Nucleolar Dominance and Replicative Dominance in Drosophila Interspecific Hybrids

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ABSTRACT

The replication of the rDNA complement of only one nucleolus organizer region during polytene chromosome formation (replicative dominance) was initially observed in Drosophila melanogaster. Here we demonstrate replicative dominance in Drosophila simulans and D. melanogaster/D. simulans interspecific hybrids. A second nucleolar phenomenon, nucleolar dominance, is observed in the diploid tissue of interspecific hybrids. In this case only one of two nucleolus organizer regions forms a nucleolus. However, reorganizations of the X chromosome heterochromatin which eliminate nucleolar dominance have no apparent effect on the expression of replicative dominance. These observations lead us to conclude that nucleolar dominance and replicative dominance are operationally separable functions influencing the rDNAs, and may be determined by differing regulatory events.

WHILE the structure of the ribosomal RNA cistrons (rDNA) of Drosophila has been physically well characterized, less is known about the factors which regulate their replication and expression. The redundancy of the tandem repeats changes in response to genetic background [see Ritossa (1978) for review] and is autonomously established relative to other genomic components during the formation of polytene cells (Spear and Gall 1973; Endow and Glover 1979). In many cases, the ribosomal RNA cistrons of one nucleolus organizer (NO) region are preferentially replicated during polyteneization (replicative dominance) (Endow 1980). In addition, rDNA repeats which bear one class of intervening sequence (ivs+) are under-replicated relative to repeats that lack this sequence (Endow and Glover 1979). This phenomenon will be referred to as replicative selection. Replicative dominance in Drosophila melanogaster was shown to be independent of the heterochromatic sequences which surround the ribosomal RNA cistrons (Endow 1983). Partial deletions of the ribosomal genes of the dominant NO region, however, appeared to result in the replication of both NO regions (Endow 1983).

Nucleolar dominance is defined cytologically as the formation of nucleoli at the nucleolus organizer regions of only one parental genome in interspecific hybrids. It was first described in plants (Navishan 1934) and has since been demonstrated in a variety of animal and amphibian hybrids (see Reeder (1984) for review), including Drosophila larval diploid cells (Durica and Krider 1977). Evidence that nucleolar dominance is the result of a transcriptional effect has now been presented for hybrids of Xenopus (Reeder and Roan 1984) and mouse-human cells (Onishi, Bergland and Reeder 1984; Miesfeld et al. 1984). In vitro studies are consistent with the notion that nucleolar dominance may be due to competition for transcription factors between the enhancer-like elements found within rDNA repeats (Reeder and Roan 1984) or to the absence of species-restricted transcription factors in the hybrid cells (Grummt, Roth and Paule 1984; Miesfeld et al. 1984; Onishi, Bergland and Reeder 1984). In Drosophila melanogaster/Drosophila simulans interspecific hybrids melanogaster was shown to be dominant over simulans (Durica and Krider 1977). Deletions of the heterochromatic region between the sc51 and sc6 breakpoints on the melanogaster X chromosome abolishes nucleolar dominance, and the NO regions of both species are associated with a nucleolus (Durica and Krider 1978). The mechanism by which this region, which we call ND, influences nucleolar dominance and the relationship of this mechanism to those described in Xenopus and mouse-human cell hybrids is unknown.

Replicative dominance is observed in polytene cells, while nucleolar dominance is normally demonstrated in mitotic cells of interspecific hybrids. Both preferentially affect one of the two rDNA complements. A polytene analogue of nucleolar dominance has been reported in interspecific hybrids of the Drosophila mulleri complex (Bicudo 1981). In interspecific hybrids of the D. melanogaster species subgroup such observations cannot be made because the proximal
end of the X chromosome is obscured in the polytene cell chromocenter. Using inverted X chromosomes in which the rDNA has been separated from the proximal block of the flanking heterochromatin, Hilliker and Appels (1982) were able to study the activity of the rDNA in polytene nuclei of intraspecific crosses.  

In situ hybridization of rDNA probes to salivary glands allowed dominance relationships to be established in terms of nucleolar formation and rDNA replication. NO regions which were scored as dominant showed both nucleolar association and rDNA replication. Interestingly, deletions heterochromatin immediately distal to, but outside of the rDNA, affected the dominance relationships observed.

