Differential Chromosomal Distribution of Ribonucleoprotein Antigens in Nuclei of *Drosophila* Spermatocytes

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Abstract. The ribonucleoprotein (RNP) composition of the active Y chromosomal structures in spermatocyte nuclei of *Drosophila hydei* has been investigated using the anti–RNP antibodies Dm 28K2 and pp60 as a probe. Antibody Dm 28K2 was raised against an RNP protein of cytoplasmic RNP particles in *D. melanogaster* cells, while antibody pp60 was raised against a pre-messenger RNP fraction from oocytes of *Xenopus laevis*. Both antibodies detect nuclear RNP (nRNP) antigens of *D. hydei*. This is shown by CsCl density centrifugation of nRNP from *D. hydei* cells and immunoblotting across the density gradient. Dm 28K2 and pp60 recognize antigens of nRNP complexes which band at a characteristic buoyant density of ∼1.4 g/cm³ in CsCl. By indirect immunofluorescence we observe that the nRNP complexes identified by Dm 28K2 are localized at only two of the five Y chromosomal loop structures which are named according to their distinct morphology. Dm 28K2 decorates RNPs within the "clubs," within the cones, and within the matrix of the "pseudonucleolus." Ultrastructural bodies that are candidates for this immuno-reaction are RNP granules that resemble the so-called perichromatin granules. Antibody pp60 recognizes RNP complexes close to the axes of the active Y chromatin. In the "pseudonucleolus" it can be shown that the structures recognized by pp60 are quite distinct from those detected by Dm 28K2. Thus, the "pseudonucleolus" is a striking example for the presence of different RNP populations within a same defined nuclear compartment. Together with previous results (Glätzer, K. H., 1984, *Mol. Gen. Genet.*, 196:236–243), our data represent evidence that the morphological and apparently functional differences between the active Y chromosomal loops, which are involved in male fertility, are caused by the presence of qualitatively and possibly also functionally different RNP populations within these nuclear compartments. Because both RNP antigens are discussed in the literature in connection with repressed mRNP the observed cross-reaction of the respective antibodies in *D. hydei* suggests a more general and important function of these proteins in the RNA metabolism of eukaryotic cells.

IN eukaryotes the nuclei harbor a heterogeneous mixture of ribonucleoprotein (RNP)[1] complexes. With respect to their specific RNA and protein composition several classes of nuclear RNPs (nRNP) such as heterogeneous nRNP (hnRNP), pre-ribosomal RNP, pre-messenger RNP (pre-mRNP), and small nuclear RNP can be distinguished (reference 3 for review). There is now increasing evidence that the protein constituent of the RNP complexes plays a major role in determining the function of certain RNP particles or strongly influence the metabolic fate of individual transcripts in events such as processing, transport, and storage.

With the availability of antibodies directed against nRNP it soon became evident that the distribution of nRNP complexes within the nucleus is not random but in many cases is restricted to specific nuclear sites (e.g., 4, 22, 24).

In nuclei of primary spermatocytes of *Drosophila hydei* at least three different populations of nRNP complexes have so far been discriminated. Two of them, specified by the monoclonal antibodies S5 and X4 which are directed against hnRNP of *Drosophila melanogaster* cells (18, 19), are shown to accumulate at two of the five characteristic Y chromosomal sites necessary for male fertility (9).

In this paper we describe a novel group of nonribosomal RNP antigens and their relation to nuclear structures in primary spermatocytes of *D. hydei*. Our data represent clear evidence that the distinct morphological differences between the Y chromosomal compartments must partly be due to the presence of qualitatively different RNP populations. The two antibodies used in this study are Dm 28K2 and pp60. Dm 28K2 was raised against a 28-kD protein of the cytoplasmic 19S ring-type particles of *D. melanogaster* cells: these particles probably have a regulatory function at the translational level (20, 21). The other antibody pp60 was raised against an RNP protein that has been shown to be tightly bound to pre-

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1. *Abbreviations used in this paper:* Cl, clubs; Co, cones; hnRNP, heterogeneous, nuclear RNP; Ns, nooses; nRNP, nuclear RNP; Ps, pseudonucleolus; RNP, RNA-protein complexes, ribonucleoprotein; Th, threads; Tr, tubular ribbons.

mRNP located in oocyte nuclei of the vertebrate *Xenopus laevis*, but is also present in cytoplasmic mRNP and appears to be involved in the generation of a specialized class of maternally inherited RNPs (7). Both antibodies recognize nRNP antigens in *D. hydei*, which in a CsCl density gradient occupy the same buoyant density position as the previously characterized hnRNP antigen S5 (18).

Materials and Methods

Animals

Drosophila hydei stocks from our institute's collection were kept at 23°C on a medium containing cornmeal, malt, molasses, soja flour, agar, and yeast. Males with specific Y chromosomal fragments were constructed as described earlier (8). In short, males of XY-translocation stocks were mated to XX/O females (1). The sterile male offspring then carry only the translocated Y fragment. In other crosses combination stocks designated COM-stocks (12) were used. The fragments are labeled according to the loop-forming site carried. The arrangement of these sites on the Y chromosome is, from the short arm to the tip of the long arm: nooses (Ns)-clubs (Cl)-tubular ribbons (Tr)-pseudonucleolus (Ps) with cones (Co)-threads (Th).

