Y chromosomal fertility genes in *Drosophila*

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In *Drosophila*, as in many other organisms, the Y chromosome usually appears heterochromatic and only few genes have been discovered. The known genes are essentially for male fertility but their function is unknown. They appear not to code for proteins although this cannot yet be entirely excluded. As an alternative function, protein binding to the growing transcripts is considered, which may be important in the context of nuclear domain formation.

THE male fertility genes in the Y chromosome of *Drosophila* have attracted attention since the early days of genetics. This interest is based on the fact that these were the only known genes in the Y chromosome, which could be identified with conventional genetic methods besides those for ribosomal RNA. No markers were available for precise mapping of Y chromosomal genes. Only crude information regarding their number and localization could be obtained with the aid of translocations and deletions. Much of the Y chromosome appears to be devoid of genes as it can be deleted without consequences regarding fertility, viability or even on the phenotype. The first substantiated information on the number and location of the fertility genes were obtained by Neuhaus[1]. Later Gatti and Pimpinelli[2] inferred the existence of 6 fertility genes on the Y-chromosome of *D. melanogaster* on basis of a combined cytological and genetic mapping on DAPI stained somatic prophase chromosomes. These chromosomes allowed visualization of a banding pattern within the Y chromosome. On this basis, each fertility gene was assigned to a distinct section within the Y chromosome. The result of this analysis[2] confirmed earlier conclusions[3-5] that the fertility genes include large sections of the DNA and each exceeds 1000 kb of DNA. Much of this and the subsequent work on the Y-chromosomal fertility genes has been carried out with *Drosophila hydei*. The cytology of meiotic cells in this species is superior to that of *D. melanogaster* and allows easy distinction of the fertility genes in the cells where they are expressed, viz. the primary spermatocytes. It was first recognized by Meyer *et al.*[6] that expression of the fertility genes is accompanied by formation of large lampbrush loops. A direct homology of the intranuclear structures with lampbrush loops as found in amphibian oocytes was demonstrated by Hennig[7] who showed transcription of RNA in these chromosomal structures by ^{3}H-uridine autoradiography. A detailed mapping of the fertility genes on the Y-chromosomal lampbrush loops was carried out by Hess[8] on the basis of the cytology of deletion strains. Finally, Hennig[9] and Hennig *et al.*[3] documented, by *in situ* hybridization experiments, that middle repetitive DNA sequences are transcribed in the Y chromosomal fertility genes. A critical review of details of these earlier studies is found in Hennig[10]. The application of microdissection technique to Y chromosomal lampbrush loops from primary spermatocytes[11] initiated a series of investigations on the DNA associated with the fertility genes. These studies allowed formulation of a model which applies in principle to all of the Y-chromosomal fertility genes. They are composed of blocks of short satellite-like DNA sequences which are interspersed with fragments of transposable element[12-14]. A remarkable detail, although not yet understood in its significance, is the observation that the satellite DNA blocks occur always in the same orientation within the transcription unit although the same sequence, if located in non-transcribed regions, may have either orientations. Moreover, the transposons within one lampbrush loop appear to be of one type, which is characteristic for that loop, or may belong to two types of transposons, e.g. *micropia* and *rally*, but again these are also characteristic for the particular loop[15-17]. *An important conclusion from these studies, which included extensive DNA sequencing data, was that there is no evidence for the presence of any protein coding sequences*. But it has also been clearly emphasized that the existence of such protein coding sections cannot be excluded as long as the DNA sequence of the entire transcription unit is not known. There is also *a priori* no reason to exclude the presence of conventional protein coding genes in the Y chromosome as Leoncini[18] has shown that Y-chromosome-linked temperature sensitive mutations can be obtained, which may be within or closely linked to some of the fertility genes. *On the other hand, the extraordinary size and the abnormal cytology of the fertility genes during their expression in the primary spermatocyte nucleus imply that other unusual functions must be connected with these genes.* That led to the conclusion that the fertility genes in the Y chromosome

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represent a new type of eukaryotic genes. Their function appears to be related to the accumulation of nuclear proteins as suggested by Hennig[10]. The giant transcripts appear to serve as targets for the binding of some proteins although this binding is most likely not specifically related to a particular RNA sequence. Subsequently[19–24] various proteins have been identified to be bound to particular lampbrush loops. The proteins identified include histone variants, a leucine zipper protein and tectin.