We infer from these observations that the expression of nucleolar dominance and replicative dominance may result from a common mechanism. To test this notion we have first examined the rDNAs of D. simulans polytene and diploid cells. Our observations indicate that the polytene rDNAs of D. simulans are affected by replicative dominance and selection like that seen in D. melanogaster. We have then examined genotypes in which the appearance of either replicative dominance or nucleolar dominance is altered in D. simulans/D. melanogaster hybrids to determine if the two events are concordant in their expression.

MATERIALS AND METHODS

Drosophila stocks and culture conditions: The D. melanogaster stocks O5A, O8A, O10C, O11B, C8B and C7C stocks carry X chromosomes cloned from the Oregon R and Canton S strains as described in Dutton and Krider (1984a, b). D. simulans was described in Durica and Krider (1977). The African strains of D. simulans (Szpb 5A) and D. melanogaster (MGR-1) were obtained from Dr. Agnew (Union of South Africa).

The rDNA contents, as a percent of the total DNA, of the Oregon R derivative O11B is 0.383 ± 0.008 SEM, while the rDNA content of the Canton S derivative C7C is 0.414 ± 0.007 SEM (Dutton and Krider 1984a, b). The D. simulans stock, 8DS, has an rDNA content of 0.397 ± 0.014 (Durica and Krider 1977). All of the bobbed D. melanogaster stocks and the D. simulans stock 8DS were tested for the expression of the bobbed phenotype in XO males. O5A, O8A, and C8B were bobbed as XO males, while 8DS XO males were not. Their values (±SEM) are: O5A, 0.188 ± 0.007; C8B, 0.210 ± 0.012; and O8A, 0.243 ± 0.017.

Experimental crosses: D. melanogaster/D. simulans interspecific hybrid females were obtained by mating 20 virgin females with 20 males as described below. Successful matings were infrequent [see Durica and Krider (1977) for details], but larval populations sufficient for tissue dissection could usually be obtained from eight independent matings in each cross. The mating of melanogaster females and simulans males produces exclusively female adult progeny with these stocks (Sturtevant 1929). While some male larvae appear in the very early instars, no males are found in the migrating third instars used for the isolation of tissues. Hybrids with an X chromosomal rearrangement were obtained by mating D. melanogaster females homozygous for the altered X chromosomes with D. simulans.

Extraction of DNA from third instar larval ganglia (diploid tissue) and salivary glands (polytene tissue): The procedure used is a modification of one used by S. Daniels (personal communication) for whole flies. Ganglia or salivary glands were dissected from 60 to 80 third instar larvae and placed directly into 250 μl grind buffer. The grind buffer is 4 parts Drosophila homogenization buffer (0.1 M NaCl, 0.2 M sucrose, 0.01 M EDTA, 0.05 M Tris-HCl, pH 8.0) and 1 part phage lysis buffer (0.25 M EDTA, 2.5% (w/v) SDS, 0.5 M Tris-HCl, pH 9.2). The salivary glands were drawn twice through a 25-G needle and the needle was rinsed with an additional 250 μl of grind buffer. The neuroblast tissue was ground in a glass-on-glass micro homogenizer. The homogenates were then transferred to 1.5-ml Eppendorf tubes. Potassium acetate was added to a final concentration of 1 M. The mixture was incubated on ice for ten minutes before centrifuging for ten minutes in a microfuge. The supernatant was extracted with an equal volume of phenol saturated with TE (100 mM Tris, 10 mM EDTA, pH 7.5). Following the addition of an equal volume of sevag (24:1 chloroform/isooamy alcohol), the mixture was briefly extracted before the emulsion was broken by centrifugation for 1 min in a microfuge. The supernatant was then reextracted with an equal volume of sevag. The aqueous phase was then transferred to a clean 1.5-ml Eppendorf tube and the nucleic acids were precipitated by the addition of 100% ethanol at room temperature. The precipitate was recovered immediately by centrifugation in a microfuge for 3 min. After briefly air drying, the DNA pellet was resuspended in 200 μl of TE, pH 7.5. This was adjusted to 0.5 M sodium acetate and DNA was precipitated by the addition of two volumes of ice cold 95% ethanol. The DNA was recovered by centrifugation and the pellet was gently washed with 70% ethanol. After air drying the pellet was resuspended in 30 μl of TE.