The following stocks were analyzed, the cytological data given in parentheses: Wild type (Ns, Cl, Tr, Ps-Co, Th; complete Y present); XO (no Y structures; Y absent); COM 290/2 × 700/97 (Ns, Cl, Ps-Co, Th); 290/2 (Ns, Cl, Tr); 697/16 (Th, Ps-Co); 717/5 (Th, "mutant" Ps-Co); 340/2 (Ns, Cl); 290/1 (Ns).

Antibodies

The antibody Dm 28K2 was raised in rabbit against a 28-kD protein band of the 19S ring-type small, cytoplasmic RNP particles of *D. melanogaster* culture cells (21). Particle proteins were separated on preparative 7.5–15% SDS polyacrylamide gel gradients according to Laemmli (16). The proteins were stained with Coomassie Brilliant Blue. The 28-kD band was excised and the protein eluted by diffusion in 50 mM EDTA, 10 mM Tris/HCl, pH 7.5, 10 mM dithiothreitol, 0.5% SDS. The eluted proteins were freeze dried and solubilized in a buffer containing 80 mM potassium acetate and 20 mM Hepes, pH 7.5. For immunization an emulsion was formed using 50% incomplete Freund's adjuvant. Control serum was taken from the ear vein before the first injection. The rabbit was bled from the ear vein 10 d after the last booster injection.

Antibody pp60 was raised in rabbit against a 60-kD polypeptide isolated from the pre-mRNP fraction of oocytes of *Xenopus laevis*. The protein was separated on a preparative SDS polyacrylamide gel, extracted from gel slices with 8 M urea, 0.2 M KCl, 2 mM EDTA, 10 mM Tris/HCl, pH 7.5, and renatured in the presence of an equal mass of poly(U). The pp60 poly(U)RNP construct was then used as an immunogen for injection into rabbits (7).

Isolation of nRNP and Density Determination

The *D. hydei* tissue culture cell line H33 (25) was used. The cells were grown at 25°C in a medium as described by Shields and Sang (23) containing 10% fetal calf serum and adjusted to concentrations between 2×10^6 and 4×10^6/ml. Labeling conditions and isolation of nRNP were performed as described earlier (15). For density centrifugation in CsCl nRNP complexes were fixed with 4% neutralized formaldehyde in RNP isolation buffer (100 mM NaCl, 1.5 mM MgCl₂, 20 mM Tris/HCl, pH 7.2). The formaldehyde-fixed RNP complexes were applied to 20–50% CsCl step gradients in RNP isolation buffer and centrifuged at 35,000 rpm for 24 h at 20°C in an SW 60 Ti swing-out rotor. 200-μl fractions were collected from the top. Labeled material was detected after precipitation of gradient fractions with trichloric acid on glass-fiber filters. Radioactivity was determined by counting washed and dried filters in toluene-based scintillation cocktails.

Immunoblotting

For immunoblotting 50-μl aliquots of each gradient fraction were applied to nitrocellulose filters using a slot blot apparatus. The filters were preincubated for 1 h in a blocking solution (5% non-fat dry milk, 10 mM Tris/HCl, pH 7.2, 140 mM NaCl, 0.5% Tween 20) adapted from Billingsley et al. (2). After preincubation the filter for the monoclonal antibody S5 was

washed for 15 min in the same solution but with the reduction of non-fat dry milk to 1% (140 mM washing solution). For the polyclonal antibodies Dm 28K2 and pp60 the NaCl molarity was raised to 1 M (1 M washing solution). The antiserum incubation was performed in the 1 M washing solution with the antibodies Dm 28K2 or pp60 (1:1,000) or in the 140 mM washing solution with antibody S5 (0.5 μg/ml) for at least 6 h. After the incubation the filters were washed with three changes of the respective washing solutions within 1 h. The second antibodies were alkaline phosphatase conjugated–goat anti–rabbit IgG or goat anti–mouse F(ab')₂ (Dianova, Hamburg, FRG) at a dilution of 1:5,000 in the respective washing solution. The filters were incubated for at least 6 h and then washed three times, 15 min for each wash. The color reaction was carried out with nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indoxyl phosphate potassium salt (SERVA, Heidelberg, FRG) (17).

Indirect Immunofluorescence

Testes from males were dissected in *Drosophila* Ringer's solution (18 mM KCl, 46 mM NaCl, 3 mM CaCl₂, 10 mM Tris/HCl, pH 7.2) and transferred into a drop of the same solution on a clean glass slide. The testes were squashed under a siliconized coverslip and frozen in liquid nitrogen. After removal of the coverslip the preparations were fixed first in 96% ethanol for 2 min and then 5 min in 3.7% unbuffered formaldehyde solution. The slides were washed twice in PBS for 10 min. The concentration of the first antibodies was adjusted to 20–40 μg/ml. As second antibodies 1:80 dilutions of fluorescein isothiocyanate–labeled goat anti–mouse IgG F(ab')₂ (Dianova, Hamburg), Texas Red–labeled goat anti–mouse IgG F(ab')₂ (Medac, Hamburg, FRG), and a 1:40 dilution of fluorescein isothiocyanate–labeled goat anti–rabbit IgG (Dianova) were used. Incubations were carried out at room temperature for 45 min each with both first and second antibody in a moist chamber. For double immunofluorescence the preparations were incubated with both first antibodies simultaneously. After washing for 20 min in PBS the two, differently labeled, second antibodies were applied. The preparations were then washed again in PBS and mounted in *p*-phenylenediamine/glycerol (13). They were examined and photographed under phase contrast or epifluorescent illumination using a Zeiss photomicroscope III equipped with an oil immersion objective (Plan-Neofluar 63/1.25). The filter combination for fluorescein isothiocyanate was 450–490 nm/520 nm, and 546 nm/590 nm for Texas Red. Micrographs were taken on Ilford HP5.

