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Spermatogenesis in *Drosophila*

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ABSTRACT A short summary on the present knowledge on spermatogenesis in *Drosophila* is given which also points out particular questions of interest in the context of this morphogenetic process. Such points of interest are the formation of lampbrush loops in primary spermatocytes, the chromosomal events during meiosis, the occurrence of chromatin rearrangements and the regulation of gene activities at the posttranscriptional level. The activities and some major conclusions from my laboratory are subsequently described. They include studies of the expression of histone variants, the structure and function of lampbrush loops and the expression of genes participating in sperm morphogenesis.

KEY WORDS: *Drosophila, spermatogenesis, germ line, Y chromosome, histone*

What is special about male germ cell development?

In higher organisms the development of germ cells is one the prerequisites to assure the continuity of the species throughout successive generations. Even though it is accepted that the genetic material of different cells in general is identical or similar – except for some specialized cell types like those of the mammalian immune system – germ cells of some organisms show a constitution of their genetic material different from that of their somatic cells. The best known case has been discovered by Boveri as early as 1896 when he observed that in *Ascaris* parts of the chromosomes are lost (eliminated) during the early embryonic development from all somatic cells. Comparable situations of the genetic constitution of germ cells have been described subsequently in different groups of lower and higher eukaryotes (Hennig, 1986). The biological significance of chromatin elimination, however, is unclear. Such cases of elimination of parts of the genome from somatic cells are exceptions and it is believed that in most organisms the germ cell genome and that of the other cells of an organism is identical. A special situation might exist for telomeres which may gradually shorten in somatic cells but must be maintained or are extended in germ cells to assure the inheritance of complete chromosomes. The shortening of telomeres is a consequence of the molecular mechanism of replication which proceeds exclusively from free 3'-OH groups (review Biessmann and Mason, 1994).

Some remarkable principal differences between the genome of germ cells and of somatic cells have more recently been found at the level of gene regulation. For example, in *Drosophila* the regulation of sex determination of germs cells occurs relatively independent of that of the somatic cells (see, for example, Steinmann-Zwicky, 1993). For mammalian germ cell differentiation is has become clear that the paternal and the maternal genomes during the germ cell development receive different patterns of imprinting which results in a specific expression pattern of parental genes in the embryo. That is, genes are differently regulated in the embryo dependent on whether they are derived from the paternal or from the maternal germ cells (see, for example, Surani *et al.*, 1993). So far it not clear whether such imprinting occurs specifically in mammals or whether it is a general phenomenon. Some arguments in favour of the latter may be derived from observations in *Drosophila* also suggesting paternal imprinting of some genes (Spofford, 1976; G. Reuter, personal communication). Just as for chromatin elimination, also for parental imprinting the biological relevance is not understood.

A more general phenomenon related to the differentiation specifically of male germ cells is the chromatin reorganization which usually is initiated during the meiotic prophase I (primary spermatocyte stage) and results, in a complex series of substitution steps, in the replacement of somatic, cell-cycle-regulated histones by more basic chromosomal proteins, usually of a protamine type (Hecht, 1987). Again, also for this process the biological relevance is unclear. Arguments have been made that the chromatin reorganization, which leads to a tighter packaging of the chromatin, is required to accommodate the genome in the small sperm head or to protect the sperm genome from mutations which might especially occur in organisms without internal fertilization; in such cases the environment might expose the sperm genome to increased levels of mutagens as compared with an internal fertilization. However, both arguments are not necessarily convincing. It is by no means obvious why the penetration into an egg of a sperm may should require a particularly compact genome. In some organisms sperm heads are large (Jamieson, 1987). It is rather unlikely that an additional condensation of chromatin, which may result in a 30% compaction in a mature sperm as compared to a normal mitotic metaphase chromosome condensation (Grond, 1984; Hennig and Kremer, 1991),

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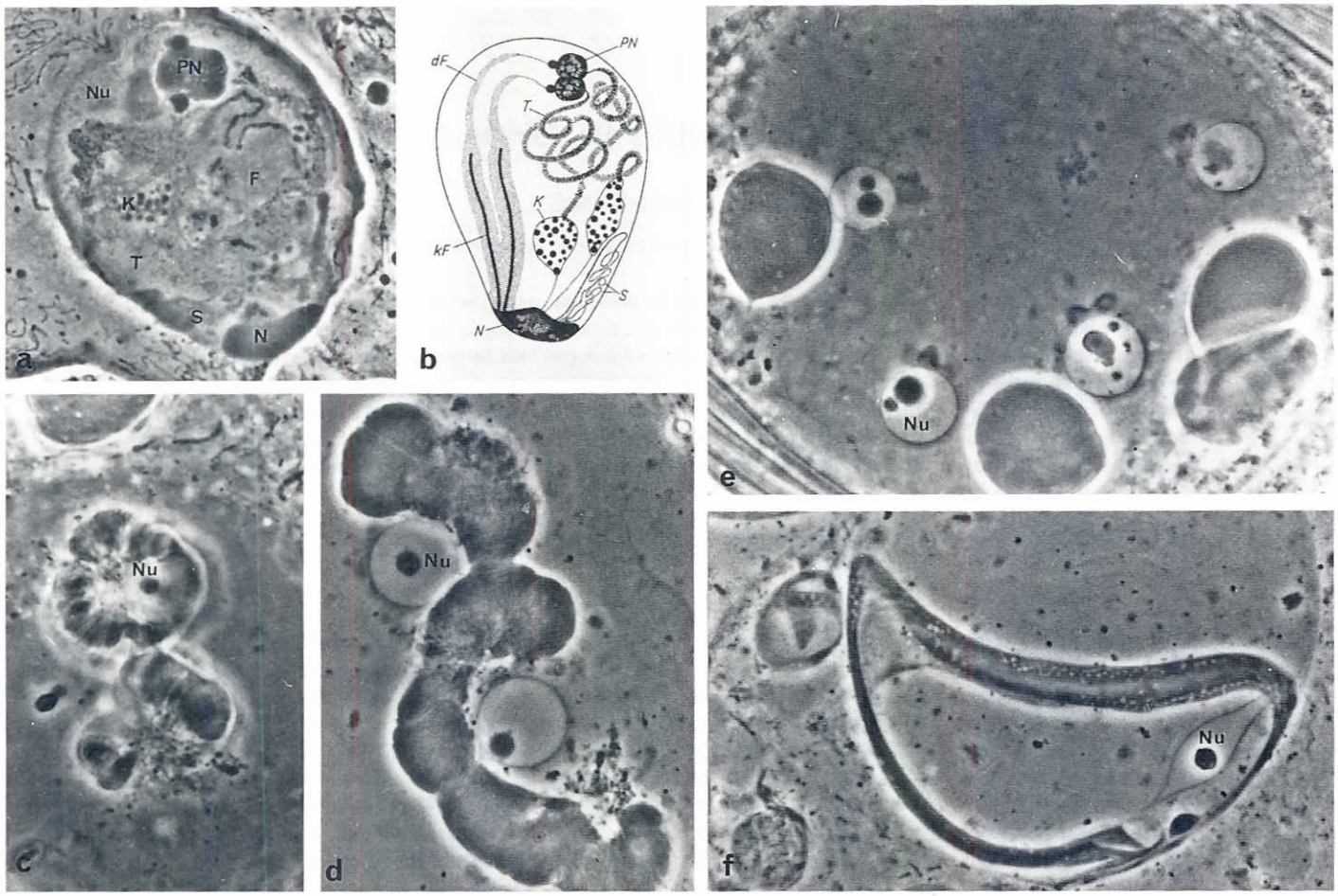