Southern blot analysis: Restriction of DNA with EcoRI, fractionation on 0.8% agarose gels, transfer to nitrocellulose filter paper and hybridization with 32P-labeled rDNA were carried out as described by Dutton and Krider (1984). The Drosophila rDNA probe was pDm ra.51 which contains an uninterrupted rDNA repeat (Dawid, Wellauer and Long 1978). The D. melanogaster type I insert probe was a 0.9-kb BamHI fragment from pDm ra.56 (provided by I. B. Dawid) which was subcloned into M13 by F. L. Dutton. A gel-purified 3.96-kb BamHI/HindIII fragment from pDm ra.56 was also used as a type I sequence probe for D. simulans rDNA. Separate EcoRI and HindIII digests of pDm ra.51 were included on the gels as size markers.

Cytology: The dissection, fixation, and acridine orange staining of third-instar dorsal and ventral ganglia have been described elsewhere (Durica and Krider 1977).

RESULTS

Representation of rDNA repeats in diploid and polytene cells of D. simulans: We examined the polytene and diploid representation of rDNA repeats of D. simulans to determine if D. simulans expresses...
replicative dominance and selection. Since there is a single recognition site for the restriction endonuclease EcoRI in ivs-repeats of *D. melanogaster*, this enzyme is commonly chosen to display the array of repeat classes existing in this species. The major repeat classes as defined by EcoRI digestion are the 11.5-kb uninserted repeat and the 17-kb repeat containing an insert in the 28S rRNA cistron (Glover and Hogness 1977). In *D. melanogaster* a comparison of the rDNA EcoRI restriction patterns of DNA extracted from diploid (larval brain) and polytene (larval salivary gland) tissues reveals that the 17-kb repeat is relatively under-represented in the polytene tissue (Endow and Glover 1979). This observation is the operational definition of replicative selection in *D. melanogaster* and is illustrated in Figure 1a, lanes A and B. A comparison of the rDNA EcoRI restriction patterns of diploid and polytene DNA from *D. simulans* also reveals that certain sequences are under-represented in the polytene tissue of this species (Figure 1a, lanes C and D). The two bands consistently observed in the polytene pattern of *D. simulans* are 9 kb and 2.9 kb. The band at approximately 11.5 kb was not always observed. Since a definitive restriction map is not yet available for the *simulans* rDNA repeats, the *simulans* Southern blot shown in Figure 1a was rehybridized using conditions identical to those of the initial hybridization with a probe containing the *melanogaster* type I insert (Figure 1b). In the diploid tissue, the band showing the greatest amount of hybridization to the *D. melanogaster* type I insert corresponds to the 12-kb rDNA EcoRI fragment which is under-represented in *D. simulans* salivary glands. The additional EcoRI fragments which are under-represented in the *D. simulans* polytene DNA (i.e., those between 9 kb and 2.9 kb) may contain inserts homologous to the *D. melanogaster* type II insert or to sequences which are unique to *D. simulans*.

To determine if replicative dominance occurs in *D. simulans* different strains of this species were screened for variant rDNA EcoRI restriction patterns. Strains in which diploid and polytene rDNA restriction patterns contained at least one fragment which was not common to both strains were used for interstrain matings. Figure 2 shows such a case using the *D. simulans* strains, 8DS and Szpb. The distinguishing rDNA restriction fragments for this pair are the 9-kb fragment present only in 8DS diploid and polytene tissue (Figure 2, lanes A and B) and the 9.5-kb fragment present in Szpb (Figure 2, lanes E and F). In the diploid tissue of the 8DS/Szpb interstrain hybrid both the 9-kb and 9.5-kb fragments are visible (lane C). In the polytene tissue, only the 9-kb fragment of 8DS is visible (lane D). 8DS, therefore, is replicationally dominant over Szpb. Thus, in terms of replicative dominance and replicative selection, *D. simulans* appears to replicate rDNA during polytenization in a manner similar to *D. melanogaster*.