Electron Microscopy

Testes for thin sections of spermatocytes were fixed for 1 h in 2.5% glutaraldehyde, 0.1 M phosphate buffer, pH 7.4, at 4°C. The fixed material was rinsed in phosphate buffer, dehydrated with a graded series of ethanol, washed with propylene oxide, and embedded in Epon 812. Sections were stained for 30 s in 5% uranyl acetate, and contrasted with 3% lead citrate additional 30 s (26).

Results

Ultrastructural Data on Y Chromosomal Chromatin

Because of the complicated ultrastructure of the Y chromosomal chromatin seen in ultrathin sections with the electron microscope it seems appropriate to summarize the latest findings about the morphology of these structures so that localization of the antigens may be understood precisely. A more detailed analysis has been carried out previously (10), so that only the main characteristics of the nuclear structures are given. The nooses and the tubular ribbons are characterized by fields of either granules or tubules, whereas the threads are differentiated into a compact fibrillar axis and a surrounding granular matrix. Both the pseudonucleolus and the clubs represent active chromatin in which more than two elements contribute to their overall appearance. Because these are the elements that predominantly react with the antibodies as shown below, some further information on their structure is given.

The body or the pseudonucleolus (Fig. 1 *a*) is differentiated into a fibrillar matrix which is penetrated by a system
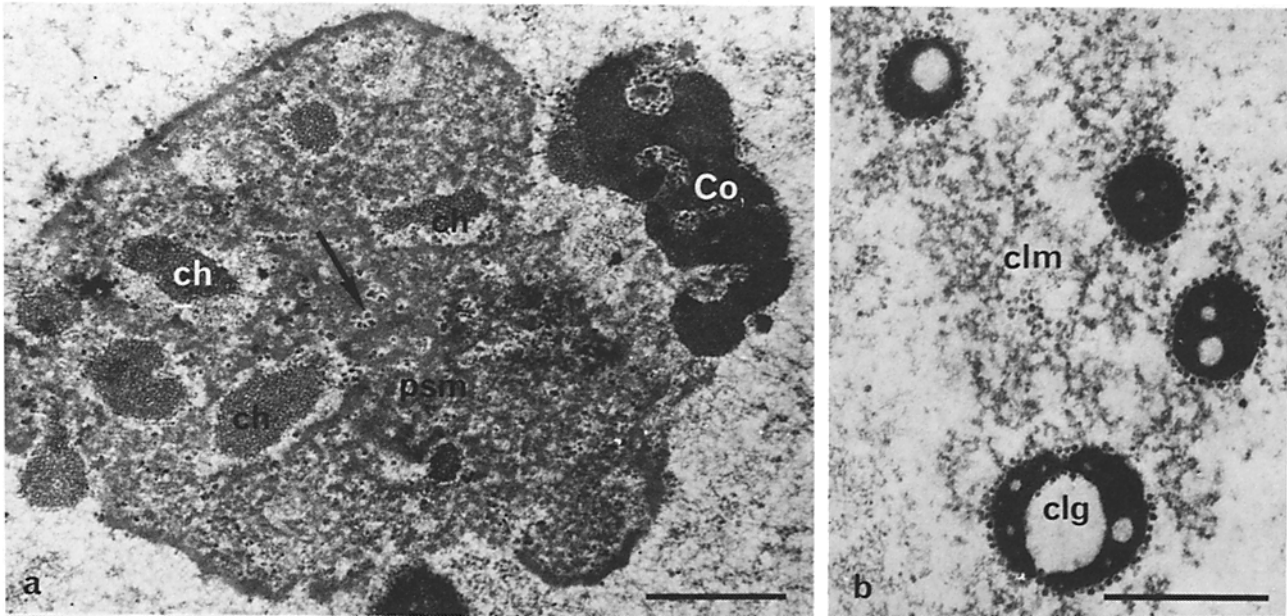
Figure 1. Ultrathin sections of Y chromosomal structures in the nucleus of *D. hydei* spermatocytes. (*a*) The pseudonucleolus is composed of different elements. In the fibrillar matrix (*psm*) RNP granules are embedded which are surrounded by a "halo" of low electron density (*arrow*). Granules of similar size are found in the cones (*Co*). The pseudonucleolus is interspersed with channels (*ch*) which are filled with RNP material. This material is continuous with the cones. (*b*) The clubs are composed of large spheres, called club grana (*clg*), which are interspersed in the fibrillar network of the club matrix (*clm*). The club grana are characteristically surrounded by an array of smaller RNP granules and often exhibit an "empty" center. Bars: (*a*) 1 μm; (*b*) 0.5 μm.

of "channels." The electron-dense fibrillar material, which fills the more or less continuous channels, leads to the cones, which appear to be composed of the same but slightly denser material. The cones are the links to the thread axis. In the matrix of the pseudonucleolus as well as in the cones, large granules of ∼35 nm are detectable. With their clear "halo" these granules resemble the perichromatin granules that have been described in the nuclei of a number of other organisms (reference 6 for review).