Fig. 1. Characteristic stages of the male germ cell differentiation in *Drosophila hydei*. **(a)** *Primary spermatocyte nucleus with Y chromosomal lampbrush loops, left cytology;* **(b)** *schematic identification of the different components of the spermatocyte nucleus;* **(c)** *early postmeiotic cells with mitochondrial aggregates around the nuclei;* **(d)** *fusing mitochondria of young spermatids;* **(e)** *"onion" Neberkern stage spermatids. The mitochondria are fused.* **(f)** *Nebenkern and nucleus are in the process of elongation.*

will be of major relevance. The argument of protection against mutations it at least partially invalidated by the fact that the mutation rates in *Drosophila* sperm are not particularly low. Since chromatin reorganization is a general property of male germ cell development, it very likely has other biological reasons.

The gene activities in germ cells serve the main purpose to supply the information necessary for the development of the germ cell. Oocytes, or cells related to oocytes in their developmental origin (such as nurse cells in *Drosophila*), may, in addition, supply genomic information ("maternal" information) for the early development of the embryo where no or little transcription takes place. In the male germ line the central event is the differentiation of the spermatozoon. Parental information for embryonic development is not produced as no relevant amounts of paternal cytoplasm are introduced into the egg which could transfer mRNA or proteins into the egg at fertilization. In the male germ cell development specific gene activities for the production of the sperm constituents and for the regulation of the morphogenetic process are required. In contrast to differentiation processes in somatic cell lines, where cellular differentiation is accompanied by an irreversible programming of the cell, the developmental program of the male germ cell is abolished after the genetic information has become available to the cell and the genome is cleared from all its regulatory signals for this specific differentiation pathway to facilitate the development of a new organism. The specific regulatory signals may be substituted for by some limited amount of regulatory signals of those genes which show paternal imprinting effects in the embryo (see Hennig. 1988).

A wealth of information is available on morphology and ultrastructure of male germ cells of higher organisms. Despite of all the diversity, it is intriguing that the principal organization of spermatozoa is very similar throughout the animal kingdom. Usually three main components are found in a spermatozoon: the nucleus with the genetic information constituting the head, the flagellum, which is in charge of the movement of the germ cell, and mitochondria or their derivatives. The function of the mitochondria is not clear in all details. They are suspected to be involved in the energy metabolisms required for the flagellar movement. However, in particular in insects, mitochondria fuse (Fig. 1) and are transformed into large mitochondrial derivatives (Nebenkern) which neither show of the characteristic components of mito-

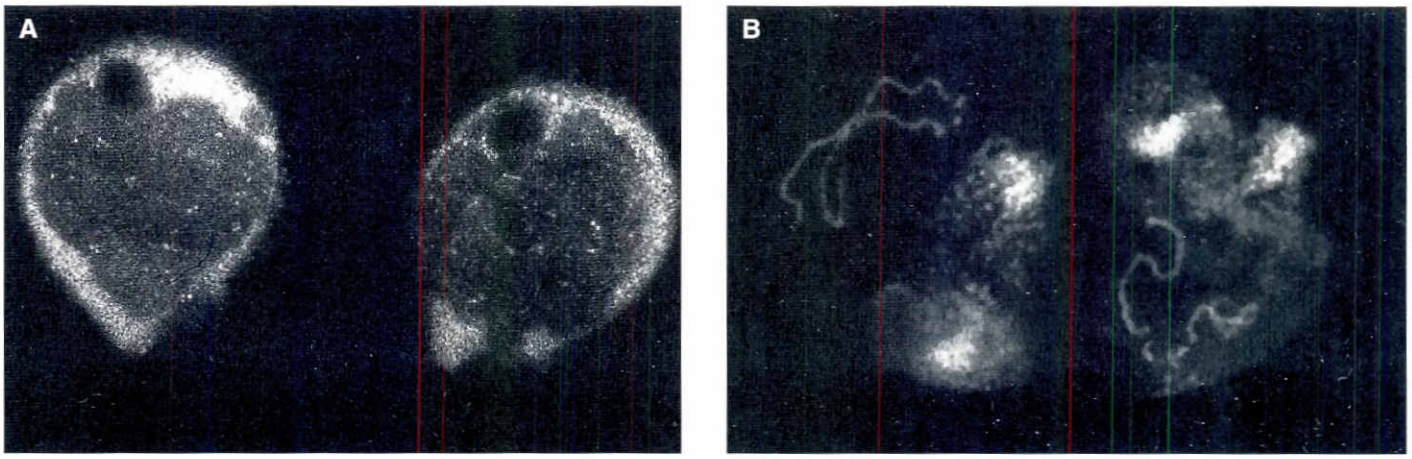

Fig. 2. Primary spermatocytes after immunoreaction with anti-histone H1 antiserum and subsequent incubation with FITC labeled secondary antiserum (A). *The level of reaction is low and is restricted to the periphery of the nucleus where the autosomes are located. Also the nucleolus does not react with the antiserum. In particular the lampbrush loops remain unlabeled (cf. Fig. 7) which becomes particularly evident by a comparison with the reaction of another antiserum which specifically binds to antigens in two of the loop pairs* **(B)**.

chondria nor display signs of an energy metabolism. In these cases, the mitochondrial derivatives may be stabilizing elements of the sperm tail. Also in organisms showing no comparable transition of the mitochondria into large mitochondrial derivatives, for example in mammals, the mitochondria are partially fused. Their location in the "neck" region of the spermatozoon, i.e. between head (nucleus) and tail (flagellum), and their structure are more compatible with the requirements posed by a participation in the energy metabolism essential for the movement of the flagellum.