**Replicative dominance in interspecific hybrids:**

In interspecific hybrids of *D. melanogaster* and *D. simulans*, a secondary constriction always appears at the NO of the *melanogaster* X chromosome. We have observed no case in which the *simulans* organizer functions exclusively when a *melanogaster* X or Y chro-
mosome is present in the hybrid. Cases in which both the melanogaster and simulans organizer regions function are rare and have been used to define one region required for sustaining nucleolar dominance [see DURICA and KRIDER (1978) and below]. There is no comparable study of polytene cell rDNA replication in these hybrids. Therefore, we determined if replicative dominance was expressed in hybrids, and if the rDNAs of melanogaster strains were preferentially represented in polytene tissues. The pronounced differences in the rDNA EcoRI restriction patterns of D. melanogaster and D. simulans facilitates the study in hybrids. We have restricted the study to X/X female hybrids. Since all matings use D. melanogaster females, the role of the maternal cytoplasm in establishing the preferential replication of polytene cell rDNAs has not been assessed. Nucleolar dominance is observed when D. simulans provides the maternal cytoplasm (DURICA and KRIDER 1977). In most of the figures which follow, the Southern transfer hybridizations were overexposed to visualize the less abundant rDNA repeats of the diploid and polytene preparations. In actual use, several exposures of each preparation were made. In all cases, the 9-kb and 2.9-kb fragments of the simulans rDNAs were used to evaluate the expression of replicative dominance since they were easily distinguished from the melanogaster fragments, and least variable in their apparent abundance between repeated preparations. The D. melanogaster X chromosome lines used in the following analysis were originally isolated from laboratory populations of the Oregon R and Canton S stocks. Their origin and characteristics have been described elsewhere (DUTTON and KRIDER 1984a, b).

Figure 2.—Replicative dominance in D. simulans. Southern transfer with 32P-labeled rDNA (see Figure 1a). The filled circle and the star denote the distinguishing EcoRI restriction fragments of simulans 8DS and simulans Szpb, respectively.

Figure 3.—Replicative dominance in Drosophila interspecific hybrids. The melanogaster strain 011B was derived from Oregon R and is wild-type in rDNA content. O8A is a bobbed derivative of Oregon R. 8DS is the D. simulans strain used in these hybrids. Lanes E and H are over-exposures of lanes D and G, respectively. The arrow denotes the 4 kb band indicative of D. simulans rDNA.
mosomes ceased to be replicationally dominant when they carried extensive rDNA deletions sufficient to cause the bobbed phenotype. Any X chromosome that bears a deletion of 50% or greater of the rDNA repeats is bobbed. In X/Y male flies the phenotype is characterized by delayed development, shortened bristles, and etched abdominal cuticle (RITossa 1978).

In order to study the effect of rDNA content on replicative dominance in interspecific hybrids, the bobbed melanogaster stocks 03A, 08A (derived from Oregon R) and C8B (derived from Canton S) were used in the formation of hybrids with D. simulans. The D. simulans stocks used in this study (8DS) are wild-type with respect to rDNA content (see MATERIALS AND METHODS and Durica and KRIDER 1977). In DNA preparations from D. melanogaster (08A)/D. simulans (8DS) polytene tissue, the rDNA EcoRI restriction pattern of D. melanogaster predominates (Figure 3, lane G) although bands indicative of the simulans polytene pattern (i.e., 9 kb, 4 kb, and 2.9 kb) are visible. These bands are faint, however, and an overexposure of the lane is necessary in order to see them clearly (lane H). The Canton S bobbed line, C8B, gives a similar result in hybrids with simulans 8DS (data not shown).

However, we observe that when the bobbed X chromosome 03A is used, the polytene pattern of the hybrids (Figure 4, lane D) now contains restriction fragments of both the parental polytene patterns. Hybrids which include the 03A chromosome are the only constructs in which the 2.9-kb simulans band appears with comparable intensity in the polytene DNAs of both the hybrid and parental simulans stock (as in Figure 4, lanes D and F). The hybrid polytene patterns now resemble that expected from the sum-

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**TABLE 1**

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<tr>
<th>Hybrids</th>
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<tr>
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<td>ENDOW (1980)</td>
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<tr>
<td>C7C/011B</td>
<td>C7C</td>
<td>GOODRICH (1987)</td>
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<tr>
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<td>011B/8DS</td>
<td>011B</td>
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<tr>
<td>08A/8DS*</td>
<td>08A</td>
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<td>C8B/8DS*</td>
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<td>In(1)wexc/8DS</td>
<td>In(1)wexc</td>
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* Denotes X chromosomes with rDNA deletions sufficient to produce a bobbed phenotype.