An essentially new situation for our understanding the fertility genes appeared to be created by the report of Gepner and Hays[25] that a dynein coding gene is located in the Y chromosomal region encoding one of the fertility genes in *D. melanogaster*. However, as was pointed out by Hennig[26], the data supplied were not sufficient to exclude that a dynein gene was located actually close to, rather than within, the respective fertility gene. These observations, therefore, do not provide a final answer to the question whether the fertility genes simply represent conventional protein coding genes. More recently an extension of the work of Gepner and Hays[25] has been published by Kurek *et al.*[27] to support the claim that one of the fertility gene-related lampbrush loops in *D. hydei* as well as in *D. melanogaster* encodes dynein. Unfortunately, however, this paper too is inconclusive, and it is rather surprising that it could pass peer-reviewing without additional data. Besides other shortcomings, the main objection is that the authors were not able to demonstrate the location of transcripts encoding dynein within the respective lampbrush loop by *in situ* hybridization technique. This failure to localize the dynein gene transcripts on the loop is sufficient to question the validity of their conclusions. Akhmanova and Hennig (unpublished) have also carried out *in situ* hybridization experiments with labelled oligonucleotides derived from the dynein sequence published by Kurek *et al.*[27], but could not get any signal on lampbrush loops of *D. hydei* despite the application of a highly sensitive technique. How can these contradictions be explained? In a critical discussion of the data of Gepner and Hays[25], Hennig[26] pointed out that all the experiments to locate the dynein sequence within the Y chromosome have been carried out on basis of the deletion mapping technique (markers for conventional gene mapping are not available for the Y chromosome). All available deletions cover exceedingly large sections of DNA, usually several megabases. Consequently, no fine mapping, as is standard for other genes in *Drosophila*, can be carried out. It has been further pointed out by Hennig[26] that the mutation frequencies in the Y chromosome may vary widely between different regions of this (normally heterochromatic) chromosome. In addition, none of the mutation experiments carried out for the Y chromosome so far is extensive enough to approach even half-saturation with mutations. Therefore, it is most likely that any 'normal', i.e. protein coding, gene would have escaped our attention. Taking all the data together, a straightforward interpretation is that the Y chromosome of *Drosophila* may harbour conventional protein coding genes, which may or may not be located close to the established fertility genes, but which do not overlap with them. This conclusion does not provide an answer to the functions of the fertility genes but appears to answer the contradictory observations.

The data available strongly argue in favour of a function of the transcripts of the Y chromosomal fertility genes which is not related to protein coding. It is a most attractive idea that the growing RNA molecules are functional in the context of the formation of distinct nuclear domains in the spermatocyte nucleus. They apparently play a structural role as targets for nuclear proteins. Such a structural function of RNA has been established much earlier when it was shown that ribosomal RNA molecules are of structural importance for the correct assembly of ribosomes.

The exciting story of the Y chromosomal lampbrush loop-forming fertility genes in *Drosophila* as a special type of eukaryotic genes is still awaiting its final answer.

1. Neuhaus, M. J., *Genetics*, 1939, **37**, 229–254.
2. Gatti, M. and Pimpinelli, S., *Chromosoma*, 1983, **88**, 351–373.
3. Hennig, W., Meyer, G. F., Hennig, I. and Leoncini, O., *Cold Spring Harbor Symp. Quant. Biol.*, 1974, **38**, 673–683.
4. Grond, C. J., Siegmund, I. and Hennig, W., *Chromosoma*, 1983, **88**, 50–56.
5. de Loos, D., Dijhof, R., Grond, C. J. and Hennig, W., *EMBO J.*, 1984, **3**, 2845–2849.
6. Meyer, G. F., Hess, O. and Beermann, W., *Chromosoma*, 1961, **12**, 676–716.
7. Hennig, W., *Chromosoma*, 1967, **22**, 294–357.
8. Hess, O., *Chromosoma*, 1965, **16**, 222–248.
9. Hennig, W., *J. Mol. Biol.*, 1968, **38**, 227–239.
10. Hennig, W. *Adv. Genet.*, 1985 **23**, 179–234.
11. Hennig, W., Huijser, P., Vogt, P., Jackle, H., Edstrom, J.-E., *EMBO J.*, 1987, **2**, 1741–1746.
12. Hennig, W., Brand, R. C., Hackstein, J., Hochstenbach, R., Kremer, H., Lankenau, D. H., Lankenau, S., Miedema, K. and Potgens, A., *Genome*, 1989, **31**, 561–571.
13. Hochstenbach, R., Potgens, A., Meijer, H., Dijkhof, R., Knops, M., Schouren, K. and Hennig, W., *Chromosoma*, 1993, **102**, 526–545.
14. Hennig, W., *Int. J. Dev. Biol.*, 1996, **40**, 167–176.
15. Huijser, P. and Hennig, W., *Mol. Gen. Genet.*, 1987, **206**, 441–451.
16. Huijser, P., Kirchhoff, C., Lankenau, D-H. and Hennig, W., *J. Mol. Biol.*, 1989, **203**, 689–697.
17. Trapitz, P., Glatzer, K. H. and Bunemann, H., *Mol. Gen. Genet.*, 1992, **235**, 221–234.
18. Leoncini, O., *Chromosoma*, 1977, **62**, 329–357.
19. Hulsebos, T. J., Hackstein, J. H. and Hennig, W. *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 3404–3408.
20. Pisano, C., Bonaccorsi, S. and Gatti, M., *Genetics*, 1993, **133**, 569–579.
21. Heatwole, V. M. and Haynes, S. R., *Chromosoma*, 1996, **105**, 285–292.

22. Akhmanova, A., Miedema, K., Wang, Y., van Bruggen, M., Berden, J. H., Moudrianakis, E. N. and Hennig, W., *Chromosoma*, 1997, **10**, 335–347.
23. Sun, X., Ph D Thesis, University of Nijmegen, 1994.
24. Harhangi, H, Sun, X., Wang, Y. X., Akhmanova, A., Miedema, K, Heyting, C. and Hennig, W., *Chromosoma*, 1999, **108**, 235–242.
25. Gepner, J. and Hays, T. S., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 11132–11136.
26. Hennig, W., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 10904–10906.
27. Kurek, R., Reugels, A. M., Glatzer, K. H. and Bunemann, H., *Genetics*, 1998, **149**, 1363–1367.