In sharp contrast to the overwhelming amount of descriptive data on sperm morphology, our knowledge on the molecular aspects of sperm morphogenesis and its regulation is very restricted. One reason for this relates to the difficulties to use genetic methods to get insight into the successive steps in the regulatory events guiding sperm morphogenesis. As pointed out before, the genomic activity during spermatogenesis is found mainly in cells up to the first meiotic division while the actual morphogenetic processes occur postmeiotically. Therefore, no genetic dissection of sperm morphogenesis on the basis of phenotypes is possible (see Hennig and Kremer, 1991) as it has been so successfully applied to early embryonic development in *Drosophila* (Johnston and Nüsslein, 1992). Consequently, the extensive approaches to obtain – with the aid of studies of male sterile mutants - insight into the successive genetic and molecular steps required in *Drosophila* sperm development has been essentially unsuccessful (Lindsley and Tokuyasu, 1980).

From the genetic point of view it has been an interesting observation that in *Drosophila* the number of genes giving male sterile phenotypes if mutated, is unexpectedly large (Lindsley and Tokuyasu, 1980). Does that imply that a large proportion of all genes of the genome is involved in spermatogenesis? We cannot answer this question yet. However, it is plausible to assume that pleiotropic effects of many genes may affect sperm development. Defective spermiogenesis could then be considered as a sensible selection mechanism in evolution to prevent defective alleles to be passed on to the next generation.

Another remarkable observation is that the number of genes known to be specifically and exclusively active during male germ cell development is very small. Many of the genes required for sperm development appear to be required also in other cellular differentiation pathways. This creates additional problems for a genetic dissection of spermatogenesis as many important genes may induce lethality if mutated and therefore their function in germ cell development would be difficult if not impossible to be recognized by genetic studies. An example is the muscular myosin heavy chain, which we found expressed in testes (Miedema *et al.*, 1995). Mutations impairing the function of this protein would probably always result in embryonic or larval lethality.

The mechanism of sperm morphogenesis is likely to display some features not frequently used in other differentiation pathways due to the exceptionally high degree of structural differentiation of these cells and due to their particular (posttranscriptional) regulatory mechanisms. Spermatogenesis is also important for the studies of meiotic mechanisms. At the molecular level our knowledge of meiosis is very limited. Processes such as chromosome pairing, recombination and chromosome segregation are far from being understood although they are of central importance to the genetics of eukaryotes.

The work in my laboratory deals with some of the questions raised before. To this purpose we are investigating three groups of genes which in different ways are relevant for spermatogenesis in *Drosophila*. The main effort in the past has been directed towards an understanding of male fertility genes in the Y chromosome of *Drosophila* which were known since the early days of *Drosophila* genetics but are still obscure with respect to their biological function. This work has been recently extended into questions on the mechanisms of meiosis. Another part of our interest has been to obtain more insight into the process and into the reasons for chromatin reorganization in the male germ line. Finally we have investigated some genes which we recognized to be expressed in the germ line as well as in other tissues. A major part of our data are summarized and discussed from different points of view in several reviews (Hennig, 1985, 1988; Hennig and Kremer, 1990; Hennig *et al.*, 1989). Some main points will be addressed subsequently.

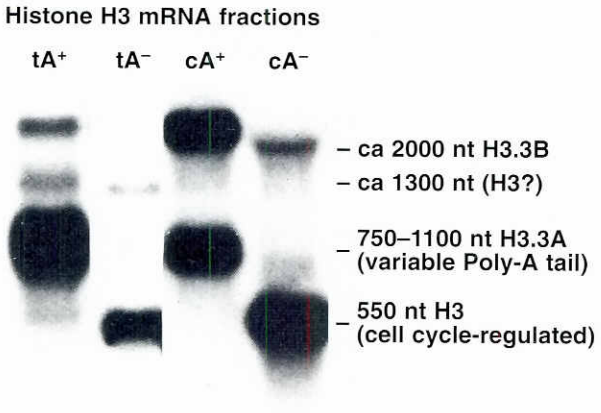

Fig. 3. Expression of the histone H3.3 variant in testes of *D. hydei*. *Northern blots of testis (t) and carcass (flies without gonads) (c) RNA. Histone H3.3 mRNAs are found in poly[A]+- RNA (A+) as well as in not polyadenylated (A-) RNA. The various mRNA sizes are assigned to the different genes (right).*

Chromatin constitution in the *Drosophila* male germ line

It was more than 100 years ago when Friedrich Miescher recognized that during sperm development the nuclear proteins undergo a transition which results in the substitution of the normal chromatin-associated proteins by another type of proteins. These substitution proteins, which were called protamines, are highly positively charged because of their high content of basic amino acids. Subsequently it was recognized that protamines are found in the sperm of most if not all animals.

In *Drosophila* only some cytological evidence has indicated that basic proteins substitute the normal somatic histones (Hauschteck-Jungen and Hartl, 1982). Is difficult to biochemically identify such proteins since sufficient numbers of sperm heads to extract enough material for biochemical studies are not easily available. On the other hand, *Drosophila* would permit studies of the biological effects of a replacement of such protamine-like nuclear proteins in sperm. One could, for example, be studying flies with the genes coding for protamine-like proteins inactive but substituted for by somatic histones. Such a genetic constitution could be created with the aid of the technique of reverse genetics where experimentally modified genes are introduced into the genome by transformation.

