma elimination of the Ks in *A. lucidus*. Su et al. (1998) demonstrated that, for nuclei in syncytial embryos of *Drosophila*, the exit from mitosis requires proteolysis and cyclin degradation. They suggested "that localized cyclin destruction is a specialization of early syncytial cycles that allows local control of exit from mitosis despite the absence of cell membranes" and "that exit from mitosis in syncytial cycles is modified to allow nuclear autonomy within a common cytoplasm".

Syncytial nuclei in *Acricotopus* early embryos also seem to regulate their entrance into mitosis and into elimination mitosis autonomously. This assumption is strongly supported by the confocal image shown in Fig. 5d, in which one of two daughter nuclei is already in anaphase of elimination mitosis, while the other is still in interphase. Both nuclei are connected by a chromatin bridge, indicating that they are daughter nuclei of a previous mitosis.

In the complex chromosome cycle of the fungus gnat *Sciara coprophila*, a programmed elimination of paternal X chromosomes takes place in the course of early cleavage divisions (Metz 1938; Gerbi 1986). Using confocal microscopy and FISH, de Saint Phalle and Sullivan (1996) demonstrated that the X chromosome is eliminated by a failure of sister chromatid separation. The chromatids remain joined at a region on the long arm of the X chromosome. But the centromeres of the X chromosomes remain attached to the spindle during anaphase of an elimination mitosis, as demonstrated by the fact that the chromatids are stretched towards the poles. De Saint Phalle and Sullivan (1996) also observed the loss of L chromosomes in the divisions preceding the loss of the X chromosomes, and they reported that the L chromosomes are eliminated probably in the same way as the X chromosomes by a failure of sister chromatids to separate completely. They observed that during the elimination process the L chromosomes are stretched toward the opposite poles of the spindle. Their centromeres have been separated and remain attached to the kinetochore microtubules, but the sister chromatids do not separate completely and so the L chromosomes cannot move away from the equatorial plane.

In *Acricotopus* the centromeres of the K sister chromatids must be attached to spindle microtubules during prometaphase and metaphase of an elimination mitosis in order to arrange the Ks into the equatorial plane together with the Ss. But in contrast to *Sciara*, the K sister chromatids are not stretched in direction of the opposite poles, and the centromeres of the Ks seem not to separate while the Ss move to the poles. Thus one can conclude that the K sister chromatids remain joined at their centromeric regions rather than at the chromosome arms or the telomeres. In *Drosophila* a specific centromeric protein, MEI-332, was identified that is required for the maintenance of centromeric sister chromatid cohesion (Kerrebrock et al. 1995). MEI-S332 delocalizes from the centromeres at mitotic and second meiotic metaphase/anaphase transition when the centromere cohesion is released (Tang et al. 1998; LeBlanc et al. 1999).

Possibly a protein (or proteins) like MEI-S332, a member of a predicted class of centromeric proteins responsible for centromeric cohesion (LeBlanc et al. 1999), is involved in the irreversible K sister chromatid attachment in the soma elimination of the Ks in *A. lucidus*.

For the programmed X chromosome elimination in *Sciara*, de Saint Phalle and Sullivan (1996) suppose that "the decision to eliminate is made at the level of the individual nucleus". The L chromosomes are lost in an earlier division cycle than the X chromosomes. To explain this sequential elimination, de Saint Phalle and Sullivan propose a model in which maternally supplied factors necessary for sister chromatid separation are titrated by elements on the L and X chromosomes at each division cycle. The L chromosomes are lost earlier because they require much more of the factors than the X chromosomes.

It is not always the case that all Ks of a nucleus are eliminated during one mitosis. Especially K9 was found to be eliminated, sometimes in a later mitosis than the other Ks (Fig. 5d). K9 is the only K that contains no elements of the germ line-specific highly repeated AlKe DNA sequence family in its centromeric region, as demonstrated by FISH (Staiber et al. 1997). In contrast to the other Ks, K9 contains high amounts of repetitive AlSo sequences in its centromere which are characteristic for the centromeric regions of the three Ss. Its similarity to the Ss is also indicated by the fact that K9 is completely painted by a soma DNA painting probe, while the other Ks are only partially painted (Staiber and Schiffkowski 2000).

