Chromosoma (2000) 109:343–349
DOI: 10.1007/s004120000084

Structural evolution of the germ line-limited chromosomes in *Acricotopus*

Wolfgang Staiber, Claudia Schiffkowski

Institute of Genetics,
University of Hohenheim,
D-70593 Stuttgart, Germany
Email: wstaiber@uni-hohenheim.de

Abstract

The elimination of chromatin or whole chromosomes from the future somatic nuclei during germ line-soma differentiation in early embryogenesis is a genetic phenomenon found in a wide variety of animal species. Less is known about the origin, structure, and function of the germ line-limited chromosomes. In the chironomid *Acricotopus lucidus* fluorescence in situ hybridization (FISH) with labeled soma DNA to "Keimbahn" chromosomes (Ks) and soma chromosomes (Ss) of spermatogonial mitoses revealed that each of the nine different K types possess large S-homologous sections, mostly in the distal parts of both chromosome arms. Painting probes of the three Ss and of each of their chromosome arms were generated by microdissection of polytene salivary gland chromosomes and subsequent degenerate-oligonucleotide-primed PCR (DOP-PCR). Multicolour FISH demonstrated that each of the Ks, with exception of one K type, was painted by only one of the three S probes. Furthermore, in seven Ks, one chromosome arm was painted by the long-arm probe and the other by the short-arm probe of the S concerned. The hybridization pattern strongly suggests that each of these K types is derived from a specific S. One function of the S-homologous K sections is thought to be determination of the regular occurrence of crossover events, with resulting chiasmata in these sections ensuring correct segregation of the K homologs during meiosis. Reverse chromosome painting on polytene S sets with a probe generated from metaphasic Ks corroborates the above results and produces conclusive evidence for the hypothesis that during evolution the Ks have developed from the Ss by endopolyploidization and rearrangements followed by the accumulation of germ line-specific repetitive DNA sequences in the centromeric regions.

Introduction

Germ line-soma differentiation is a fundamental process during early embryonic development. In various groups of animals - Protozoa, Nematoda, Crustacea, Insecta and Vertebrata - this differentiation is occasionally associated with the programmed elimination of chromatin or whole chromosomes from the future somatic nuclei (Tobler 1986; Kohno et al. 1986; Goto et al. 1998).

In the dipteran families of cecidomyiids, chironomids and sciarids the germ line-limited chromosomes (called E = eliminated, K = "Keimbahn" or L = limited chromosomes) pass through complex chromosome cycles together with the soma chromosomes (Ss) (Bauer and Beermann 1952; White 1973; Gerbi 1986; De Saint Phalle and Sullivan 1996). Experiments with the cecidomyiids *Wachtliella* and *Mayetiola* produced strong evidence that the Ks have essential functions during the differentiation of germ cells in both sexes (Geyer-Duszynska 1966; Bantock 1970).

Knowledge about the origin, structure and differentiation of the Ks is very limited (Hennig 1986). Painter (1966) has suggested, in referring to Nicklas (1959, 1960), that the germ line supernumeraries be interpreted as descendants of the S complement by way of "one or more endomitotic division cycles" or "one or more incomplete cell divisions". A characteristic feature of the germ line extra chromosomes established in the recent years in the hagfish species, *Eptatretus okinoseanus* and *Eptatretus chirratus* (Vertebrata, Cyclostomata), and in the chironomid *Acricotopus lucidus*, is the accumulation of germ line-specific repetitive DNA sequences (Kubota et al. 1993; Staiber et al. 1997; Goto et al. 1998). Furthermore, in *Acricotopus*, analysis in the polytene salivary gland of X-ray-induced K-S-rearrangements has demonstrated that the Ks contain segments of heterochromatin and sections with S-homologous banding patterns (Staiber and Thudium 1986, Staiber 1991b). The latter observation corroborates the suggestion that the Ks originate from the Ss.

In this study chromosome painting with probes generated by microdissection of Ss and Ks and by degenerate oligonucleotide-primed PCR (DOP-PCR) (Carter et al. 1992; Meltzer et al. 1992; Telenius et al. 1992) was employed to determine the questions of the descent of the Ks from the Ss and the pathway of differentiation of the K complement.

