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A stochastic mechanism controls the relative replication of equally competent ribosomal RNA gene sets in individual dipteran polyploid nuclei

(nurse cells/salivary glands/*Calliphora erythrocephala*/nontranscribed spacer polymorphisms/relative allele activity)

ESTHER J. BELIKOFF AND KATHY BECKINGHAM*

Department of Biochemistry, Rice University, P.O. Box 1892, Houston, TX 77251

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ABSTRACT **The endoreplication of the two nucleolar organizers (NOs) of the diploid genome has been examined in individual polyploid nuclei of the dipteran *Calliphora erythrocephala*. Crosses between two strains with diagnostic nontranscribed spacer polymorphisms in their rRNA genes were used to provide progeny with distinguishable NOs, and single nuclei of two highly polyploid cell types—salivary gland and nurse cells—were examined from individual F_1 animals. Initially the representation of the two NOs in total polyploid tissue DNA was determined. This revealed that, although the NO regions present in one of the strains (Tom) were very similar in spacer composition, they displayed two types of behavior in the hybrids containing the single NO region typical of the second strain (Karla). In TW phenotype F_1 progeny, very little replication of the Tom NO relative to the Karla NO occurred, whereas in TS phenotype progeny replication of the Tom and Karla NOs was approximately equivalent. When individual polyploid nuclei of the TS phenotype animals were examined, however, the relative replication of the Tom and Karla NOs was found not to be a fixed genetic property but to vary dramatically from cell to cell. This was true even for the nurse cell nuclei within a single ovarian follicle, which are the products of only four mitotic divisions of a single germ-line cell. These findings indicate that for NOs of similar replicative competence, a stochastic mechanism governs the relative usage of each NO for endoreplication and that the relative activity of the two NOs is not stably determined through the mitotic divisions preceding polyploidization. Stochastic selection after mitotic DNA replication could be a general phenomenon governing the relative usage (transcription) of different, but equally competent, alleles of any gene in individual cells, if the required factors are in short supply.**

Within the Diptera, the replication of the rRNA genes (rDNA) during polyploidization has long been known to be independent of the replication of the euchromatic genome (1–4). The mechanisms underlying this independent regulation of rDNA polyploidization are not understood but the studies of Spear and Gall (2, 3) have established that in polytene salivary gland tissue, the rDNA is autonomously replicated to a constant level that is independent of both the amount of rDNA initially present in the diploid genome and the number of nucleolar organizer (NO) regions within the genome in which this rDNA is distributed. It has been suggested that endoreplication of rDNA cistrons from only one NO may in part explain these observations (1, 5–7).

Recent studies have established that, at the level of the total polyploid tissue DNA, the overall contribution of the two NOs to the polyploidization of the rDNA is a function of the pair of NOs examined (6, 8–12). In some cases, one NO is replicated to the virtual exclusion of the other, whereas for other NO pairs, cistrons from both NOs are equally well represented in the total polyploid tissue DNA. Such findings cannot be fully interpreted, however, without an examination of NO replication in individual polyploid cells, for, given the hypothesis that only one NO is polyploidized in any individual cell, these results could reflect differences for particular NO pairs in the number of cells choosing to replicate one or the other of the two NOs of the genome.

Using the dipteran *Calliphora erythrocephala*, we have examined the polyploidization of pairs of distinguishable NOs in individual polyploid nuclei from two very different tissues—the somatic salivary gland cells and the germ-line nurse cells of the ovarian follicles. These experiments and others we have performed (13) have established that in individual cells, cistrons from both NOs are used for polyploidization. They have also revealed an additional unexpected phenomenon. For NO regions with an overall similar capacity for endoreplication at the level of total tissue DNA, dramatic variation in their relative polyploidization nevertheless occurs in the individual nuclei of the polyploid tissue.