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**FIGURE 4.** rDNA polytenization in O3A/8DS interspecific hybrids. O3A is a bobbed derivative of Oregon R. 8DS is the simulans strain used.
normal (Figure 5a). In mitotic preparations of melanogaster/simulans interspecific hybrids, however, the X chromosome of the melanogaster parent bears a secondary constriction, while the simulans X chromosome does not. This configuration operationally defines nucleolar dominance. Such a case is demonstrated in a hybrid generated between the C7C (D. melanogaster) and 8DS (D. simulans) stocks shown in Figure 5b. When interspecific hybrids were produced which contained any of the bobbed melanogaster X chromosomes (03A, 08A and C8B), constrictions appeared at nucleolus organizers of X chromosomes from both of the parental species. For instance, secondary constrictions are visible at both the C8B (D. melanogaster) and 8DS (D. simulans) X chromosomes of the preparation of Figure 5c. In all of the interspecific hybrid combinations at least 20 cells were examined, and all showed the described behavior. Thus, replicative and nucleolar dominance are concordant to the degree that both respond to reduced rDNA content of the dominant complement by allowing expression and replication of other rDNAs. They are not equally sensitive, all the bobbed X chromosomes failed to sustain nucleolar dominance, while only the 03A X chromosome lost its replicative dominance.

The heterochromatic element necessary for nucleolar dominance is not necessary for replicative dominance: Although the expression of both replicative and nucleolar dominance showed sensitivity to rDNA content, albeit to different degrees, rDNA repeat number could influence these phenomena by different mechanisms. In order to investigate the possible relationship of replicative and nucleolar dominance further, we asked if these two phenomena respond similarly to perturbations of the surrounding heterochromatin. Figure 6 diagrammatically shows the normally arrayed X chromosome and the sites of breakage used in this study. Deletion of the proximal heterochromatic region lying between the se and se breakpoints on the melanogaster X chromosome (as In(1)scs1sc8R) results in the loss of nucleolar dominance in hybrids with simulans (Durica and Krider 1978). However, the In(1)sc8 X chromosome does sustain nucleolar dominance in interspecific hybrids (Durica and Krider 1978, and our observations).

To determine if this function influences replicative dominance, the rDNA EcoRI restriction patterns of DNA from the larval diploid and polytene tissues of In(1)sc8 (melanogaster)/8DS (simulans) and In(1)scs1sc8R (melanogaster)/8DS simulans hybrids were examined. The results summarized in Table 1 show that the melanogaster NO region always shows replicative dominance in hybrids with simulans regardless of the melanogaster strain from which it is derived. If the nucleolar dominance function is also involved in the expression of replicative dominance, then we would
expect that the rDNA complement on the \textit{In}((1)\textit{scu}/8DS) \textit{X} chromosome would fail to show replicative dominance. The results of this analysis are presented in Figure 7a. We note first that the array of rDNA repeats of the interspecific hybrid diploid DNA preparations (lane E) appear to be a composite of the two parental patterns (lanes D and G). In the DNA from the polytene tissue (lane F), the 11.5-kb repeat of the \textit{In}((1)\textit{scu}/8DS) \textit{melanogaster} rDNA predominates. The 9-kb and 2.9-kb bands indicative of \textit{simulans} polytene rDNA are not visible. An over-exposure of this autoradiograph does not reveal any \textit{simulans} specific bands (i.e., 9 kb, 4 kb and 2.9 kb) (data not shown). Thus, a genotype which fails to show nucleolar dominance (i.e., \textit{In}((1)\textit{scu}/8DS) \textit{melanogaster}/\textit{simulans}) still expresses replicative dominance. The replicational dominance of the \textit{In}((1)\textit{scu}/8DS) \textit{melanogaster} chromosome in hybrids with \textit{D. simulans} is also shown in Figure 7a (lanes B and C).