Materials and methods

Chromosome microdissection

Squash preparation of chromosomes of salivary glands and gonads from larvae of a laboratory stock of *Acricotopus lucidus* (Diptera, Chironomidae) were made in 45% acetic acid, frozen on dry ice, dehydrated in an ethanol series and air-dried (Staiber 1988; Staiber et al. 1997). Microdissection of selected chromosomes was performed with an inverted microscope (Zeiss) using phase contrast with a mechanical micromanipulator (Leitz), and with glass microneedles produced by a horizontal pipette puller (Bachofer) and a De Fonbrune microforge (Beaudouin, Paris). Dissected chromosomes were transferred to a 0.5 ml microcentrifuge tube in a 5 µl drop

of 10 mM Tris-HCl buffer, pH 8.5. After sampling two to five polytene or ten metaphase chromosomes 5 µl proteinase K (2 mg/ml, Boehringer, Mannheim) were added. Following digestion for about 48 h at room temperature proteinase K was inactivated by heating the probe to 94°C for 30 min.

Amplification and labeling of probes

Premixed reagents for DOP-PCR containing 1.25 U Taq were added to the tube to a final volume of 50 µl (DOP-PCR Master Kit, Boehringer, Mannheim). For amplification in a Progene cycler (Techne) a slightly modified version of the manufacturer's protocol was used: After an initial denaturation step at 95°C for 5 min, eight cycles were performed at 94°C for 1 min, at 30°C for 1 min 30 s with a 4 min transition from 30°C to 72°C, and at 72°C for 3 min, then followed by 35-38 cycles with denaturation at 94°C for 1 min, annealing at 62°C for 1 min and synthesis at 72°C for 3 min adding 5 s at each cycle. Final extension was carried out at 72°C for 7 min. In a second 50 µl labeling PCR a 2,5-5 µl aliquot of the PCR-generated probe pool was amplified for 38-40 cycles under the above conditions, except that 40 µM digoxigenin-11-dUTP or 66 µM fluorescein-12-dUTP (Boehringer, Mannheim) was added. A nick translation mix was used for labeling painting probes and the plasmid Ct111 with biotin-16-dUTP (Boehringer, Mannheim). Ct111 contains a repeat unit of 18S and 28S rDNA from *Chironomus tentans* (Degelmann et al. 1979). Soma DNA of *A. lucidus* obtained from dissected thoraces of larvae and prepupae using a QIAamp tissue kit (Qiagen) was labeled by a digoxigenin random-primed DNA labeling reaction (Boehringer, Mannheim).

Fluorescence in situ hybridization

Labeled PCR or nick translation products were cleaned by using spin columns (Qiagen) or by ethanol precipitation. The hybridization mixture consisted of 5x SSC (pH 7.0) with 0.1% SDS or of 50% deionized formamide, 10% dextrane sulfate, 2x SSC, 1x Denhardt solution and 0.1% SDS in 40 mM phosphate buffer pH 7.0. Chromosome preparations, treatment of slides and denaturation was done as described in Staiber et al. (1997). Hybridization was performed in a humidified chamber overnight at 58°C or 37°C.

For post-hybridization washing, slides were placed for 5 min once each in 2x SSC at room temperature and twice in 2x SSC at 37°C. Probe detection and signal amplification were performed for digoxigenin-labeled probes by applying alterating layers of anti-digoxigenin mouse IgG1, anti-mouse-Ig-digoxigenin F(ab')2 fragments and finally anti-digoxigenin-fluorescein Fab fragments according to the manufacturer's instructions (Boehringer, Mannheim). Fluorescein-labeled probes were detected with a 1:200 dilution of a rabbit anti-FITC IgG (Dako) and with 1:50 dilution of a goat anti-rabbit IgG conjugated with fluorescein (Vector, Laboratories). Biotin-labeled probes were alternatively visualized by layers of Cy5 or FITC conjugated streptavidin, dilution 1:10 (Dako, Boehringer Mannheim), biotinylated goat anti-streptavidin antibody, dilution 1:50 (Vector Laboratories), and finally Cy5 or FITC conjugated

streptavidin. Preparations were then mounted in Vectashield fluorescence antifade solution (Vector Laboratories) containing 2,5 µg/ml propidium iodide and were examined under a fluorescence Axiophot microscope (Zeiss). Digital images were captured with a confocal laser scanning microscope (MRC 1024, Biorad) and processed with a Corel Draw software package.

