Genetic and molecular dissection of the *kl-3* Y-chromosome loop of *Drosophila melanogaster*.

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Primary spermatocyte nuclei of *D. melanogaster* exhibit three giant lampbrush-like loops formed by the *kl-5, kl-3* and *ks-1* Y-chromosome fertility factors. These structures contain and abundantly transcribe highly repetitive, simple sequence DNAs and accumulate large amounts of non-Y encoded proteins. The biological role of the Y-chromosome loops is still largely unknown. They may accumulate non-Y-encoded proteins involved in spermiogenesis, harbor genes encoding for axonemal components, or both.

To elucidate the biological role of the Y loops we used two complementary approaches aimed at the identification and characterization of the proteins bound to these structures: (1) we raised antibodies against loop proteins and cloned the corresponding gene; (2) we isolated mutations affecting the formation and the morphology of the Y loops.

By immunizing mice with the 53kDa fraction of *Drosophila* testis proteins we raised a polyclonal antibody, designated as T53-1, which decorates the *kl-3* loop and the sperm flagellum. Immunological and biochemical analyses showed that this antibody reacts with a single polypeptide with characteristics similar to the tektins, a class of axonemal proteins known to form Sarkosyl-urea insoluble filaments in the wall of flagellar microtubules. By screening a testis-specific expression library with the T53-1 antibody we have cloned the gene encoding this polypeptide. The gene, designated as *kl3 lp-1* (*kl-3 loop protein 1*), maps to region 53 C9-10 of the polytene map, is expressed only in testes and produces a single polyadenylated message of about 2.2 kb. Sequence analysis revealed that *kl3 lp1* encodes a protein of 402 amino acids. This protein does not show sequence homology with tektin A1, a recently cloned sea urchin tektin. However, it is strongly homologous (40% identity and 60% similarity) to leucine aminopeptidase from bovine eye lenses. This homology is quite surprising in that the abundance and the localization of the *kl3 lp1* product strongly suggests that this protein plays a structural role. We speculate that *kl3 lp1* may represent another case of evolutionary recruitment of an enzyme for structural functions. Such cases are well documented for several components of vertebrate and invertebrate eye lenses.

To isolate mutants defective in loop formation we screened 210 male sterile mutants for the presence and normality of the Y loops. This analysis led to the identification of 5 mutants that specifically affect the formation of the *kl-3* loop. Two of these mutants [*ms(3)HB267* and *ms(3)HB223*] completely lack the *kl-3* loop, while the other three mutants [*ms(3)HB933*, *ms(2)HA30* and *ms(2)HB108*] exhibit extremely reduced *kl-3* loops; in all these mutants the *kl-5* and *ks-1* loops are normal. Complementation tests showed that *ms(3)HB267* and *ms(3)HB933* are allelic, indicating that the five mutants analyzed identify four loci necessary for proper development of the *kl-3* loop. All these mutations have been statistically mapped using a variety of marked chromosomes. In addition, two mutations, *ms(2)HA30* and *ms(3)HB267* have been mapped over deficiency. In both cases mutant/deficiency males were normally viable, completely sterile and devoid of the *kl-3* loop. This suggests that the genes specified by these mutants are specifically required for the formation of the *kl-3* loop in the male germ line.

Taken together our screens have led to the identification of four genes involved in the formation of the *kl-3* loop. In addition, we have identified a protein, the kl-3 loop protein-1, accumulated on this structure. We believe that these findings open the way to the molecular dissection of the *kl-3* loop that must be viewed as a complex organelle whose formation is controlled by a region of the Y chromosome and at least five different autosomal products.