The rDNA is separated from the more distal heterochromatic elements of the \textit{melanogaster} \textit{X} chromosome by the right break of the \textit{In}((1)\textit{wm4}) \textit{chromosome} (Figure 6). Previous studies have indicated that several \textit{cis}-acting functions influencing the redundancy of the rDNA are localized to this heterochromatic region (PROCUNIER and TARTOF 1978; YEDVOBNIK, KRIDER and LEVINE 1980). In addition, HILLIKER and APPELS (1982) have demonstrated that deletions of the distal heterochromatic block can influence the expression of replicative dominance in some intraspecific crosses. Although no nucleolar dominance related functions are found in the distal heterochromatic block (DURICA and KRIDER 1978), we examined rDNA replication in \textit{In}((1)\textit{wm4} \textit{melanogaster}/\textit{simulans}) (8DS) hybrids to see if a \textit{cis}-acting element influences replicative dominance. The failure to observe replicative dominance in these hybrids would indicate the presence of a \textit{cis}-acting function in this region which specifically influences rDNA polypetenization in \textit{D. simulans}/\textit{D. melanogaster} hybrids. The restriction patterns of hybrid diploid tissue (Figure 7, lane J) contains both the parental \textit{D. simulans} and \textit{D. melanogaster} \textit{In}((1)\textit{wm4}) \textit{chromosome} rDNA repeats (compare with lanes H and L). In the hybrid polytene tissue, the \textit{melanogaster} \textit{In}((1)\textit{wm4}) rDNA restriction pattern predominates (lane K). Bands indicative of the \textit{simulans} rDNA polytene restriction pattern (9 kb and 2.9 kb) are not visible. Thus, we were unable to detect the presence of a \textit{cis}-acting function which influences rDNA replication in Drosophila interspecific hybrids.

DISCUSSION

Replicative dominance, the preferential replication of one of two rDNA complements, occurs in the polytene cells of Drosophila salivary glands (ENDOW and GLOVER 1979). Nucleolar dominance, the function of only one nucleolus organizer, has been demonstrated in diploid tissues of interspecific Drosophila hybrids (DURICA and KRIDER 1977, 1978). We assayed both phenomena for a number of pairs of \textit{X} chromosomes to determine whether they are concordant in expression. Such a relationship may reflect common determinants shared by the two events.
We first established that *D. simulans* replicates its rDNA during polytenization in a manner similar to *D. melanogaster* in that replicative dominance and replicative selection can be demonstrated in this species. In interspecific hybrids, *D. melanogaster* always shows replicative dominance over *D. simulans* when both X chromosomes bear wild-type levels of rDNA (summarized in Table 1). Interspecific hybrids offer the advantage of easily distinguished rDNA repeat types between the two rDNA complements, and provide the only context in which diploid cell nucleolar dominance can be compared to rDNA replication in polytene tissues. We note that using interspecific hybrids we have repeatedly substantiated the observations of Endow and Glover (1979) and Endow (1980) which defined replicative dominance in *D. melanogaster*. Recently, Belikoff and Beckingham (1985a, b) have argued that the replication of rDNA complements is stochastic in individual Caliphora polytoid nuclei. Although whole salivary glands were used as the source of DNA in our studies, the consistent demonstration of one species' rDNA showing replicative dominance over another suggests a nonrandom process for determining dominance. It is possible that Caliphora and Drosophila differ significantly in the manner in which nondiploid rDNA replication occurs. Alternatively, Belikoff and Beckingham may have detailed a case of codominance. Such combinations have been observed in *D. melanogaster* (Endow 1982) and in *D. simulans* (C. Goodrich-Young, unpublished observations). However, we concur with Belikoff and Beckingham that replicative dominance need not result in the exclusive replication of one rDNA complement. For example, although the *melanogaster* rDNAs are preferentially represented in the polytene tissues of O11B/8DS hybrids, our data is consistent with the replication of a subset of the *simulans* repeats as well. Endow (1983) has argued that the elements mediating replicative dominance must lie within the array of rDNA repeats. She observed that normally dominant rDNA complements are unaffected by proximal or distal deletions of heterochromatin. We also note that translocation of the heterochromatic elements from either the proximal (In(1)scstl,sc86) or distal (In(1)w*54) side of the rDNAs has no impact on replicative dominance in interspecific hybrids. However, Hilliker and Appels (1982) observed that removal of the heterochromatic regions from one X chromosome resulted in the reversal of dominance with Y chromosomal rDNAs. And, Karpen, Schaefer and Laird (1988) have shown that when a single rDNA repeat is inserted into euchromatic domains by P element mediated transformation, its replication resembles that of the surrounding chromatin. Thus, rDNAs lacking heterochromatin may be unable to respond to the mechanisms that normally mediate dominance.