Results

Identification of S-homologous sections in the Ks

Fluorescence in situ hybridization with whole soma DNA as a probe onto spermatogonial metaphases allows detection of those sections in the Ks containing S-homologous sequences. Figure 1A presents the hybridization patterns of Ks and Ss in a normal gonial mitosis, and Fig. 1C in a differential mitosis, that is the last gonial mitosis prior to meiosis, where all Ks move undivided, with unseparated chromatids, to only one pole, while the paired Ss stay behind in the equatorial plate. The digital inversion of the propidium iodide fluorescence patterns (Fig. 1B), corresponding to C-banding patterns of Ss and Ks, facilitates the identification of the chromosomes. As expected, the Ss are completely painted, with most intensive fluorescent signals in the centromeric regions. All Ks, except K9, exhibit a characteristic hybridization pattern with painted sections in distal parts in both chromosome arms. The centromeric regions of the Ks do not show any hybridization. The hybridization-free sections include the para-centromeric heterochromatin bands characteristic for all Ks apart from K9 (Staiber 1991a), and the terminal C-banding positive bands in K1 and K2. In the long arm of K4 an additional large bulb-like section adjacent to paracentromeric bands remained unpainted. The special K9 is completely painted like the Ss.

Vizualization of relations between the different S and K types by using chromosome-specific S painting probes

For the characterization of specific homologies between the different S and K types, painting probes for each of the three Ss were generated by microdissection of polytene salivary gland Ss and DOP-PCR. The salivary gland chromosomes of *A. lucidus* can be easily distinguished from each other by specific cytogenetic landmarks as centromeric regions and different Balbiani rings (Mechelke 1953; Staiber and Behnke 1985). The chromosome specifity of each of the paints was checked by hybridization onto polytene S sets prior to their application to gonial mitoses.

Initially, painting probes were produced from entire polytene Ss, including the large heterochromatic centromere blocks that contain large amounts of repetitive DNA sequences, especially of the highly repeated AlSo sequence family (Staiber et al. 1997). Hybridization such probes to spermatogonial metaphases resulted in strong hybridization signals at the centromeric regions of all Ss. Hence, in Fig. 1D, not only do the centromeres of the painted SIIs fluoresce very brightly, but also those of the SIs and SIIIs, and that of K9. This made it difficult to capture clear images of the weaker fluorescence in the arms of the painted S and in the S-homologous

sections of the Ks. Therefore, the heterochromatic blocks were removed prior to microdissection in order to reduce the amount of repetitive sequences in the painting probes.