The clubs generally appear as two bodies which do not fuse as does the bipartite pseudonucleolus. Large grana of ∼0.5 μm in diameter are interspersed in the fibrillar matrix of the clubs (Fig. 1 *b*). During spermatocyte development these grana increase in volume and concommitantly one to several "vacuoles" or "empty centers" appear. The club grana are characteristically surrounded by smaller granules.

Immunofluorescence in Spermatocyte Nuclei Using Antibody Dm 28K2

In wild-type spermatocytes only two of the five Y chromosomal loop structures, namely the clubs and the pseudonucleolus, contain antigens that react with antibody Dm 28K2 (Fig. 2 *a*). In spermatocyte nuclei of males with the XO genotype, however, only one to several grana in the vicinity of the nucleolus (NO) give positive immunofluorescence (Fig. 2 *d*). The remaining nuclear content shows no concentrations of antigenic sites above a low positive background level. More detailed information is obtained when genotypes with appropriate Y chromosomal deficiencies are used for immunofluorescence analysis. In micrographs of nuclei containing only the threads and the pseudonucleolus it becomes evident that the cones are stained more weakly

than the body of the pseudonucleolus (cp. Figs. 2, *a–c*). Although the pseudonucleolus exhibits a mostly uniform immunofluorescence, the material in the channels of this chromosomal structure remains undecorated (Fig. 2 *c*). Remarkably the adjacent nucleus of a cyst cell shows no detectable immunofluorescence (Fig. 2 *b*, arrowhead).

The clubs show the most intense reaction with antibody Dm 28K2. The club grana as well as elements of the club matrix are decorated. This becomes particularly obvious in genotypes where the clubs have lost their compact consistency (Fig. 2 *c*). The high specificity of the immunoreaction in a spermatocyte nucleus is also demonstrated by double immunofluorescence (Fig. 3). While Dm 28K2 identifies antigens on the clubs, the monoclonal antibody X4 only decorates the RNP material of the tubular ribbons as previously described (9). Neither antibody shows any cross-reaction with the Y chromosomal structure recognized by the other antibody.