MATERIALS AND METHODS

Fly Strains Studied. The fly strains studied were selected for containing NO regions with highly diagnostic rDNA nontranscribed spacer (NTS) polymorphisms, identified by hybridization of a cloned NTS-containing rDNA fragment, pBW1 (14), to blots of *Rsa* I-cleaved genomic DNA. The Tom strain was derived from a single pair mating of individuals from a fly stock obtained from E. Thomsen, Copenhagen, Denmark. The pattern of hybridization of pBW1 to *Rsa* I-cleaved DNA from individuals of the original Thomsen stock indicated the presence of two NO types in the population—one carrying the diagnostic Tom NTS fragment (see *Results*) but lacking *Rsa* I fragments A and B common to all other stocks examined (Fig. 1) and one containing both the Tom band and fragments A and B. Both parents used to generate the Tom strain were homozygous for NOs containing only the Tom NTS fragment. The Karla strain was isolated from a mixed stock of flies generated by interbreeding individuals from three different stocks—Cambridge (from the Zoology Department, Cambridge, UK), Karlson (from M. Jamrich), and Levenbook (from L. Levenbook)—which contained, therefore, NOs typical of these three stocks. By using the progeny from a single pair mating in which the male parent was homozygous for NOs of the Cambridge stock (Cam NO) and the female parent was heterozygous for a Cam NO and an NO typical of the Karlson stock, two further

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Abbreviations: bp, base pair(s); NO, nucleolar organizer; NTS, nontranscribed spacer; rDNA, genes for ribosomal RNA.
*To whom reprint requests should be addressed.

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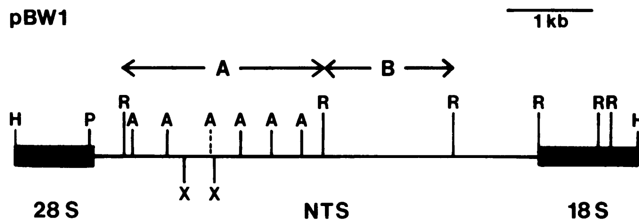

FIG. 1. Restriction enzyme site map for the rDNA *Hin*dIII fragment present in clone pBW1. This clone was originally isolated from the Clever strain of *C. erythrocephala* (16). Sites for *Alu* I (A), *Hin*dIII (H), *Pvu* II (P), *Rsa* I (R), and *Xba* I (X) are shown. Only the *Alu* I and *Xba* I sites within *Rsa* I fragment A are shown. The *Alu* I sites in the right-hand region of fragment A are within the four 350-bp repeating units present in this clone (14). The *Alu* I site indicated by a dashed line represents a site seen in one of the 350-bp units in other cloned spacers (14) but not detected by us in pBW1. kb, Kilobase.

rounds of single pair matings were performed. A single pair homozygous for the Karla NO was found among the third round matings and its progeny mass-mated to yield the Karla strain. The Karla strain is therefore homozygous for the single Karlson-type NO region present in the female parent of the first round single pair mating. The Tom strain, however, contains four NO regions, which, although essentially indistinguishable when judged by Southern hybridization, could contain genetic differences (see *Results*).

DNA Preparations and Digestions. Preparations of nurse cell nuclei from whole ovaries were performed as described (15). DNA from these preparations and from brains and salivary glands was prepared, quantitated, digested, and subjected to electrophoresis as described (13). The preparation, digestion, and electrophoresis of DNA from individual salivary gland and nurse cell nuclei have also been described (13).

Other Methods. Southern blots, filter hybridizations, plasmid preparations, and radiolabelings are described elsewhere (13).

RESULTS

Diagnostic NTS Polymorphisms Used to Monitor Endoreplication of Individual NO Regions. The cleavage sites for the enzyme *Rsa* I within clone pBW1 (a *Hin*dIII fragment of the rDNA spanning a NTS region) are shown in Fig. 1. The largest *Rsa* I fragment, fragment A, spans a region of the NTS that contains four repeats of a 350-base-pair (bp) unit (14). The number of these repeats varies in different NTS regions and thus, on hybridization of pBW1 to *Rsa* I-cleaved DNA from different strains of *C. erythrocephala*, variants of this fragment are frequently seen dependent upon the NTS types present in the NOs typical of the strain (16).