In an earlier study investigating the K complement compositions in gonial mitoses of *A. lucidus* by G-banding, it was established that both gonads of an animal can have different numbers and compositions of K types in their K complements (Staiber 1988). Until now it has been assumed that these differences result from the germ line elimination of about half of the Ks during the first mitoses of the primordial germ cells that proceeds in both gonads in newly hatched larvae (= germ line elimination; Bauer and Beermann 1952; Staiber 1988). But, as is to be seen in the nondisjunction of a K in the first mitosis of the pole nucleus in Fig. 3b, these differences can arise already during pole cell formation and therefore much earlier in development.

In the cecidomyiid *Wachtliella persicariae*, a species also having additional chromosomes in the germ line, the migration of nuclei into the pole plasm was experimentally delayed by ligation of early embryos (Geyer-Duszynska 1966). Resulting from this, the Ks were eliminated from all nuclei. In this experiment, nuclei without Ks reached the posterior pole plasm, pole cells were formed and the embryos developed normally. But both sexes were not able to produce fertile gametes. This indicates that in *Wachtliella* the nuclei in the syncytial part of the embryos eliminate their Ks independently from pole cell formation, perhaps in a critical period of time in the embryonic development.

Raff and Glover (1989) reported that aphidicolin injected into *Drosophila* embryos at nuclear cycle 7-8 inhibited DNA synthesis, nuclear division and nuclear migration, and causes centrosomes to be separated from nuclei. In such embryos centrosomes continued to migrate, also into the germ plasm, and were able to initiate pole cell formation in the absence of nuclei.

Whether this remarkable event is also inducible in *Acricotopus* and whether then pole cell formation without nuclei would then accompany elimination mitoses in the syncytial compartment of the embryo, remains to be examined.

One may conclude that the evolution of the elimination mitoses occurring in *Acricotopus* and in *Sciara*, representing an unusual mode of chromosome loss, is achived by the co-oping of normal cellular factors rather than by the evolution of a complete new system. Such factors with specific roles in the process of soma elimination of the Ks remain to be identified.

Acknowledgments The author would like to thank Prof. Anette Preiss for helpful discussions and support, and Bernd Johannes and Dr. Anja C. Nagel for advice in laser scanning microscopy and computer programs. This work was supported by a grant of the DFG (STA 462/2-2).

References

Baker J, Theurkauf WE, Schubiger G (1993) Dynamic changes in microtubule configuration correlate with nuclear migration in the preblastoderm *Drosophila* embryo. J Cell Biol 122: 113-121

Bantock CR (1970) Experiments on the chromosome elimination in the gall midge, *Mayetiola destructor*. J Embryol Exp Morphol 24: 257-286

Bauer H (1970) Rearrangements between germ-line limited and somatic chromosomes in *Smittia parthenogenetica* (Chironomidae, Diptera). Chromosoma 32: 1-10.

Bauer H, Beermann W (1952) Der Chromosomencyclus der Orthocladiinen (Nematocera, Diptera). Z Naturforsch 7b: 557-563.

De Saint Phalle B, Sullivan W (1996) Incomplete sister chromatid separation is the mechanism of programmed chromosome elimination during *Sciara coprophila* embryogenesis. Development 122: 3775-3784.

Foe VE, Odell GM, Edgar BA. Mitosis and morphogenesis in the *Drosophila* embryo: point and counterpoint. In: Bate M and Martines Arias A (eds) The development of *Drosophila melanogaster*. Vol I. Cold Spring Harbor Laboratory Press, New York, pp 149 - 300

Gerbi SA (1986) Unusual chromosome movements in Sciarid flies. In: Hennig W (ed) Germ line-soma differentiation. Results and problems in cell differentiation, vol 13. Springer, Berlin Heidelberg New York Tokyo, pp 71-104

Geyer-Duszynska I (1966) Genetic factors in oögenesis and spermatogenesis in Cecidomyiidae. In: Darlington CD, Lewis KR (eds) Chromosomes today, vol 1. Oliver and Boyd, Edinburgh, pp 174-178