Immunofluorescence in Spermatocyte Nuclei Using Antibody pp60

The pp60 antigen is detected in the cytoplasm as well as in the nuclei of primary spermatocytes by indirect immunofluorescence (Fig. 4). In the cytoplasm immunolabeled structures can be observed that measure up to 1 μm in diameter. There is no obvious correlation between the concentration of mitochondria and these elements (e.g. Fig. 4 *c*). In some squashes the RNP material that is found in pouches of the nuclear envelope (10) appears to be stained (Fig. 4 *a*). The pseudonucleolus and the cones are the structures predominantly labeled by the antibody pp60 in spermatocyte nuclei. A comparison with light microscopical and ultrastructural
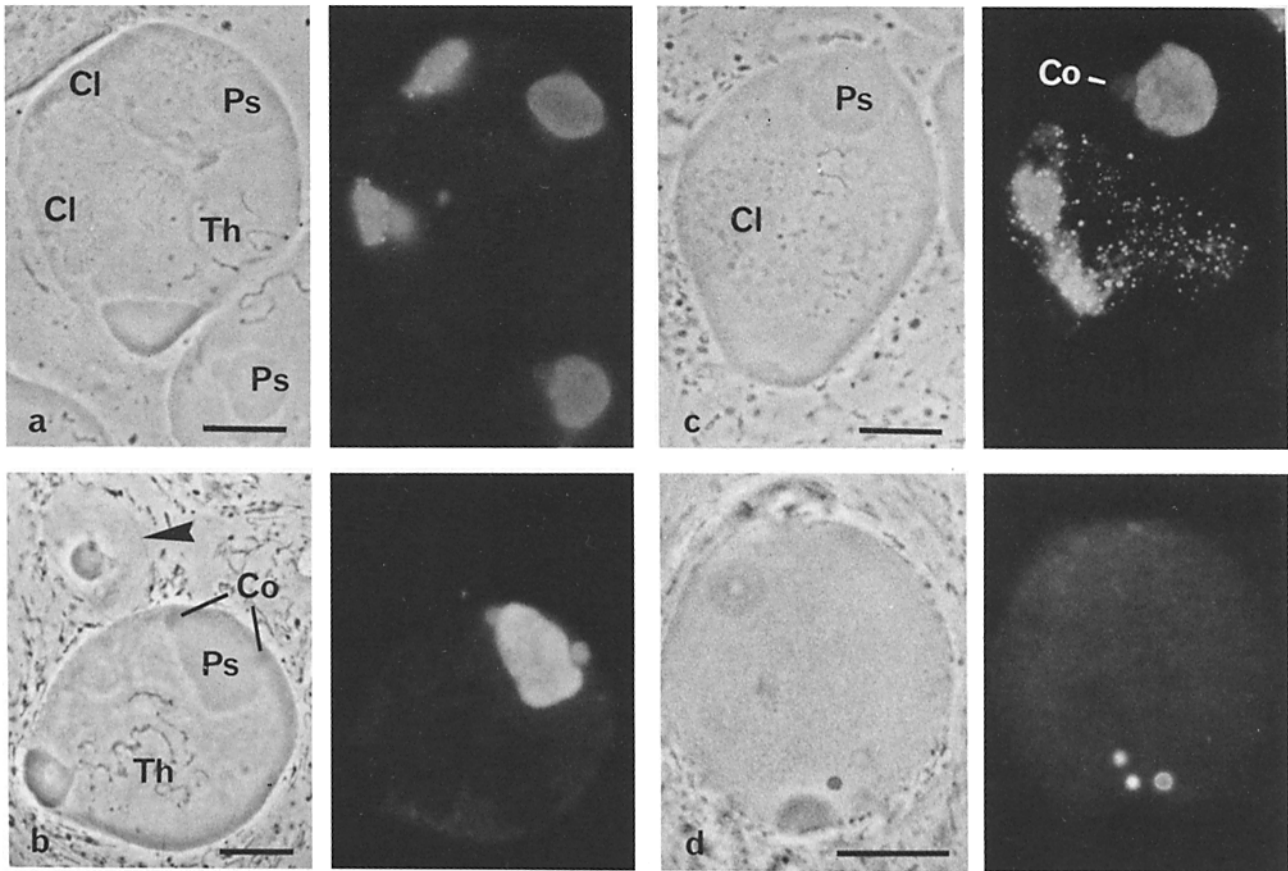
Figure 2. Spermatocyte nuclei of various genotypes of *D. hydei* in phase contrast (*left*) and immunofluorescence after reaction with antibody Dm 28K2 (*right*). (*a*) Wild type. The pair of the clubs (*Cl*) and the single pseudonucleolus (*Ps*) are decorated. (*b*) Th Ps genotype. Only the pseudonucleolus is immunostained. No detectable fluorescence is found in the adjacent nucleus of a cyst cell (*arrowhead*). Note the weaker reaction of the cones (*Co*). (*c*) Ns Cl Ps Th genotype. In this genotype the Tr region is missing, resulting in an altered topological arrangement of the clubs. The micrograph shows that not only the club grana but also the club matrix is decorated. In the pseudonucleolus the channels are clearly not decorated. (*d*) XO genotype. The nucleus shows no concentration of Dm 28K2 antigens with the exception of a few granules near the nucleolus, which presumably represent X chromosomal activity. Bars, 10 µm.
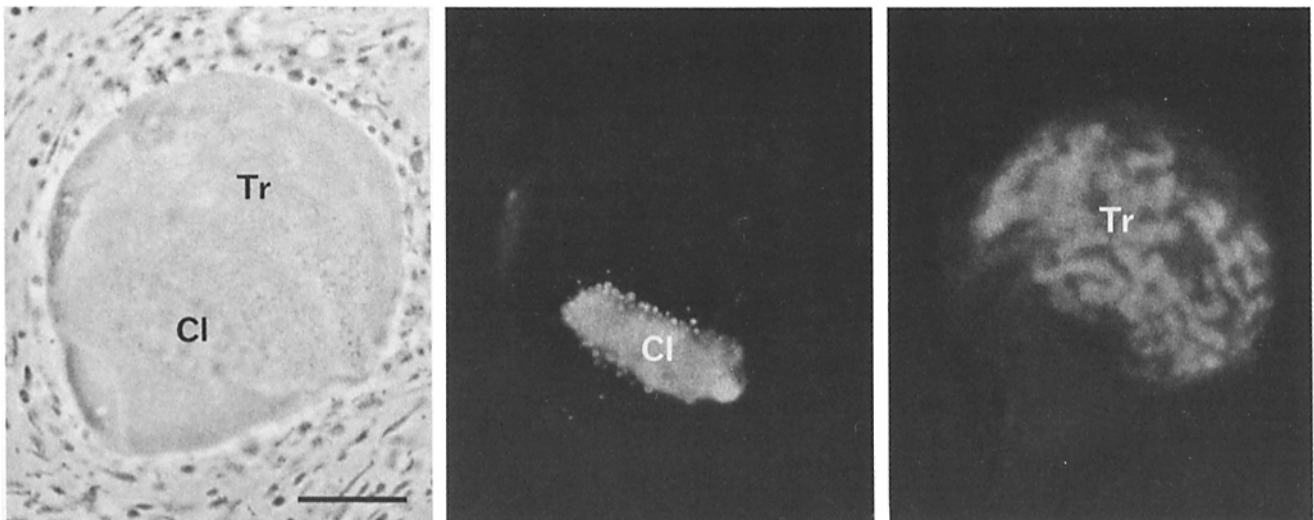