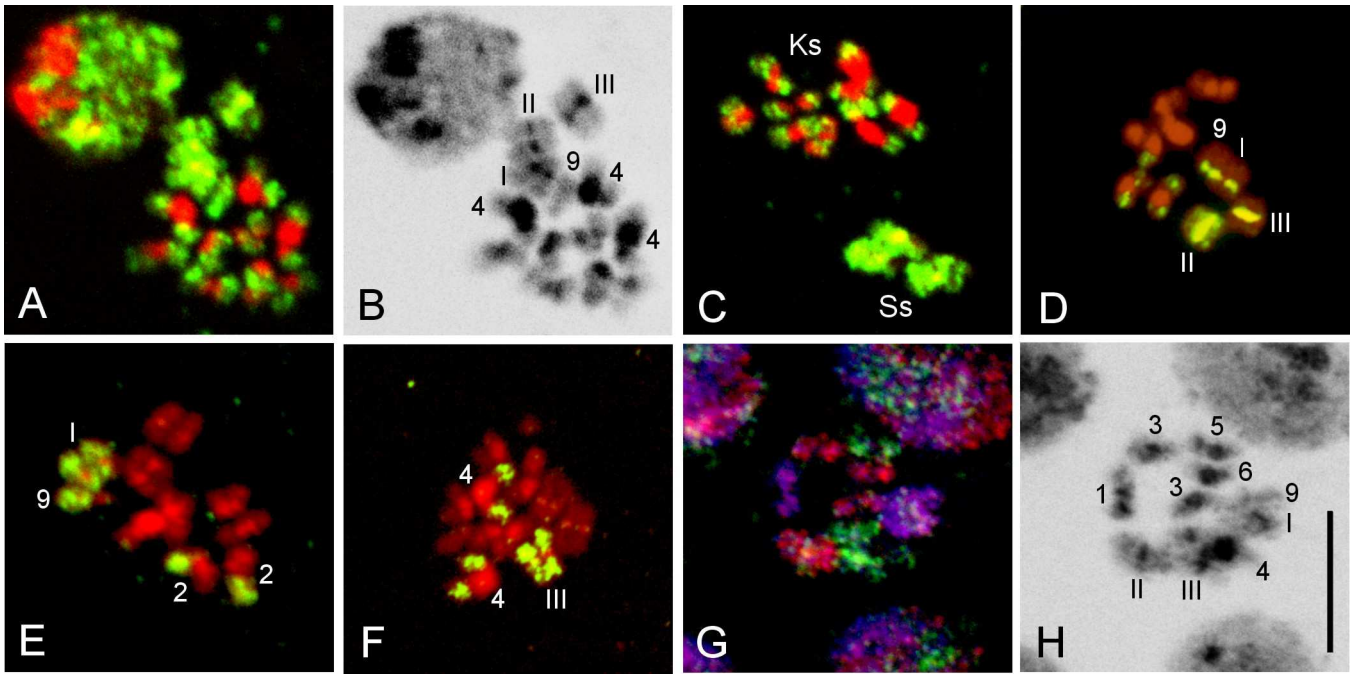

Fig. 1. Identification of S-homologous sections in the Ks of *A. lucidus*. **A** FISH with labeled soma DNA as probe (*green*) to a spermatogonial metaphase and an interphase nucleus. Chromosomes are counterstained with propidium iodide (*red*). **B** Reverse presentation of the propidium iodide fluorescence. **C** Hybridization of soma DNA to a differential gonial mitosis. **D** FISH with a SII painting probe generated from polytene SIIs containing the centromeric heterochromatin. Strong signals also at the centromeric regions of the SIs, SIIIs and K9. **E** Chromosome painting with a SI probe and **F** a SIII probe without centromeric repetitive DNA sequences. **G** Three-color chromosome painting with probes of SI (*purple*), SII (*red*) and SIII (*green*). **H** Digital inversion of the propidium iodide fluorescence. *Bar* represents 10 µm. *I - III*, soma chromosomes SI - SIII. *1 - 9*, germ line limited chromosomes K1 - K9.

Fluorescence in situ hybridization with PCR products generated from SI onto a spermatogonial metaphase is shown in Fig. 1E. The paired SIs and the spatial associated K9 are completely painted, except for the centromeric regions, while in both homologs of K2 only the long arms are painted. Using an SIII painting probe, as can be seen in the metaphase in Fig. 1F, the K4s fluoresce brightly in the distal parts of both arms.

The use of differently labeled probes of the three Ss combined for multicolor FISH provides the potential to determine the homologies between Ss and Ks in one hybridization experiment. In Fig. 1G the biotinylated SI probe was visualized by Cy5 (blue) and the digoxigenated SII probe by rhodamine (red); the fluorescein-labeled SIII probe fluoresces green. For orientation Fig. 1H shows the digital inversion of the propidium iodide fluorescence of the chromosomes. Each of the seven Ks present in this metaphase was painted only by one of the S probes. FISH-positive sites were assigned to more distally located euchromatic (=C-band negative) sections in both arms of each K, except for K9, where both arms are completely painted.

Identification of chromosomal rearrangements within the Ks by using chromosome arm-specific S painting probes and by reverse painting of K probes on polytene Ss

Use of the painting approach to determine the homologies between Ss and Ks was extended to FISH with combinations of S chromosome arm probes. Results of dual-color FISH with arm-specific painting probes to gonial metaphases are shown for SI in Fig. 2A, for SII in Fig. 2B, and for SIII in Fig. 2C, and are summarized in Table 1. The short arm (p) probes of the Ss were visualized by Cy5 (blue), and long arm (q) probes by fluorescein (green). Chromosomes were counterstained with propidium iodide (red).