The Karla strain that was prepared for these studies is homozygous for a single NO region derived originally from a fly stock from P. Karlson's laboratory. Hybridization of pBW1 to *Rsa* I-cleaved DNA from this strain reveals that some of the NTS regions within this NO contain four repeats and thus generate fragment A but that most of the spacers present generate a fragment ≈350 bp shorter than A, which probably therefore contains only three repeats. Thus, a prominent, diagnostic band of hybridization (the Karla band, Fig. 2) is seen on hybridization of pBW1 to diploid DNA from animals carrying this NO region. The second strain used in this study (Tom strain) contains four related NOs that are very similar in terms of their NTS composition and contain a more unusual spacer polymorphism. The *Rsa* I site that separates fragments A and B in pBW1 and all other spacers examined (Fig. 1) appears to be absent in the NTS regions of these NOs. Thus, fragments A and B are missing from the hybridization patterns for Tom strain individuals, and, instead, a major fragment of their combined size (the Tom band, Fig. 2), which is highly diagnostic for these NOs, is present. An additional minor band that is ≈350 bp larger than the Tom band is also detected in all Tom strain NOs (Fig. 2). This minor band could derive from spacers also lacking the A–B junction *Rsa* I site but that contain five, not four, 350-bp repeats.

Cistrons Containing the Diagnostic Polymorphisms Are Well Replicated in Parental Strain Polyploid Tissues. Differential replication of cistrons within a single NO region during polyploidization has been noted in previous studies (6). Diploid (brain) and polyploid DNA samples (salivary gland or nurse cell nuclear) from individual larvae and adult females of both strains were therefore examined to ensure that the diagnostic NTS fragments are well represented after polyploidization. Within the Karla NO, cistrons containing the diagnostic fragment proved to be the only genes selected for endoreplication since the Karla fragment is the only representative of this region of the NTS seen in both types of polyploid tissue (Fig. 2 *a* and *b*). The Karla band is therefore a very clear marker for monitoring the replication of the Karla NO in polyploid tissues of hybrid animals. The polyploid DNA samples from Tom strain individuals established that the Tom band-containing genes remain the major component of the rDNA after endoreplication (Fig. 2 *c* and *d*) and thus that the NOs present in the strain are also appropriate for examination of individual NO polyploidization in hybrid progeny.

The Four Tom Strain NOs Show Differences in Minor Cistron Composition and Endoreplication. Comparison of diploid DNA samples from different Tom strain individuals revealed barely detectable differences in minor bands of hybridization (Fig. 2 *c* and *d*), indicating that small differences in cistron composition exist between the four NOs

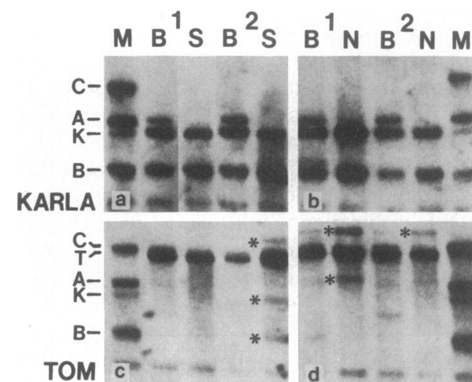

FIG. 2. Diagnostic NTS polymorphisms of the two NO types studied and their polyploidization in salivary gland and nurse cell nuclear DNA. The patterns of hybridization of pBW1 DNA to blots of *Rsa* I digests of diploid and polyploid DNA from individual animals of the Karla and Tom strains are shown. (*a* and *c*) DNA from the brains (lanes B) and salivary glands (lanes S) of two individual third instar larvae of the Karla and Tom strains, respectively. Brain DNA samples, 0.05 μg; salivary gland DNA samples, 0.2 μg (to compensate for rDNA underreplication). (*b* and *d*) DNA from the brains (lanes B) and whole ovary nurse cell nuclear preparations (lanes N) of two individual adult females of the Karla and Tom strains, respectively. DNA samples, 0.2 μg. A and B, bands of hybridization to fragments equivalent to *Rsa* I fragments A and B of pBW1 (Fig. 1). T and K, the diagnostic Tom and Karla bands (see text). Asterisks mark minor bands that are overreplicated in polyploid DNA samples from Tom strain individuals (see text). Lane M (marker DNA), *Rsa* I digest of diploid DNA from a mixed fly population, which contains fragments A and B, the diagnostic Karla fragment, and a NTS fragment (C) typical of another strain (Cambridge). DNA in marker lanes of different autoradiograms was as follows: *a*, 0.05 μg; *b*, 0.1 μg; *c*, 0.05 μg; *d*, 0.2 μg.