Hiraoka Y, Dernburg AF, Parmelee SJ, Rykowski MC, Agard DA, Sedat JW (1993) The onset of homologous chromosome pairing during *Drosophila melanogaster* embryogenesis. J Cell Biol 120: 591-600

Kerebrock AW, Moore DP, Wu JS, Orr-Weaver TL (1995) MEI-S332, a *Drosophila* protein required for sister-chromatid cohesion, can localize to the meiotic centromere region. Cell 83: 247-841

Lantz VA, Clemens SE, Miller KG (1999) The actin cytoskeleton is required for maintainance of posterior pole plasm components in the *Drosophila* embryo. Mech Dev 85: 111-122

LeBlanc HN, Tang TT-L, Wu JS, Orr-Weaver TL (1999) The mitotic centromeric protein MEI-S332 and its role in sister-chromatid cohesion. Chromosoma 108: 401-411

Lehmann R, Ephrussi A (1994) Germ plasm formation and germ cell determination in *Drosophila*. Ciba Found Symp 182: 282-296

Mahowald AP (1992) Germ plasm revisted and illuminated. Science 255: 1216-1217

Metz CW (1938) Chromosome behavior, inheritance and sex determination in *Sciara*. Am Nat 72: 485-520

Okada M (1998) Germline cell formation in *Drosophila* embryogenesis. Genes Genet Syst 73: 1-8

Raff JW, Glover DM (1989) Centrosomes, and not nuclei, initiate pole cell formation in *Drosophila* embryos. Cell 57: 611-619

Staiber W (1988) G-banding of germ line limited chromosomes in *Acricotopus lucidus* (Diptera, Chironomidae). Chromosoma 97: 231-234

Staiber W, Schiffkowski C (2000) Structural evolution of the germ line-limited chromosomes in *Acricotopus*. Chromosoma 109: 343-349

Staiber W, Wech I, Preiss A (1997) Isolation and chromosomal localization of a germ line-specific highly repetitive DNA family in *Acricotopus lucidus* (Diptera, Chironomidae). Chromosoma 106: 267-275

St Johnston D (1993) Pole plasm and posterior group genes. In: Bate M, Martines Arias A (eds) The development of *Drosophila melanogaster*, vol I. Cold Spring Harbor Laboratory Press, New York, pp 325-363

Su TT, Sprenger F, DiGregorio PJ, Campbell SD, O'Farrall PH (1998) Exit from mitosis in *Drosophila* syncytial embryos requires proteolysis and cyclin degradation, and is associated with localized dephosphorylation. Genes Dev 12: 1495-1503

Tang TT-L, Bickel SE, Young LM, Orr-Weaver TL (1998) Maintainance of sister chromatid cohesion at the centromere by *Drosophila* MEI-S33 protein. Genes Dev 12: 3843-3856

Tautz D, Pfeifle C (1989) A nonradioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals a translation control of the segmentation gene *hunchback*. Chromosoma 98: 81-85

Tobler H (1986) The differentiation of germ and somatic cell lines in nematodes. In: Hennig W (ed) Germ line-soma differentiation. Results and problems in cell differentiation, vol 13. Springer, Berlin Heidelberg New York Tokyo, pp 1-69

Thudium D (1974) Das Verhalten von Soma- und Keimbahnchromosomen bei *Acricotopus lucidus* STAEGER (Orthocladiinae, Diptera). Doctoral thesis. University of Hohenheim.

White MJD (1973) Animal cytology and evolution, 3rd edn. Cambridge Univ Press, Cambridge, pp 500-546

Wolf KW, Bastmeyer M (1991) Cytology of Lepidoptera. V. The microtubule cytoskeleton in eupyrene spermatocytes of *Ephestia kuehniella* (Pyralidae), *Inachis io* (Nymphalidae), and *Orgyia antiqua* (Lymantriidae). Eur J Cell Biol 55: 225-237

Zissler D (1992) From egg to pole cells: Ultrastructural aspects of early cleavage and germ cell determination in insects. Micr Res Tech 22: 49-74