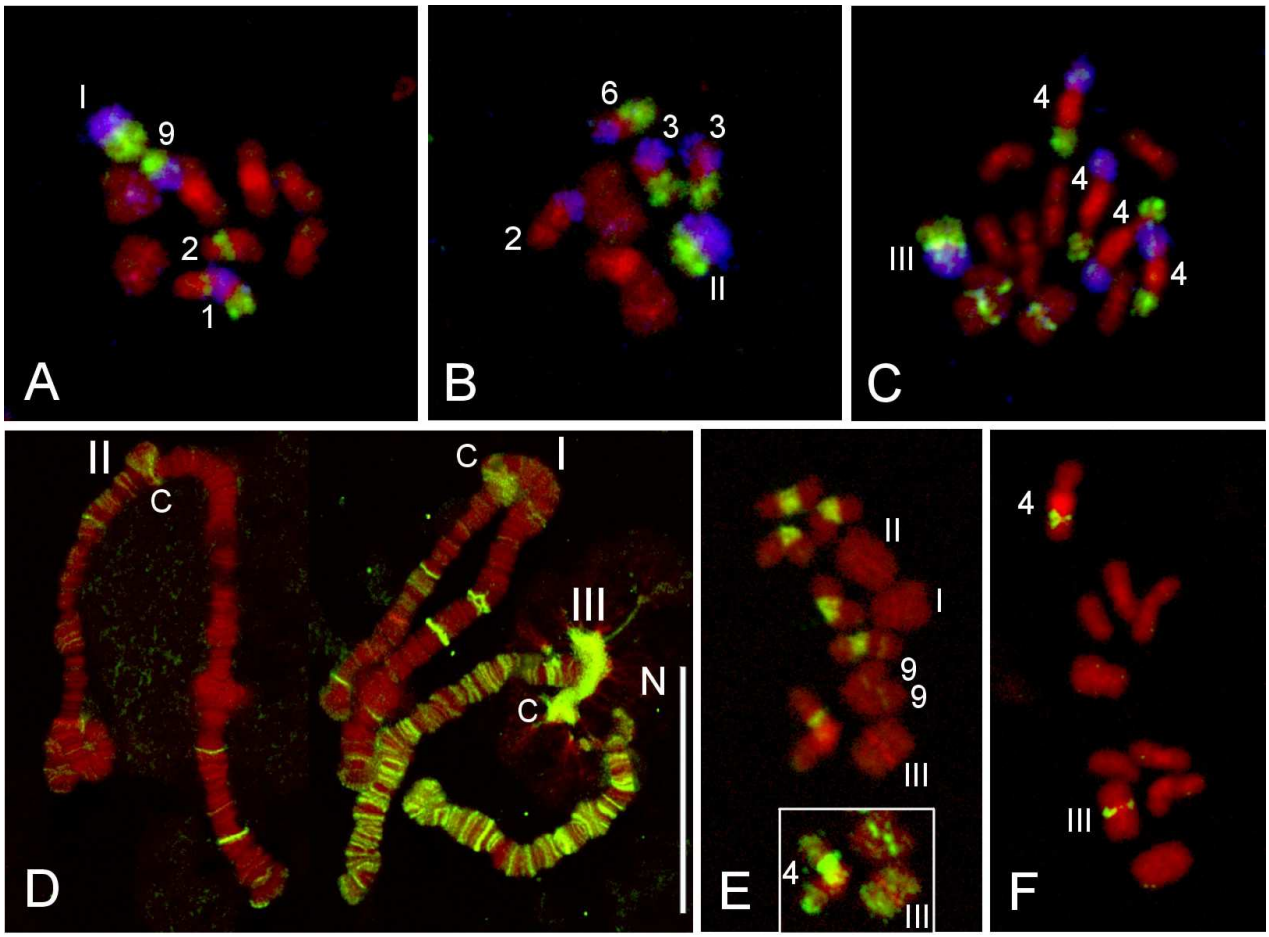

Fig. 2. Structural analysis of the Ks. Two-color FISH with combinations of probes from long (*green*) and short (*blue*) chromosome arms of (**A**) SI, (**B**) SII and (**C**) SIII. **D** Reverse chromosome painting with a probe generated from metaphasic K4s onto a polytene salivary gland S set. Distinct painting of both arms of SIII. *C*, heterochromatic centromeric regions. *N*, nucleolus. Bar represents 50 µm. **E** Control-hybridization of the K4 probe to a spermatogonial metaphase. Very strong signals on the paracentromeric heterochromatin of all Ks except K9 resulting from germ line specific repetitive DNA sequences in the probe. The insert represents a section of the metaphase with digital increased signal amplification showing clear hybridization in both arms of SIII, in the central and distal parts of K4, and on an intercalary band in K9. **F** Localization of a rDNA site in K4 with clone Ct111 containing a rDNA repeat from *Chironomus tentans*.

With the exception of K1 (Fig. 2A) and K2 (Figs. 2A, 2B), in all other Ks the one chromosome arm is painted by the short-arm probe the other arm by the long-arm probe. In the long arm of K1 (Fig. 2A) there is, in addition to a section painted by the SI short-arm probe, a small section between two of the three terminal heterochromatin bands detected by the long arm probe of SI.