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present in this strain. These differences were much more obvious in the polyploid DNA samples, however, where markedly different patterns of minor fragment overreplication were seen in DNA from different animals (Fig. 2 *c* and *d*). Three different patterns of minor fragment replication were seen in salivary gland DNA and three were seen in nurse cell DNA samples. The three patterns of minor bands seen for both tissues could not have resulted from different combinations of two basic patterns of minor cistron replication. Thus, the four NO regions present in the Tom strain must be of at least three different types with respect to minor cistron classes. One of the nurse cell minor band replication patterns was identical to one of the salivary gland patterns, which suggests that, within any one of the Tom NOs, the *same* minor cistrons are selected for overreplication in both nurse cells and salivary gland cells.

Endoreplication of the Karla and Tom NOs in F_1 Interstrain Hybrids. Table 1 details the two single pair crosses of Tom and Karla individuals studied and the individual F_1 progeny examined. Whole brain and whole salivary gland DNA from individual larvae and whole brain and total ovary nurse cell nuclear DNA from individual adult females were prepared and analyzed to establish the overall tissue patterns of NO replication. In addition, individual salivary gland or nurse cell nuclei were isolated from all glands or ovaries at the time of total tissue DNA preparation, so that the replication of the two NOs in individual nuclei could be examined and compared to the total tissue pattern. This analysis revealed the following findings.

The Tom Strain NOs Show Two Kinds of Endoreplicative Behavior in Tom/Karla Heterozygotes. The analysis of the whole tissue DNA samples revealed that the pBW1 hybridization pattern for diploid (brain) DNA from all F_1 progeny was the expected composite pattern of parental bands and also that the replicative behavior of the Karla NO in both types of hybrid polyploid nuclei was identical to that seen in the homozygous parental Karla strain. However, the progeny from both of the Tom/Karla crosses proved to be of two classes with respect to polyploidization of the NO derived from the Tom parent (Fig. 3). In one class (TS, Tom Strong), the Tom fragment diagnostic of the Tom NO was strongly represented in polyploid DNA (either salivary gland or nurse cell). In the second class (TW, Tom Weak), replication of the Tom NO in the polyploid tissues was very weak, yielding a barely detectable Tom band in DNA from these tissues. Approximately half the F_1 progeny of each Tom × Karla cross was of each phenotype (see Table 1).

Two origins for these two replicative phenotypes are possible: either they represent distinct properties of the different NO regions known to be present in the Tom strain or they result from the presence of different alleles at some non-rDNA locus(i) of the genome, the products of which differ in their capacity to interact with the Tom NO regions. Our data provide some evidence in favor of the first possibility and specifically indicate that the ability of a Tom NO to undergo strong polyploidization is a function of its total rDNA content. Thus, when the hybridization patterns for the *diploid* DNA samples from the TS and TW progeny were examined closely, the relative intensities of the Tom and Karla bands of hybridization in TS animals were found to differ from those seen for TW animals (Fig. 3). Of the 9 TS