We have initiated such a study of the chromosomal proteins in the male germ line, in particular of histones. Two interesting aspects emerged from this cytological and ultrastructural study. First, the normal histone H1 appeared to be absent from all stages of spermatogenesis except only stem cells and spermatogonia (Fig. 2). Second, during the early postmeiotic development the chromatin passes through a cycle of condensation and decondensation before it is finally condensed and packed into the sperm head (Kremer, Hennig and Dijkhof, 1986). The significance of this condensation-decondensation cycle is unknown. Decondensation cannot simply be caused by a transcriptional activity of the genome as no uridine-incorporation can be observed in postmeiotic cells by autoradiography (Hennig, 1967). This indicates that transcription is either very low or fully absent in postmeiotic cells. The condensation-decondensation cycle is therefore more likely related to the rearrangement of chromosomal proteins.

Our cytological and immunological observations induced a study of histone expression in testes of *Drosophila* (Kremer *et al.*, 1986). These experiments were initially carried out with *D. hydei* as this species provides several advantages compared with *D. melanogaster*. Not only that the flies, and in particular also their testes, are much larger than those of *D. melanogaster*, which makes dissection easier and requires less efforts to collect enough material. But also the cytology of testes is superior to that of developing *D. melanogaster* male germ cells. The subsequent developmental stages in *D. hydei* testes occur in an extremely ordered pattern while in *D. melanogaster* testes cysts of germ cells of different stages are mixed and do not allow their separation during the dissection of the testis tube.

We have first isolated and characterized a repeat unit of the cell-cycle-regulated histone genes of *D. hydei* as in particular histone H1 turned out to be so much diverged in its nucleotide sequences from those of *D. melanogaster* that no cross reaction in hybridization experiments occurs (Kremer and Hennig, 1990). The isolated histone genes were used to study histone expression in testes. Normally, histone mRNAs are characteristically not polyadenylated. It emerged that polyadenylated variant forms of the mRNA for histone genes coding for the histones H2B, H3, and H4 exist in testes (Kremer, 1991). We set up to isolate such variant forms by screening cDNA libraries of testis polyadenylated RNA and recovered two histone H3.3 variant genes which are comparable to the histone H3.3 gene found in mammals. The detailed study of these genes revealed that two different histone H3.3 genes exist as single copy genes outside the histone repeat cluster as well of *D. hydei* as well as of *D. melanogaster* (Fig. 3) (Akhmanova *et al.*, 1995). Both genes (called histone H3.3A and H3.3B) are expressed in testes but also in other tissues. However, H3.3A is much stronger transcribed in testes while H3.3B is more strongly expressed in somatic cells. The nucleotide sequences of the parts of the genes not coding for proteins are entirely different, but the protein sequences derived from these DNA sequences are identical. These observations provide an excellent basis for studying histone gene function and regulation in higher eukaryotes which has so far been impossible, because of the repeated nature of the normal cell-cycle-regulated histone genes (approximately 120 copies in *Drosophila*). Only the single copy genes of yeast permitted to investigate histone function in some detail (Matsumoto and Yanagida, 1985). Our present work is directed to recover mutants of the single-copy histone H3.3 genes. This will permit us to investigate whether the function of the H3.3 variant histones is essential in different tissues, and to what extent they can be replaced functionally by the cell-cycle-regulated histones. The study of the regulation of their expression compared with the regulation of the cell-cycle-regulated histone genes will be of particular interest.

The structure and function of Y-chromosomal male fertility genes

Only few genes in *Drosophila* have been identified which are specifically and exclusively active in the male germ line. These

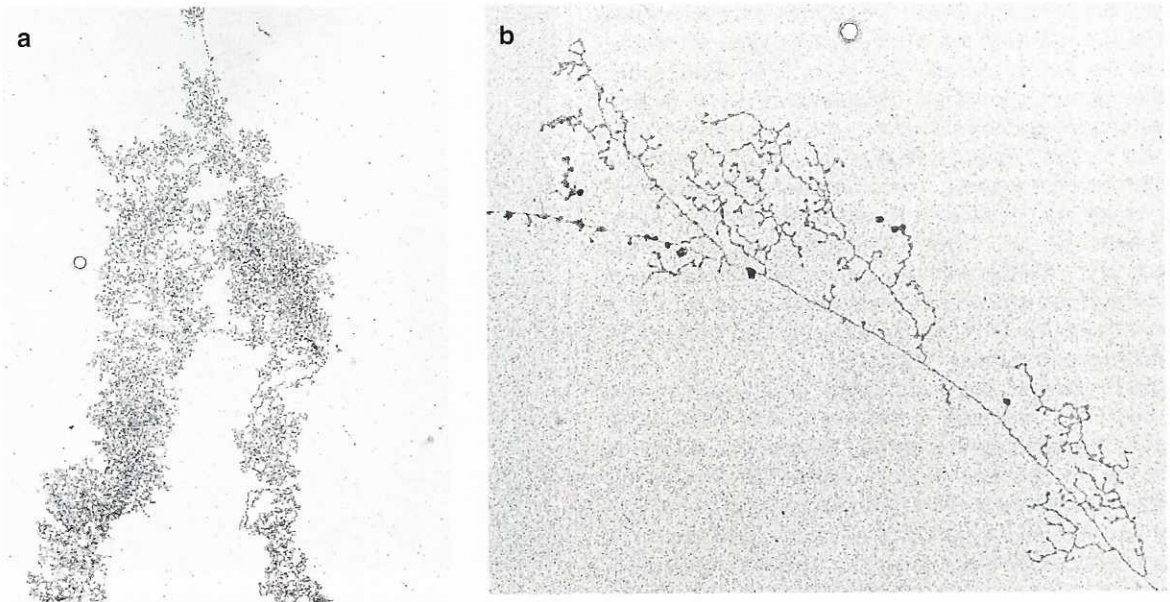

Fig. 4. Miller spreads of Y chromosomal lampbrush loop from primary spermatocytes of *D. hydei*. **(a)** *A complete loop ("nooses") is visualized. The length of the transcription unit is approximately 260 kb.* **(b)** *Details of the transcript structure of one of the lampbrush loops. A high degree of secondary structure is displayed by the growing RNA molecules.*

genes include the β2-Tubulin (Kemphues *et al.*, 1979), a group of seven genes of unknown function specifically expressed in the male germ line (Schäfer *et al.*, 1986), a histone H5-like gene (Russell and Kaiser, 1993), the Stellate locus (Livak, 1990), Janus B (Yanicostas *et al.*, 1989) and a number of Y chromosomal fertility genes (Bridges, 1916; Review Hennig, 1988). Some of these genes, even though active exclusively in the male germ line, do not lead to sterility if mutated.