In Fig. 2A, a section in the long arm of K2 is painted by the long-arm probe of SI, while in Fig. 2B the short arm of K2 is painted by the short-arm probe of SII. In the metaphase in Fig. 2C, the short arms of the four K4s are painted by the short arm probe of SIII, and more distal parts of their long arms by the long-arm probe of SIII. Fluorescein signals on the centromeric regions of SI, SII and K9 resulted from sequences of the centromeric, highly repetitive AlSo family (Staiber et al. 1997) present to some extent in the SIII long arm probe.

To complement the characterization strategy of the S-homologous K sections with specific paints from Ss or S arms, the reverse path was taken: the generation of probes from metaphase Ks also by using microdissection, DOP-PCR and FISH onto Ss. In clinical genetics this micro-FISH approach, applied to characterize cytogenetically unclassifiable marker chromosomes, has been termed reverse chromosome painting, in which one uses normal chromosomes as probes onto metaphases containing the chromosomes of interest (Carter et al. 1992; Carter 1994; Xu et al. 1995). In *A. lucidus* advantage can be taken of the high cytogenetic resolution of the polytene salivary gland chromosomes for hybridization of the K probe. K4 was chosen for generating a probe from a distinct metaphasic K because K4 can be easily identified in metaphases without banding by its characteristic morphology. Ten metaphasic K4s were dissected for the probe used for the reverse painting onto the polytene S set shown in Fig. 2D. In both arms of SIII and its centromeric region, distinct strong hybridization was visualized compared with SI and SII. In SI and SII the K4 probe only hybridized on a few bands and only slightly in the centromeric regions.

To check whether the K4 PCR probe really originates from K4, the probe was hybridized to Ks and Ss of spermatogonial metaphases (Fig. 4E). Very strong signals on the paracentromeric bands of all Ks, with the exception of K9, indicate that the probe contains sequences of the germ line specific highly repetitive AlKe DNA family located in those bands (Staiber et. al. 1997). In addition, clear hybridization was ascertained in the central part of K4, in some intercalary bands of other Ks, among them K9, and in the terminal S-homologous sections of K4 as well as in both arms of the SIIIs, as can be seen in the insert of Fig. 2E, with increased digital signal amplification. The latter corresponds to the painting of both arms of the polytene SIII by the K4 probe in Fig. 2D.

Localization of rDNA sites in the Ks

In the polytene salivary gland S set of *A. lucidus* the only NOR has been localized in the short arm of SIII adjacent to the heterochromatic centromeric block (Mechelke 1953; Staiber and Behnke 1985), forming one large nucleolus during larval and prepupal stages (Fig. 2D).

In order to identify and localize rDNA sites in the K complement of A. *lucidus*, labeled Ct111 plasmid containing a repeat unit of 18S and 28S rDNA from *Chironomus tentans* (Degelmann et al. 1979) was hybridized to spermatogonial chromosome spreads. One rDNA site was detected in the Ks and mapped to the short arm of K4 adjacent to the paracentromeric heterochromatin (Fig. 2F). This corresponds to the observation that the short arm of K4 was painted by the short-arm

probe of SIII, and, conversely, K4 contains sections of the short arm of SIII as determined by reverse painting.

Table 1 Summary of fluorescence in situ hybridization analysis of the germ line-limited chromosomes of *Acricotopus lucidus*. (K1–K9, germline-limited (Keimbahn) chromosomes; SI–SIII, soma chromosomes; p, q, short and long chromosome arm. For nomenclature of Ks see Staiber 1988)

Painting probe	Hybridization																	
	K1		K2		K3		K4		K5		K6		K7		K8		K9	
	p	q	p	q	p	q	p	q	p	q	p	q	p	q	p	q	p	q
SI p		+													+		+	
q	+	+[a]		+												+		+
SII p			+		+						+		+					
q						+						+		+				
SIII p							+		+									
q								+		+								

[a] One small section in K1q (see K1 in Fig. 2A)

Discussion

In this study a chromosome painting strategy was used to answer the question about the origin of the germ line-limited chromosomes. Some authors see the Ks as descendants of the S complement by way of endopolyploidy (Nicklas 1960; Painter 1966; Kunz et al. 1970). First evidence for this hypothesis came from the analysis of X-ray induced K-S-rearrangements in polytene salivary gland chromosomes of *A. lucidus*, demonstrating that the Ks contain sections with S-homologous banding patterns (Staiber and Thudium 1986; Staiber 1991b).