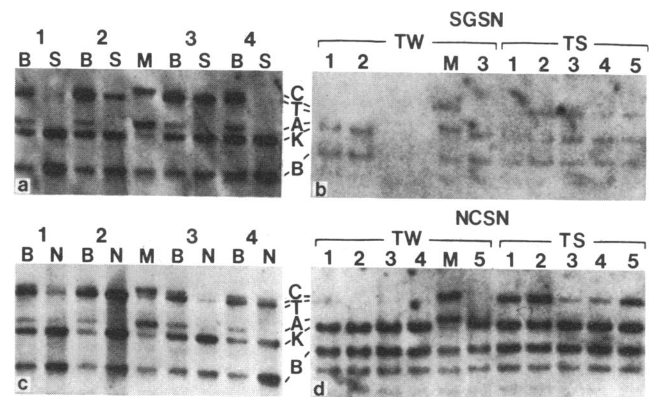

FIG. 3. Representation of the Tom and Karla NOs in diploid and polyploid nuclei of individual F_1 progeny from Tom × Karla crosses. The hybridization of pBW1 to *Rsa* I digests is shown. (*a*) DNA from brains (lanes B) and salivary glands (lanes S) of four F_1 larvae of the KT1 cross (Table 1). Animals 2 and 3 show the TS phenotype; animals 1 and 4 show the TW phenotype (see text). Note that diploid DNA of the two TS phenotype animals shows greater hybridization to the Tom band relative to the Karla band than diploid DNA from the TW phenotype individuals. DNA sample sizes as Fig. 2. (*b*) DNA from three individual salivary gland nuclei (SGSN, salivary gland single nuclei) from a single TW phenotype larva and five individual salivary gland nuclei from a TS phenotype larva of the TK16 cross. Four of the five TS phenotype nuclei contain both the Tom and Karla bands and differ from one another in the relative intensities of these bands. (*c*) DNA from brains (lanes B) and whole ovary nurse cell nuclear preparations (lanes N) for four individual F_1 adult females of the TK16 cross. Animals 2 and 4 show the TS phenotype and animals 1 and 3 show the TW phenotype. Animal 4 was the only TS phenotype animal examined in which the Tom band of diploid DNA did not show greater hybridization than the Karla band. DNA sample sizes as Fig. 2. (*d*) DNA from five individual nurse cell nuclei (NCSN, nurse cell single nuclei) of both a TW and a TS phenotype female. The Tom band can be detected in two of the TW nuclei. Note differences in intensity of Tom band among TS nuclei. Marker DNA (M) and band designations (C, T, A, K, and B) as in Fig. 2. Marker DNA in different autoradiograms: *a*, 0.05 μg; *b*, 0.85 ng; *c*, 0.2 μg; *d*, 3.4 ng.

Table 1. Single pair strain crosses used in this study and individual F_1 progeny examined

Cross number	Parental strain: Female parent	Male parent	Progeny examined,* no.: Third instar larvae	Adult females	Successful hybridizations for single nuclei,† no.: Salivary gland nuclei	Original larvae	Nurse cell nuclei	Original females	Related nurse cell nuclei‡	Original follicles
KT1	Karla	Tom	4 (2 TS, 2 TW)	5 (1 TS, 4 TW)	5 (1 TS, 4 TW)	3 (1 TS, 2 TW)	25 (5 TS, 20 TW)	5 (1 TS, 4 TW)	—	—
TK16	Tom	Karla	4 (2 TS, 2 TW)	7 (4 TS, 3 TW)	14 (7 TS, 7 TW)	4 (2 TS, 2 TW)	55 (35 TS, 20 TW)	6 (4 TS, 2 TW)	40 (25 TS, 15 TW)	14 (10 TS, 4 TW)

The designations TS and TW in parentheses indicate number of animals, follicles, or nuclei of the "Tom Strong" and "Tom Weak" phenotypes, respectively, in each group.
*For all progeny, total DNA from both the diploid and the appropriate polyploid tissue was examined.
†Individual nuclei were derived from progeny listed in columns to the left.
‡These nuclei are a subset of the nurse cell nuclei listed in the column to the left.

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phenotype animals examined, all but 1, which could not be decided unequivocally (animal 4, Fig. 3*c*), showed stronger hybridization to the Tom band than to the Karla band, whereas for all of the 11 TW phenotype animals, the Tom band was of equal or weaker intensity than the Karla band. Given that a single Karla NO was made homozygous to form the Karla strain, the hybridization to this band is an appropriate internal standard. This suggests that Tom NOs containing less rDNA than the Karla NO show a TW phenotype.

In TS Phenotype Animals, Individual Polyploid Nuclei Replicate the Tom and Karla NOs to Different Extents. In preparing hybridization patterns for individual nurse cell nuclei, our initial approach was to examine random nuclei taken from the total ovarian nurse cell nuclear preparations from given individual F_1 females. Five nuclei were examined from each of three different TS phenotype females. All of the hybridization patterns were of high quality (Fig. 3) and demonstrated two phenomena very clearly. First, both the Tom and Karla NOs were seen to be replicated in each individual nucleus, showing conclusively, as demonstrated by other crosses we have performed (13), that the hypothesis of single NO replication within individual cells is incorrect. Second, very dramatic variation in the relative replication of the two NOs was seen among the individual nurse cell nuclei from the same animal. In the example shown in Fig. 3, replication of the Tom NO in different nuclei varies from being approximately equal to that of the Karla NO to being considerably lower, but in one of the other TS females examined an even greater range of variation was seen, with one nucleus showing much greater replication of the Tom NO relative to the Karla NO and two nuclei showing almost negligible Tom NO replication.