Figure 3. Spermatocyte nucleus of the Ns Cl Tr genotype in phase contrast (*left*), double immunolabeled with antibody Dm 28K2 (*middle*), and the class I monoclonal anti-RNP antibody X4 (*right*) (19). The primary antibodies raised in rabbit (Dm 28K2) and in mouse (X4) were identified with fluorescein isothiocyanate-labeled goat anti-rabbit IgG and Texas Red-labeled goat anti-mouse IgG F(ab')$_2$ as second antibodies. Clearly two RNP populations are accumulated at two different Y chromosomal sites without an overlapping distribution. Antibody Dm 28K2 decorates the clubs (*Cl*), and it appears that not only the club grana but also the club matrix is immunolabeled. In contrast, the antibody X4 detects antigens that are accumulated on the tubular ribbons (*Tr*) (9). The presence of X4 antigen elsewhere in the nucleus than on Y chromatin, i.e., the background fluorescence, is characteristic. Bar, 10 µm.
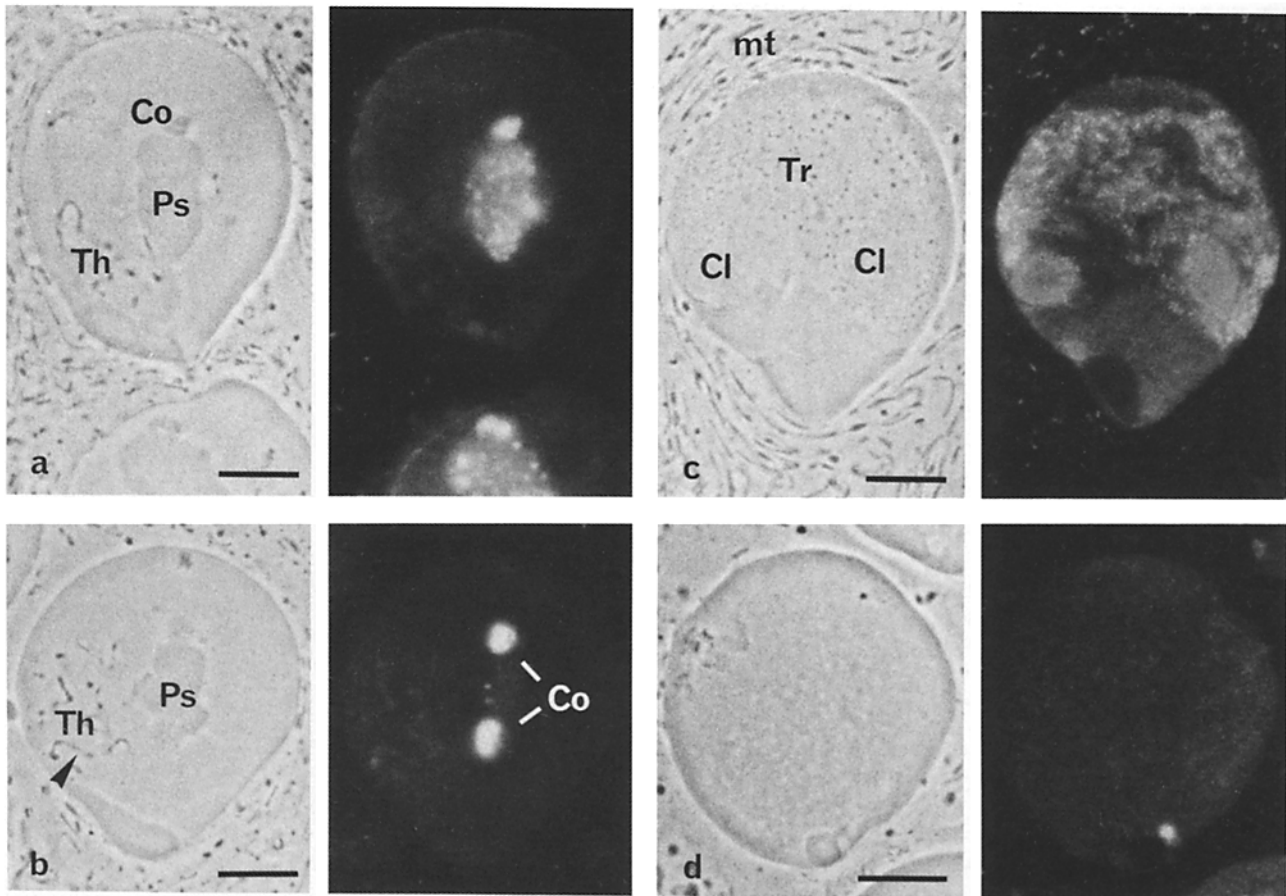
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Figure 4. Spermatocyte nuclei of various genotypes of *D. hydei* in phase contrast (*left*) and immunofluorescence after reaction with antibody pp60 (*right*). (*a*) Th Ps genotype. The cones (*Co*) and the channel material of the pseudonucleolus (*Ps*) are stained intensely. The dense part of the proximal thread axis (*arrow*) as well as RNP along the nuclear envelope show accumulations of pp60 antigen. (*b*) Th Ps genotype with a mutated pseudonucleolus. Only the cones are immunostained. The antigens normally associated with the channel material are missing. The dense thread axis is slightly positive (*arrowhead*). (*c*) Ns Cl Tr genotype. Above a positive background the clubs (*Cl*) appear as two homogeneously immunostained elliptical structures. The contour of the tubular ribbons (*Tr*) can be traced by its granularly stained axis. The region of the nooses is not detectable by immunoreaction as can also be seen in *d*. (*d*) Ns genotype. No accumulation of pp60 antigen is found. In all genotypes elements in the cytoplasm react with antibody pp60. This is clearly seen in Fig. 5 *c*. Note that the stained elements are distinct from mitochondria (*mt*). Bars, 10 μm.

data leaves no doubt that it is the channel material that is decorated by this antibody. This material is in conjunction with the cones, which therefore also show a bright fluorescence. This contrasts with the data obtained with antibody Dm 28K2, which leaves the channels free and only decorates components of the matrix.