Fluorescence in situ hybridization with soma DNA as probe demonstrated that all Ks in *A. lucidus* contain large sections with S-homologous DNA sequences exhibiting a specific distribution preferentially in the distals parts of both chromosome arms. More details as to the relations between Ks and Ss came from forward and reverse chromosome painting, methods based on chromosome microdissection, DOP-PCR and FISH have become a powerful tool in clinical genetics to ascertain the origin of cytologically unclassifiable marker chromosomes or to characterize complex chromosome rearrangements (Blennow et al. 1992; Carter et al. 1992; Lucas and Sachs 1993; Carter 1994, Müller-Navia et al. 1996). Eight of the nine K types contain S-homologous sections always detected by one S probe, and, furthermore in seven Ks the one chromosome arm was painted by the short-arm probe, the other arm by the long-arm probe of the concerning S. This strongly indicates that each of these K types has developed from one of the Ss. Only one K type, K2, has chromosome arms painted by arm probes of two different Ss, of SI and SII, probably resulting from a whole arm translocation. In contrast to the other Ks, the special K9 was completely painted by the SI probe. This result supports the idea that K9 is an evolutionarily younger K with great similarity to the Ss, but which has still not accumulated germ line-specific repetitive sequences in its centromeric region (Staiber et al. 1997).

In *A. lucidus* the K complements in gonial mitoses can strongly vary in the number of Ks (6 to 16 Ks) and in the compositions of the nine K types in larvae of different egg depositions (Staiber

1988). Each K type can be absent (i.e. K4 in Fig. 1D) or can be present one or two times (K4 in Figs. 1H and 1F), and especially K4 even three (Fig. 1B), four (Fig. 2C) or five times. Therefore, the painting results demonstrate that the portion of the S-homologous sections derived from a specific S varies strongly in the K complements of *A. lucidus.*

The results of reverse painting with the K4 probe onto polytene S sets agree with those from forward painting with SIII probes onto K4. In particular, they show that the S-homologous sections in K4 contain DNA sequences derived from both arms of SIII, as can be seen in the distinct hybridization pattern in both SIII arms in Fig. 2D. To our knowledge, this was the first application using polytene chromosomes as target in reverse painting for probes generated from metaphasic chromosomes. The signals on a few bands in the polytene SI and SII in Fig. 2D are most probably ascribable to genomic dispersed repetitive DNA sequences.

The only 18S-28S rDNA site of the Ks was mapped to the short arm of K4 adjacent to the centromeric region. This corresponds to the specific painting of the short arm of K4 by the probe from the short arm of SIII as this S arm contains the only 18S-28S rDNA locus of the S set.

In an earlier study of male meiosis in *A.lucidus*, chiasmata were observed in all K bivalents and almost exclusively in the S-homologous sections of the Ks (Staiber 1989). Therefore, one function of the S-homologous sections in the Ks may be to enable crossing over in meiosis with resulting chiasmata holding together the homologous Ks until anaphase I, thus ensuring the normal segregation of the Ks. But it seems to be a contradiction to eliminate on the one hand about one half of the Ks during first gonial mitoses (=germ line elimination), to compensate for this elimination in the last gonial mitosis (=differential mitosis) by a monopolar movement of the Ks (Bauer and Beermann 1952), then on the other hand to use mechanisms that ensure correct meiotic segregation. In addition to regular K bivalents and a few multivalents of homologous Ks, multivalents of nonhomologous Ks have also been detected in rare cases in spermatocyte metaphases I (Staiber 1989). With the painting results presented here, many of these unusual multivalents can be now explained by crossing over between homologous S sections in morphological different K types.

In summary, the forward and reverse chromosome painting analysis produces conclusive evidence for the hypothesis that the Ks have developed during evolution from the Ss by endopolyploidization and by rearrangements followed by the accumulation of germ line-specific repetitive DNA sequences in the centromeric regions.

Acknowledgements

This paper is dedicated to Prof. Friedrich Mechelke on the occasion of his 80th birthday. The authors are very grateful to Prof. Anette Preiss for her support, to Bernd Johannes and Anja C. Nagel for help in laser scanning microscopy and computer programs, to Dr. Irmgard Wech for helpful discussions and to Prof. Erwin R. Schmidt and Dr. Christiane Kraemer, University of Mainz, for kindly providing Ct111. This study was supported by a grant of the Deutsche Forschungsgemeinschaft (Sta 462/3-1).