Our interest has been concentrated on the Y chromosomal fertility genes because of their unusual properties. In 1961, Meyer *et al.* discovered that the activity of these genes in the primary spermatocytes is accompanied by the formation of large lampbrush loops. They are the only genes in *Drosophila* forming such chromosomal structures contrary to amphibian oocytes where most if not all transcribed genes form such loops. The type of loops found in *Drosophila* corresponds cytologically to a special type of lampbrush loops in amphibian oocytes called landmark loops (see Fig. 1). The cytology and genetics of these genes has been studied in much detail (reviews: Hess and Meyer, 1968; Hennig, 1985; Hennig and Kremer, 1991) but their function has remained obscure. If mutated or deleted, each of these Y chromosomal genes leads male sterility which is caused by a defective sperm development, but no specific defects can be found as it holds true for most of the other mutations inducing male sterility. This can be explained by the difficulties to recognize primary defects in a situation where complex posttranscriptional regulation processes determine the progress of differentiation of the cell (for discussion see Hennig and Kremer, 1990). Another interesting feature is that the Y chromosomal fertility genes represent genes in a heterochromatic part of the genome, as the Y chromosome is heterochromatic in most tissues. It has often been assumed that genes in a heterochromatic environment have special properties. Such special properties have also been indicated by our observation that ribosomal DNA, which is characteristically found surrounded by heterochromatin, is underreplicated in polytene tissues of *Drosophila* (Hennig and Meer, 1971).

As a first step in our study we were able to show that the transcripts of the Y chromosomal fertility genes are derived from repetitive DNA sequences (Hennig, 1968; Hennig *et al.*, 1974). For reasons which we understand only today, the isolation of the DNA has been much more difficult than expected. It became possible only after molecular cloning of DNA became available. Comparative differential screening of genomic DNA libraries from males and females (Vogt *et al.*, 1982; Vogt and Hennig 1983) as well as microcloning of lampbrush loop DNA from primary spermatocytes (Hennig *et al.*, 1983; Huijser and Hennig, 1987; Huijser, 1987) provided Y chromosomal DNA probes which reacted with lampbrush loop transcripts. Their detailed analysis revealed that they are composed of two characteristi-

cally different DNA fractions. One of these DNA sequence types is restricted in its location to the chromosome region accommodating one of the fertility genes. The other DNA type is also found in other genomic locations. Sequence analysis of both types of DNA sequences has shown that the gene-specific DNA sequence type (called Y-specific DNA) is composed of satellite-DNA-like, short tandem repeats. The other DNA class (called Y-associated) turned out to essentially represent defective transposons (Huijser *et al.*, 1988; Lankenau *et al.*, 1988; Hochstenbach *et al.*, 1993a). We concluded that both sequence types are interspersed and that the lampbrush loops consist of small blocks of satellite DNA alternating with defective transposons DNA (Vogt and Hennig, 1986a,b).

This general model of the organization of Y chromosomal lampbrush loop DNA has been confirmed and in more detail investigated for the lampbrush loop pair named nooses in the short arm of the Y chromosome of *D. hydei*. We choose this lampbrush loop pair because Miller spreading experiments permitted to derive details of the transcription of this gene (Grond *et al.*, 1983) and to conclude that it was the smallest of the Y chromosomal lampbrush loops accommodating some 260 kb of DNA in its transcription unit (de Loos *et al.*, 1984). This large amount of the DNA is transcribed as one transcription unit into single transcripts which form a complex secondary structure (Fig. 4). The DNA of this loop pair is essentially composed of a satellite-like DNA sequence type, called ay1, and of defective gypsy retrotransposons (Hochstenbach *et al.*, 1993a,b, 1994).

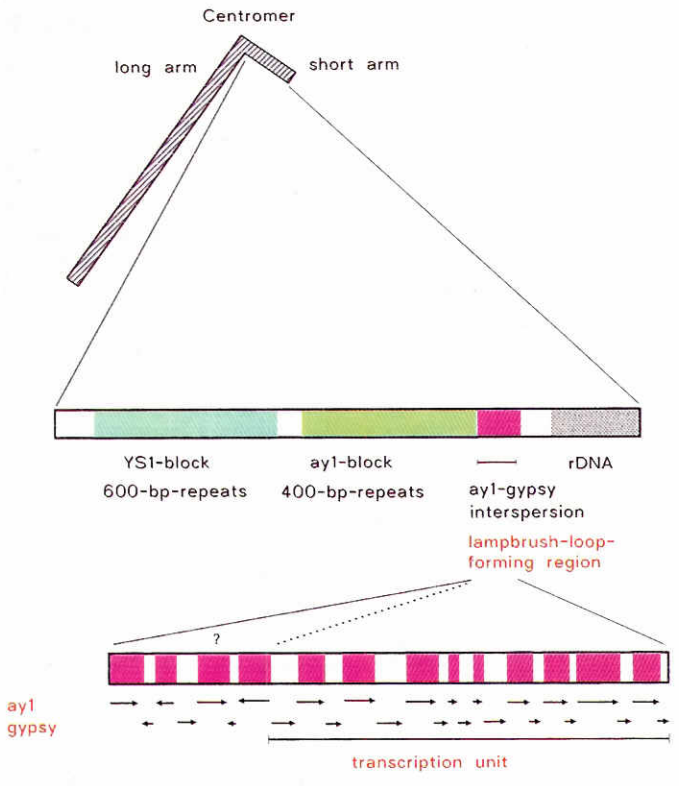

Fig. 5. Molecular fine structure of the short arm of the Y chromosome of *D. hydei*. *Two megabase pair blocks of YS1 and ay1 repeats fill a large proportion of the short arm. In an adjacent section of the short arm, short ay1-repeat blocks are interspersed with defective gypsy transposons. Within the transcription unit, ay1 and the gypsy elements are found in only one orientation (direction of arrows) while outside the transcription unit also inverted orientations of both repetitive DNA elements occur (left part of the enlarge section). The end of the short arm carries ribosomal DNA (rDNA).*