The location of the pp60 antigen in other nuclear compartments of the spermatocyte nucleus is not quite as clear. The area of the clubs is more or less uniformly decorated, whereas the label in the region of the tubular ribbons obviously follows the loop axis (Fig. 4 *c*). A higher concentration of pp60 antigen is also found along the compact axis of the threads (Fig. 5 *b*, arrowhead). Again in spermatocyte nuclei of XO and Ns genotypes there are no obvious concentrations of the pp60 antigen with the exception of some characteristic dots near the nucleolus in Ns spermatocytes, which do show a slight stain (Fig. 4 *d*). The antigen bearing structures in the cytoplasm appear to be unaffected by the loss of the Y chromosome and look like wild-type preparations.

Correlation of Dm 28K2 and pp60 Antigens with nRNP Complexes of D. hydei

The antibodies Dm 28K2 and pp60 used for the detection of antigens associated with the active Y chromosome in primary spermatocytes of *D. hydei* are both described to be directed against RNP proteins. Whereas Dm 28K2 is raised against a scRNP of somatic cells of the distantly related species *D. melanogaster*, pp60 is specific for an RNP protein of germ cells, i.e., oocytes of the amphibian *Xenopus*.

The data obtained by indirect immunofluorescence suggest that both antibodies detect RNP complexes of *D. hydei*, too. The conclusive proof, however, is to show that the antigens recognized by the antibodies are indeed constituents of RNP complexes in *D. hydei*. Because it is difficult to isolate RNP material from spermatocyte nuclei in amounts that are sufficient for biochemical analysis, *D. hydei* H33 culture cells (25) were used instead. The RNA of H33 cells was pulse labeled with [³H]uridine and the nRNP isolated. After fixa-
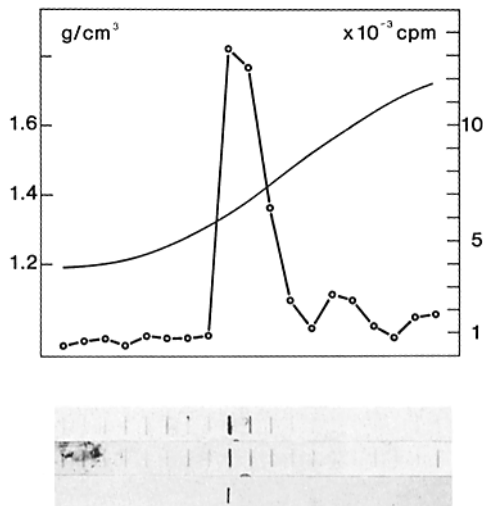
Figure 5. CsCl density gradient analysis of [³H]uridine-labeled formaldehyde-fixed nRNP of *D. hydei* H33 cells incubated in the presence of 5-fluorouridine. Aliquots of the different density gradient fractions were slotted onto nitrocellulose filters and immunoreacted with the polyclonal antibodies pp60 and Dm 28K2, and the monoclonal antibody S5 (from top to bottom). The main reaction of all three antibody stainings appears in fraction 9 and corresponds to the radioactivity peak of the RNA component of the RNP complexes at the characteristic buoyant density of ~1.4 g/cm³.

Figure 6. Tentative model on the nuclear localization of RNP antigens in spermatocytes of *D. hydei.* The scheme is modified from Glätzer (9). Both ends of the Y chromosome carry one nucleolus organizer thus bringing the tips of the chromosome together in one nucleolus (*NO*). The open chain of the active Y chromosome is drawn as a thin line. The distribution of the antigens Dm 28K2 (×) and pp60 (○) is only shown along one of the two Y chromatids. The nooses and the remaining nuclear constituents show no detectable concentrations of the RNP antigens. *Cl,* clubs; *Co,* cones; *Ns,* nooses; *Ps,* pseudonucleolus; *Th,* threads; *Tr,* tubular ribbons.

tion of the RNP with formaldehyde the RNP complexes were analyzed with respect to their buoyant density in a CsCl gradient. As shown in Fig. 5 most of the labeled RNA bands at a buoyant density of ~1.4 g/cm³, characteristic for nonribosomal nRNP. The minor radioactivity peak most likely represents residual ribosomal RNP (15).

To identify the position of the Dm 28K2 and pp60 antigens in the density gradient an immuno slot blot using the above antibodies was performed across the gradient. The previously described monoclonal anti-RNP antibody S5 (18, 19) was taken for comparison. As seen in Fig. 5 the antigens detected by the three different antibodies are predominantly positioned in the nonribosomal RNP fraction. This finding strongly suggests that the immunolabeled nuclear material in spermatocytes of *D. hydei* in fact represents ribonucleoproteins.