References

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

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

Blennow E, Telenius H, Larsson C, de-Vos D, Bajalica S, Ponder BA, Nordenskjold M (1992) Complete characterization of a large marker chromosome by reverse and forward chromosome painting. Hum Genet 90:371–374

Carter NP (1994) Cytogenetic analysis by chromosome painting. Cytometry 18:2–10

Carter NP, Ferguson-Smith MA, Perryman MT, Telenius H, Pelmear AH, Leversha MA, Glancy MT, Wood SL, Cook K, Dyson HM, Ferguson-Smith ME, Willat LR (1992) Reverse chromosome painting: a method for the rapid analysis of aberrant chromosomes in clinical cytogenetics. J Med Genet 29:299–307

Degelmann A, Royer H-D, Hollenberg CP (1979) The organization of the ribosomal RNA Genes of *Chironomus tentans* and some closely related species. Chromosoma 71:263–281

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

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

Geyer-Duszyńska 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

Goto Y, Kubota S, Kohno S (1998) Highly repetitive DNA sequences that are restricted to the germ line in the hagfish *Eptatretus cirrhatus*: a mosaic of eliminated elements. Chromosoma 107:17–32

Hennig W (1986) Heterochromatin and germ line-restricted DNA. In: Hennig W (ed) Germ line-soma differentiation. Results and problems in cell differentiation 13. Springer Berlin Heidelberg New York Tokyo, pp 175–192

Kohno S, Nakai Y, Satoh S, Yoshida M, Kobayashi H (1986) Chromosome elimination in Japanese hagfish, *Eptatretus burgeri* (Agnatha, Cyclostomata). Cytogenet Cell Genet 41:209–214

Kubota S, Kuro-o M, Mizuno S, Kohno S (1993) Germ line-restricted, highly repeated DNA sequences and their chromosomal localization in a Japanese hagfish (*Eptatretus okinoseanus*). Chromosoma 102:163–173

Kunz W, Trepte H-H, Bier K (1970) On the function of the germ line chromosomes of *Wachtliella persicariae* (Cecidomyiidae). Chromosoma 30:180–192

Lucas JN, Sachs RK (1993) Using three-color chromosome painting to test chromosome aberration models. Proc Natl Acad Sci U S A 90:1484–1487

Mechelke F (1953) Reversible Strukturmodifikationen der Speicheldrüsenchromosomen von *Acricotopus lucidus*. Chromosoma 5:511–543

Meltzer PS, Guan X-Y, Burgess A, Trent JM (1992) Rapid generation of region specific probes by chromosome microdissection and their application. Nat Genet 1:24–28

Müller-Navia J, Nebel A, Oehler D, Theile U, Zabel B, Schleiermacher E (1996) Micro-dissection and DOP-PCR-based reverse chromosome painting as a fast and reliable strategy in the analysis of various structural chromosome abnormalities. Prenat Diagn 16:915–922

Nicklas RB (1959) An experimental and descriptive study of chromosome elimination in *Miastor* spec. (Cecidomyidae; Diptera). Chromosoma 10:301–336

Nicklas RB (1960) The chromosome cycle of a primitive cecidomyiid – *Mycophila speyeri*. Chromosoma 11:402–418

Painter TS (1966) The role of the E-chromosomes in Cecidomyiidae. Proc Natl Acad Sci U S A 56:853–855

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

Staiber W (1989) Multivalent formation and pairing behavior of germ line limited chromosomes in male meiosis of *Acricotopus lucidus* (Diptera, Chironomidae). Genome 32:941–945

Staiber W (1991a) Characterization of heterochromatin of germ line limited and soma chromo-somes in *Acricotopus lucidus* (Diptera, Chironomidae) by differential banding methods. Hereditas 114:91–96

Staiber W (1991b) Structural homologies between germ line limited and soma chromosomes in *Acricotopus lucidus* (Diptera, Chironomidae). J Hered 82:247–249

Staiber W, Behnke E (1985) Developmental puffing activity in the salivary gland and Malpighian tubule chromosomes of *Acricotopus lucidus* (Diptera, Chironomidae). Chromosoma 93:1–16

Staiber W, Thudium D (1986) X-ray induced rearrangements between germ-line limited and soma chromosomes of *Acricotopus lucidus* (Diptera, Chironomidae). Genetica 69:149–156

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

Telenius H, Carter NP, Bepp CE, Nordenskjöld M, Ponder BA, Tunnacliffe A (1992) Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics 13:718–725

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

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

Xu J, Cedrone E, Roberts M, Wu G, Gershagen S, Wang N (1995) The characterization of chromosomal rearrangements by a combined micro-FISH approach in a patient with myelodysplastic syndrome. Cancer Genet Cytogenet 83:105–110