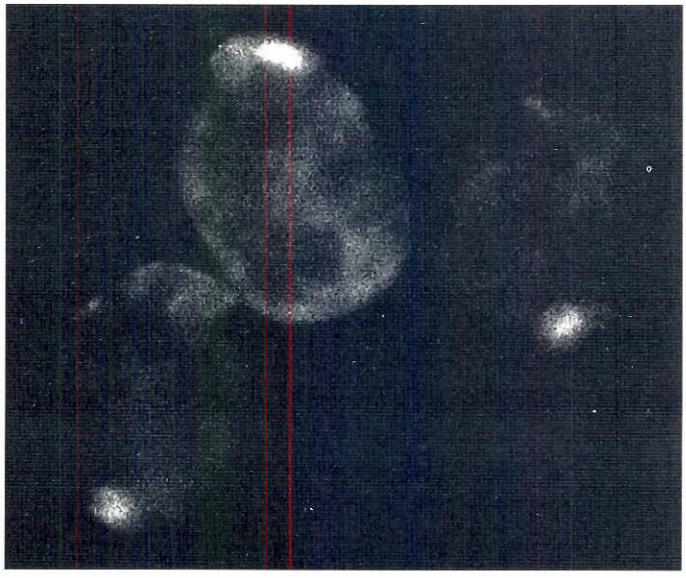

Fig. 6. Immunoreaction of primary spermatocyte nuclei with snRNP antibodies. *The lampbrush loops remain unlabeled (see Fig. 2)*

The molecular analysis of the DNA in short arm of the Y chromosome, in combination with *in situ* hybridization experiments, allowed us to reconstruct the organization of this part of the Y chromosome (Fig. 5). In its proximal section, the short arm of the Y chromosome carries two distinct, separate blocks of continuous tandem-repeats of ay1 and YsI which each extends over approximately 1 Mb. YsI is a sequence derived from ay1 by an internal partial duplication. The YsI block is closest to the centromere. It is separated from the more distal ay1 block by some DNA of unknown character. The ay1 block is, at its distal end, followed by a chromosome region with 400 to 600 kb DNA where short ay1 repeat blocks are interspersed with defective gypsy elements. Adjacent to this region, the terminal part of the short arm is composed of a nucleolus organizer region which is estimated to contain approximately 500 kb of DNA.

The lampbrush loop-forming fertility gene is located in the chromosome region carrying interspersed gypsy and ay1 elements. However, not the entire chromosome region with such an interspersion is transcribed. This can be derived from our observation that only such gypsy and ay1 DNA sequences are found in transcripts which have the same orientation within the chromosome. In the case of the defective gypsy elements only that DNA strand is transcribed which in the functional full length transposable element is used as the template for the synthesis of mRNA. In cloned DNA fragments from the chromosome region with a gypsy-ay1 interspersion pattern, however, also ay1 and gypsy elements in an orientation opposite to one another were found. Consequently, only parts of the chromosome region with this sequence pattern are parts of the transcription unit. This is consistent with our conclusion that this chromosome regions contains 400 to 600 kb of DNA while the transcription unit, as derived from Miller spreads, includes only some 260 kb of DNA.

Although other lampbrush loops have not been studied to a comparable detail, the observations available (see Hennig *et al.*, 1989) allow to assume that their general structure is very similar to that of the lampbrush loops nooses in the short arm.

The question on the function of these peculiar genes arises. Are the repetitive DNA sequences simply parts of introns or do they have a functional significance? This question has been critically discussed recently (Hennig, 1993) with the conclusion that, even though it cannot be excluded that protein coding regions are hidden within the transcription units forming lampbrush loops, the formation of such loops with their peculiar morphology implicates other biological functions than that of "normal" protein coding genes.

A key for an answer to the question what such a biological function might be, has been given by our observation that the lampbrush loops interact in a loop-specific pattern with antisera indicating a unique protein composition for each loop (Hulsebos *et al.*, 1984; Hennig, 1985;, Hennig *et al.*, 1989). This view has recently been adopted by other authors (Bonaccorsi *et al.*, 1990). These observations indicate that each loop, besides a set of common RNA-associated proteins (for example certain RNP-proteins, see Glätzer, 1984), binds one or a few proteins specific for the particular loop. It fits to such a view that an interaction with antisera recognizing snRNP-antigens (Wu *et al.*, 1991) cannot be detected in lampbrush loops (Hennig, unpublished data) (Fig. 6). Also consistent with the absence of the normal splicing machinery is the fact that opposite to common lampbrush loops (Wu *et al.*, 1991) no snRNAs can be found within *Drosophila* lampbrush loops by *in situ* hybridization (Hennig, unpublished observations). The sensitivity of the *Drosophila* lampbrush loops to the inhibition of RNA synthesis with actinomycin D (Meyer and Hess, 1963; Hennig, 1967) and the relative insensitivity against α-amantin (Hennig, 1967) supports our conclusion that the transcripts are not coding for proteins as in this case it would be expected that the transcription is highly sensitive against α-amanitin which inhibits RNA polymerase II. Actinomycin preferentially inhibits RNA polymerase I which usually transcribes rDNA.

The question on the character of the proteins bound to the Y chromosomal lampbrush loops has recently found a somewhat unexpected answer. I have studied the reaction of meiotic male germ cells with polyclonal and monoclonal antisera raised against single proteins from rat synaptonemal complexes (SCs) (Heyting *et al.*, 1988) (Fig. 7). It turned out that each antiserum reacts in a highly specific pattern with certain lampbrush loops of different *Drosophila* species including *D. hydei* and *D. melanogaster*. This observation is unexpected because *Drosophila* male germ cells in meiosis do not form SCs and they do not undergo recombination. Are the lampbrush loops morphological or functional substitutes of SCs? This would mean that at least some SC-associated proteins are not - or not only - involved in recombination only, but that they may have other functions during meiosis. Alternatively, do lampbrush loops bind proteins responsible for inducing or promoting recombination to prevent them from their normal function?