The rDNA is underreplicated in salivary glands (refs. 1 and 2; unpublished observations) but proportionally replicated in nurse cell nuclei (15) and therefore detection of the much smaller quantities of rDNA present in individual salivary gland nuclei is more difficult. Salivary gland nuclei also appeared to be frequently contaminated with a presumed endogenous DNase. For these reasons fewer successful examples of hybridization patterns for individual salivary gland nuclei than nurse cell nuclei were obtained. In total, hybridization patterns for eight individual salivary gland nuclei from three TS phenotype larvae were obtained (Table 1). Although the data from these samples were of poorer quality than for the nurse cell nuclei, both phenomena noted in nurse cells were nevertheless discernible—that is, both NOs are replicated in individual salivary gland nuclei and the relative replication of the two NOs varies between individual salivary gland cells in the same animal (Fig. 3).

Individual nurse cell and salivary gland nuclei from TW phenotype animals were also examined (Table 1 and Fig. 3) but the expected weak Tom band of hybridization proved to be at the limit of detection for these nuclei and was only seen in those samples showing greater overall hybridization signal intensity—that is, greatest DNA recovery. It was not possible therefore to determine whether any real variation in the level of Tom NO replication was occurring in the individual nuclei of this phenotype.

Individual Nurse Cell Nuclei Within a Single Ovarian Follicle Show Variation in the Replication of the Two Genomic NOs. Within the Diptera, each ovarian follicle contains a single oocyte connected by cytoplasmic bridges to 15 nurse cells (17). These 16 cells are the products of four mitotic divisions of a single germ-line cell. Each of the synchronously developing follicles within the *C. erythrocephala* ovary is derived from the germ-line cells of a different ovarian compartment (ovariole), however, and, thus, each originates from a distinctly separate germ-cell lineage. It was of interest, therefore, to determine whether the variation in Tom and Karla NO replication seen in different nurse cell nuclei reflects differences between nurse cell nuclei derived from different follicles (i.e., different germ-cell populations) or whether these differences would be present in the related nurse cell nuclei of the same ovarian follicle. Sets of nuclei from individual follicles of both ovaries were therefore prepared from three F_1 females, two of which proved to be of the TS phenotype (Table 1). The four largest (oocyte proximal) nuclei were taken from each of the follicles chosen for examination. Among the samples from the two TS females, three clear examples were found in which the relative replication of the Tom and Karla NOs by the related nurse cell nuclei of a single follicle showed pronounced variation (Fig. 4). Thus, even in nuclei that are developmentally separated by no more than four mitotic divisions, the usage of the two sets of rRNA genes for polyploidization can vary dramatically.

DISCUSSION

TS and TW Phenotypes. A surprising feature of the crosses described here was the discovery that NO regions present in the Tom strain, which in terms of NTS composition appeared very similar, showed two very different types of replicative behavior when tested in the heterozygous condition with the single NO region derived from the Karla strain. These two phenotypes would not have been recognized if pooled samples of polyploid tissue from several F_1 progeny had been used as in previous studies (6, 9–11) and thus this finding emphasizes the importance of studying replicative events in individual animals. The value of studying strains that have been made homozygous for a single NO (as is the case of our Karla strain NO) as opposed to using strains prepared from individuals with apparently identical NOs [as is the case of our Tom strain and stocks used in previous studies by others (6, 9–11)] is also apparent.

The TS and TW phenotypes could represent variable activity of any of the four Tom strain NOs as a result of differences at other loci within the genome. Our data indicate that the NOs of the Tom strain are of slightly different constitutions, however, and suggest rather that TS and TW phenotypes represent discrete properties of these different NOs, the TS phenotype being particularly associated with NOs containing physically more rDNA. Under this interpre-

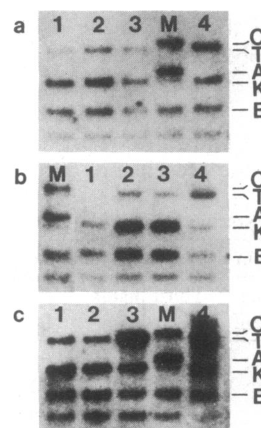

FIG. 4. Representation of the Tom and Karla NOs in related nurse cell nuclei from single ovarian follicles of the TS phenotype. The four largest nurse cell nuclei from each individual follicle were used. (*a–c*) Hybridization patterns for the four nuclei from three different follicles; *a* and *b* are derived from the same female. The DNA of nucleus 4 in *c* is incompletely digested. Differences in the representation of Tom and Karla bands among the individual nuclei of each set are clear. Marker DNA (M) (3.4 ng) and band designations (C, T, K, A, and B) as in Fig. 2.