Discussion

Our results demonstrate that the antibodies Dm 28K2 and pp60 recognize nRNP antigens of *D. hydei*, and that in nuclei of primary spermatocytes several distinct RNP populations exist with respect to their protein composition. Moreover, the different RNP complexes are not randomly distributed within the nucleus but can be attributed to specific structures of the active Y chromosome.

Previously it was shown that the Y chromosomal structures called thread matrix and tubular ribbons represent the main nuclear compartments for hnRNP complexes as specified by the hnRNP-specific monoclonal antibodies S5 and X4 (9, 18, 19). In contrast, the RNP antigens recognized by the antibodies Dm 28K2 and pp60 possess quite different nuclear specificities.

Dm 28K2 identifies RNP complexes distributed in the matrix of the pseudonucleolus, in the cones, and within the
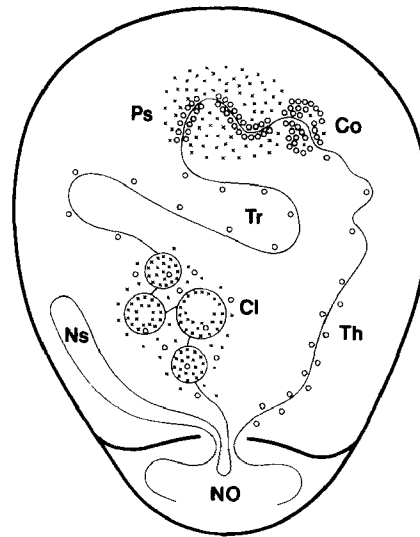
clubs. Comparison with ultrastructural data suggests that the RNP particles recognized by this antibody in the pseudonucleolus possess structural similarities with the so-called perichromatin granules (5, 6). These granules appear to be scattered throughout the matrix of the pseudonucleolus and form clusters in the cones.

The RNP granules around the large club grana initially give the impression that they are identical with the perichromatin like RNP particles observed in the pseudonucleolus matrix. However, their diameter is only half of that of the RNP granules in the pseudonucleolus. Therefore, the club granules probably represent different RNP structures, and our conclusion derived from immunostainings of several genotypes is that the Dm 28K2 antigens are associated with both the club grana and the club matrix does not seem improbable. A final answer to this question should be given by electron microscopic immunocytochemistry.

Antigen pp60 on the other hand is part of RNP material close to the axes of the Y chromosomal loops and is strongly accumulated in the channels and in the cones of the pseudonucleolus. This is a localization quite distinct from that of antigen Dm 28K2. Thus, the pseudonucleolus is a good example of the existence of different RNP populations within a same nuclear structure. A summary of the presumed location of the antigens recognized by the two antibodies in nuclei of primary spermatocytes of *D. hydei* is given in Fig. 6.

The results presented in this paper together with previous data (9) provide unequivocal evidence that the distinct morphological differences between the active Y chromosomal loops are in part caused by the presence of qualitatively and possibly also functionally different RNP populations. The latter conclusion finds its support in an earlier observation

(11) that the absence of a specific Y chromosomal loop or inactivity of a certain loop forming site cannot be compensated by an increased dosage of another, different Y loop.

It is evident from the density gradient analysis that both antibodies, which are directed against proteins from different species, i.e., *D. melanogaster* and *Xenopus laevis*, identify nRNP antigens in *D. hydei*. The pp60 antigen was first described as a tightly bound 60-kD unphosphorylated pre-mRNP protein of amphibian oocytes where it preferentially interacts with uridine-rich RNA sequences (14). In its phosphorylated state, however, this protein appears to be part of maternally stored, i.e., repressed mRNP in the cytoplasm of *Xenopus* oocytes (7). Biochemical analysis of nRNP from *D. hydei* by Western blot reveals that pp60 cross-reacts with an RNP protein of 96 kD (unpublished data). Thus, the 96-kD protein in *Drosophila* may represent the functional analogue of the pp60 antigen in amphibian oocytes and marks RNP complexes for germ line specific storage of genetic information. In addition, not only the 60-kD amphibian polypeptide but also the 96-kD protein of *D. hydei*, both of which are recognized by the same antibody pp60, can be found in the nucleus as well as in the cytoplasm. An exchange of RNP between the nuclear and the cytoplasmic compartments can be deduced from the immunoreacting material outside the nuclear envelope, which may represent the link between cytoplasmic and nRNP.

In contrast to pp60 three nRNP polypeptides with apparent molecular masses of 64, 55, and 52 kD share antigenic determinants recognized by antibody Dm 28K2 (unpublished data). This is noteworthy, since Dm 28K2 identifies proteins in the range of 28 kD, which are constituents of the morphologically distinct cytoplasmic 19S ring-type small, cytoplasmic RNP particles in *D. melanogaster* as well as in *D. hydei*. With respect to their function these cytoplasmic particles are discussed to be connected with the repression and storage of cytoplasmic RNP in the case of duck erythroblasts (20). The possible functional relationship between the nuclear and the cytoplasmic antigens detected by Dm 28K2 in *D. hydei* remains to be resolved.

The Y chromosomal loops cannot be understood in isolation. Their biological significance is the role that they play in the process of sperm development. Our data provide additional arguments that this role is to provide chromatin compartments for distinct RNP populations which most likely exert their function in post-meiotic stages during spermatogenesis.

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