We have initiated the molecular investigation of proteins from *Drosophila* testes recognized by the SC antisera of rat. So far two protein coding genes have been isolated and partially characterized (X. Sun, J. Xu, Y.X. Wang, H. Harhangi and W. Hennig, unpublished data). One of these proteins, preliminarily called tzf-protein (tzf from testis zinc finger) appears to be a typical nucleic acid binding protein comparable to those acting as transcription factors. It contains six zinc finger regions which are distributed between the terminal parts of the protein and reside in particular in the carboxyterminal end. All zinc fingers except one belong to the type identified as characteristically binding to RNA (Clemens *et al.*, 1992) rather than to DNA. This is an interesting aspect as we have earlier proposed that the transcripts of the lampbrush loops, which are characterized by a high degree of secondary structure, are used to bind specific proteins. In the case of the transcription factor TFIIIA, which is involved in the regulation of 5S rRNA synthesis in *Xenopus* (Engelke *et al.*, 1980), certain zinc fingers are able to specifically bind to double-stranded regions of the 5S rRNA.

Polyclonal antisera raised against the tzf-protein react with the same lampbrush loop also found to react with the antiserum against rat SCs which we originally used for the isolation of the

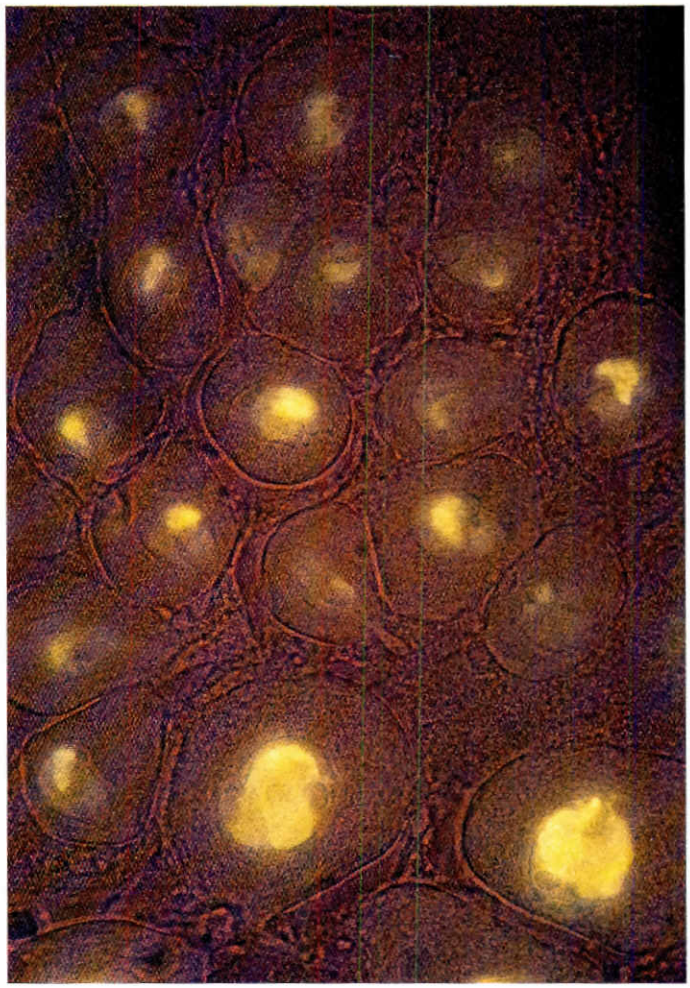

Fig. 7. Immunoreaction of testis squashes of *D. hydei* with an antiserum against rat synaptonemal complexes. *The pictures shows primary spermatocyte nuclei of different age. The amount of antigen increases with the growth of the spermatocyte and it is located in two of the lampbrush loop pairs (Figs. 2 and 6). During meiosis the antigen disappears from the nuclei.*

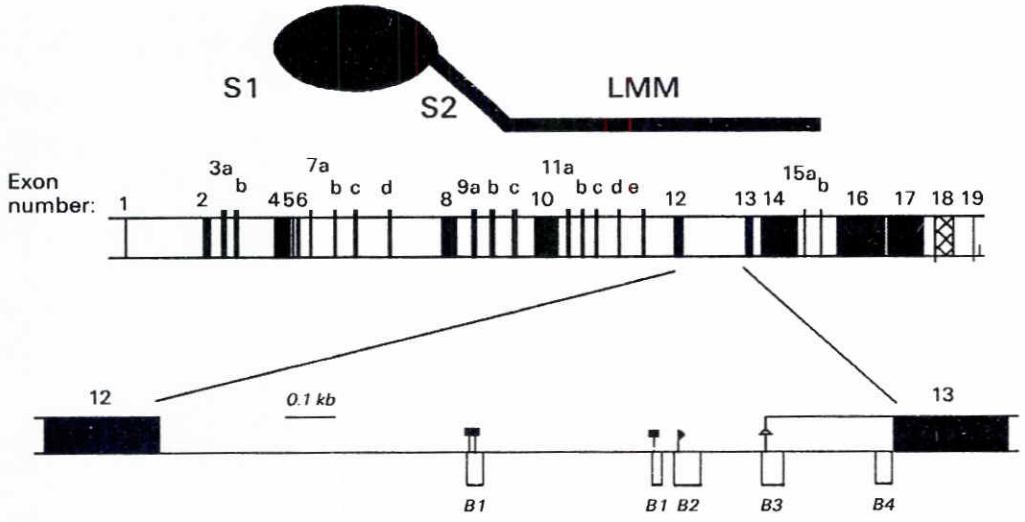

Fig. 8. Different muscular heavy chain myosin (mMHC) molecules are expressed in testes of *Drosophila*. *In the upper part of the figure, a diagram of a normal mMHC protein with the head (S1), the hinge (S2) and the tail (LMM) region is shown. Below, the molecular structure of the single-copy gene of Drosophila coding for mMHC is shown. Exons are black. Several exons can be spliced in alternative combinations (indicated by small letters and an extension of the black box) and exon 18 may be present or not. The end of the gene carries several alternative polyadenylation sites. Intron 12 (between exons 12 and 13) contains an internal promoter (small arrow above the line) which is responsible for the production of a variant mMHC protein starting at the position of the vertical arrow within the "intron". B1 to B4 are different conserved regulatory DNA sequence elements.*

gene. Moreover, a reaction is observed also in nuclei of early spermatids but in no other stage of spermatogenesis. On meiotic chromosomes from rat testes the antisera against the *Drosophila* tzf-protein show a reaction with SCs and, as in *Drosophila* testes, with nuclei of young (round) spermatids (observations of C. Heyting, unpublished). Even though we cannot exclude that we have recovered a gene for a protein with some epitopes identical to those recognized by the original SC protein antiserum, a the relationship to proteins associated with SCs exist.