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tation, each of the Tom strain parents used for the two Tom × Karla crosses examined would contain one NO of each phenotype (see Table 1, F_1 progeny). In connection with this possibility, Endow (11) has provided evidence in *Drosophila melanogaster* that the capacity of a NO derived from a Canton S strain to dominate during polyploidization resides within the Canton S NO region itself and that the ability of a NO on a *sc*^{8} X chromosome to dominate during polyploidization is lost if the NO is a strongly bobbed allele.

Differences in the Extents of Polyploidization of the Two NOs at the Level of Individual Nuclei. As judged by their approximately equal representation in polyploid tissue DNA, the Karla and Tom NOs present in our TS phenotype progeny appear to have a very similar overall capacity to interact with the endoreplication machinery of the polyploid cell. And yet, in individual polyploid cells, random and drastic variation in the replication of one NO relative to the other is seen. Such distortions in the NO replication ratio would be highly unlikely if either large numbers of replication origins within each NO were used for polyploidization or if each origin had equal access to the replication machinery after every round of replication. Rather, it would seem that a small number of origins within each NO must be used and that chance differences in the treatment of these origins occurring very early during replication must be perpetuated throughout the polyploidization process. Three mechanisms that would produce this effect seem possible. As a first possibility, origins within the replicated regions of each NO may compete initially for a limited quantity of polymerase or associated factors, but, after complex formation, the replication apparatus may remain stably associated with a given replicon. Thus, random differences in the initial distribution of a limited polymerase supply to origins within the two NOs would be maintained. Alternatively, variable replication could result from differences in the fraction of each NO that is prevented from replicating by inactivating factors. Extreme underreplication of the cluster of intron-containing rDNA cistrons of *C. erythrocephala* during polyploidization is associated with heterochromatization of these cistrons (18). If a certain fraction of the rDNA is inactivated during polyploidization as a result of production of a finite quantity of repressor protein(s), the initial extent of inactivation of the two NOs might vary randomly and thus give rise to drastic biases in the amount of rDNA from each NO available for polyploidization.

A third possibility is suggested by parallels between our findings and those for the amplified rDNA in individual oocyte nuclei of *Xenopus laevis* (19). It could be that the rDNA is excised and replicated extrachromosomally so that imbalances in replication of the two NOs would reflect random differences in the extent to which one or other NO is used for the initial excision event(s). The nurse cell nuclei of both *D. melanogaster* and *C. erythrocephala* contain multiple nucleoli (20, 21) and, in *C. erythrocephala*, Ribbert and Bier have provided evidence that these nucleoli are extrachromosomal and contain circular DNA (21). However, these observations need not necessarily imply extrachromosomal rDNA replication since multiple rounds of chromosomal replication followed by excision as outlined by Botchan *et al.* (22) could yield similar structures.

Similarity of rDNA Polyploidization in Somatic and Germ-Line Nuclei. In the salivary gland nuclei of cyclorraphous Diptera, in contrast to the situation in nurse cells, a single nucleolus attached to the NO-bearing chromosome is present. Further, the overall extent of rDNA replication is much lower in salivary glands (1, 2) of these organisms than in nurse cells (15). However, at the level of the molecular events studied here, replication of the rDNA appears very similar in nurse cell and salivary gland nuclei, suggesting that similar mechanisms of achieving polyploidization are operating in both tissues.

General Significance of Stochastic Allele Usage After Mitotic Division. Many patterns of gene expression show great stability through a large number of cell divisions, and Brown (23) has argued that these patterns are maintained through the formation of stable chromatin complexes that rapidly reform after DNA replication. To our knowledge, however, no previous study has examined the relative use of two different alleles of a given gene, or gene set, after a series of mitotic divisions. Our findings on the activity of the two rRNA gene sets in the highly related nurse cells of a single ovarian follicle indicate that, after each cell division, distribution of the required chromatin factors between the two alleles of a given gene is an entirely stochastic process. We would predict, therefore, that for any gene, if the required chromatin factors are limiting and two alleles of approximately equal strength are present in the genome, differential usage of the two alleles will occur randomly in the daughter cells of a given mitosis. If the products of the two alleles are detectably different, this would lead to a population of cells whose composition with respect to the two variant products was a continuous spectrum of values.

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