Northern blots indicate that the tzf gene is strongly expressed in testes and ovaries, but also in embryos and to a lesser extent in other somatic tissues. The distribution of mRNA in early embryos (blastoderm and early postgastrulation stages) is even throughout the entire embryo. Hence, these data indicate that the protein may represent a general transcription factor. What specific functions the tzf-protein might have during the first meiotic prophase and in young spermatid nuclei, which in *Drosophila* are essentially transcriptionally inactive, or whether they have any special functions in the germ line remains to be established. Our current approach to this question is to recover mutations of the tzf-gene which may give evidence on the function of this gene. We have first indications that a mutation in the gene results in recessive lethality indicating a fundamental function of this gene.

A second gene coding for a protein with epitopes recognized by SC antisera of the rat has been isolated. It is specifically expressed in the male germ line. Its amino acids sequence is in principle compatible with a structure expected for a chromosomal protein, as it is highly charged. However, no typical nucleic acid binding regions can be identified (H. Harhangi, X. Sun, J. Xu and W. Hennig, unpublished data). Also in this case, only the recovery of mutations will provide more insight into the function of this protein.

The identification of proteins most likely specifically bound to lampbrush loops during the first meiotic prophase may help to finally obtain an answer to the question of the biological function of the Y chromosomal lampbrush loops. We suspect that they are responsible for the binding of chromosomal proteins involved in certain so far unclear meiotic processes or in the protein substitution events characteristic for the chromatin in the male germ line. It is interesting that similar suggestions have recently been made for the *XIST* locus in the human X chromosome which is involved into the X inactivation in females and codes for transcripts not carrying information for proteins (see Goldman, 1992). Also this locus is speculated to be involved in the control of chromatin structure.

The studies of the Y chromosomal fertility genes and the concept developed by us to explain their biological function, fits exceedingly well into other recent observations on chromatin constitution and the mechanisms regulating gene activity (cf. Henikoff, 1994). Long-known effects such as position effects and transvection seem to have a similar functional basis. This common basis is the control of the local chromatin constitution by mechanisms involving the interaction of (identical or similar) DNA sequence elements (for example enhancers or silencers or repeated DNA sequences) either in cis or trans. For example, the orientation of duplicated genes or other DNA sequences within or around a gene is of considerable importance for the decision whether a particular gene becomes active or remains inactive (see Devlin *et al.*, 1990).

Our understanding of the role of the chromatin constitution in gene regulation has not passed beyond very early steps. Studies of the Y chromosomal fertility genes may contribute to the exploration of chromosomal processes at the chromatin level.

Other genes active during the male germ cell development

In the course of our studies on spermatogenesis we have identified several genes active in the male germ line. The two genes identified by us are single-copy genes with important functions in somatic tissues. This agrees with the expectations from the general observation that many mutations affecting a wide variety of genes have pleiotropic effects on the male germ

line (see Hennig, 1988 for *Drosophila*, and Handel, 1987, for mice) leading to male sterility. One of these genes is the gene for the laminin B2 chain (Wang *et al.*, 1992), the other gene codes for the muscular myosin heavy chain (mMHC) (Miedema *et al.*, 1994, 1995).

Both genes were identified on the basis of immunoscreens on expression libraries with antisera raised against protein fractions isolated from *Drosophila* testes (Hulsebos *et al.*, 1983). Their expression in testes was demonstrated by immunocytochemistry, immuno electronmicroscopy, *in situ* hybridization and on Northern blots as well as by PCR on RNA recovered from manually isolated germ cells.

The laminin B2 gene is expected to be expressed in the extracellular matrix of the testis envelope. Unexpectedly we found that it is also expressed in spermatocytes at the RNA level, and the protein is demonstrated at the ultrastructural level in the axoneme and in the spermatid nuclei by immuno electron microscopy. Its biological function in these intracellular locations is unknown but it is one possibility that the laminin has a similar function in the outgrowth of the axoneme and the elongation of the nucleus as it does in its extracellular position for the outgrowth of neurons.

The mMHC gene is transcribed in primary spermatocytes during the meiotic prophase. The mMHC protein is found within the differentiating (postmeiotic) nebenkern derivatives. Its function in this position is not clear. The myosin molecules may be constituents of the paracrystalline material which is found in the tail of the mature sperm, but another possibility is that it is involved in directing molecular components of the paracrystalline material into their final position during sperm elongation, i.e. that it controls intracellular movements of molecular components of the Nebenkern. During these studies it has been recognized that the mMHC gene is also responsible for the production of a new mMHC isoform (M_r, 155,000) which misses the head section of the complete molecule (M_r, 195,000). This isoform is produced as the consequence of the activity of a promoter located in intron 12 (Fig. 10). Although immunoelectron microscopy and immunological studies of male sterile mutants indicate that it is postmeiotically present in Nebenkern derivatives, there may be additional locations of this mMHC protein which have not yet been established. It is, however, clear that this protein variant is also present in thorax muscles.

Both genes, laminin B2 and mMHC, confirm thus the prediction that genes important in somatic tissues are also involved in sperm differentiation. Obviously, genes as laminin B2 and myosin are unlikely to be recovered in mutant screens for male sterility as they must be expected to be embryonic lethals or to have lethal effects during the larval development. This conclusion emphasizes the reasons for some of the difficulties in analysing spermatogenesis which have been experienced in the past (i.e. Kiefer, 1973; Lindsley and Tokuyasu, 1981).

In our and other laboratories, new methodologies, in particular enhancer trap experiments, have recently permitted to identify a series of other genes active in the male germ line. So far, several of the genes have been described in the molecular structure but the biological function of the respective gene products in sperm development has not yet been recognized. It can however be predicted that the investigation of the development of male germ cells will now make fast progress.

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