In intraspecific hybrids, replicative dominance is lost when the rDNA content of the dominant complement is reduced (Hilliker and Appels 1982; Endow 1983; Belikoff and Beckingham 1985a, b). This is apparently the case in interspecific hybrids as well. In this study the wildtype and bobbed lines were derived from the same *melanogaster* strains and carried X chromosomes of normal order. Thus, comparisons are less likely to be subject to background effects if they exist. The rDNA of two of the severely bobbed X chromosomes predominates in the polytene patterns of the hybrids. In these cases the appearance of *simulans* repeats may result from either an increase in the replication of the *simulans* complement, or may reflect the very low level of the diploid redundancy of the *melanogaster* rDNAs. The O3A X chromosome has the least *melanogaster* rDNA, and the contribution by *simulans* sequences is clear in this case. Reduced rDNA contents could release or reverse replicative dominance if competition for limiting factors occurs during the initiation of replication. We consider this hypothesis because it has also been suggested to account for nucleolar dominance. If this is the case, instances in which the alternate rDNA complements are both represented (codominance) could result from the limiting factors having comparable affinities for the relevant competing sites. In hybrids, replicative dominance might indicate that the factors of both species have a higher affinity for the *melanogaster* complement, or that the factors made from the *simulans* genome are in reduced supply, or that the sites to which the factors bind are most abundant in the *melanogaster* complement, or some combination of these alternatives.

In summary, it appears that replicative dominance in *D. melanogaster/D. simulans* interspecific hybrids exhibits the same characteristics as replicative dominance in *D. melanogaster* interstrain hybrids.

In diploid material all of the bobbed X chromosomes appear cytologically normal in both *melanogaster* and hybrid cytoplasms. However, none of the three X chromosomes sustained nucleolar dominance, and constrictions formed at both nucleolus organizers of hybrid mitotic preparations. It is possible that the events which produced the bobbed alleles altered the structure or activity of sequences other than the rDNAs in all three of the independently isolated X chromosomes. An alternative interpretation is that the loss of nucleolar dominance results directly from the reduction of the *melanogaster* rDNA repeats. This interpretation is consistent with the observation that in interspecific hybrids between other species, competition for transcriptional components between rDNA types contributes to the expression of nucleolar
dominance. For example, in the Xenopus nontranscribed spacer there are repetitive modules which contain imperfect copies of a 42-bp region of the polymerase I promoter (Moss, Boseley and Birnstiel 1980). A polymerase I enhancer function has been proposed for these elements, and they appear to compete for transcriptional components in oocyte injection assays (Reeder, Roan and Dunaway 1983). The dominance of the Xenopus laevis rDNA in hybrids with Xenopus borealis has been attributed to the fact that the X. laevis spacer contains more copies of the enhancer element (Reeder and Roan 1984).

Imperfect copies of the rDNA promoter region do exist in the nontranscribed spacer (Kohorn and Rae 1982), and duplication of the elements elevates transcription in competitive assays (Grimaldi and Di Noceara 1988). Thus, components of the Drosophila nontranscribed spacer may function like the Xenopus enhancer elements (Simeone, La Volpe and Bonecinnelli 1985) in the expression of nucleolar dominance. While these arguments allow us to consider that Drosophila nucleolar dominance bears mechanistic similarities to other species, they do not explain the several instances in which X and Y chromosome heterochromatic alterations (like the In(1)soSIMReR chromosome of this study) fail to sustain nucleolar dominance [see Durica (1977) and Durica and Krider (1978) for other examples]. These rearrangements define a function which is separated from the rDNAs (Durica and Krider 1978; Hilliker and Appels 1982) and require that we consider that Drosophila bears some added constraints in the expression of nucleolar organizer function.

Thus, the expression of nucleolar dominance and replicative dominance do parallel each other in some situations. First, in D. melanogaster/D. simulans interspecific hybrids the melanogaster X chromosome is always dominant regardless of the strains used in constructing the hybrids. Second, the expression of both events is sensitive to the rDNA content of the dominant NO region. These observations are consistent with the notion that in both diploid and polyploid tissue the melanogaster rDNA can sequester some limited factor(s) more efficiently than the simulans rDNA. The experiments reported here do not allow us to speculate on whether these hypothetical factors are the same in the two phenomena. It is certain, however, that the two phenomena differ in their responsiveness to the nucleolar dominance heterochromatin element. It is possible that the mechanisms supporting nucleolar dominance and replicative dominance are entirely different, or that the sensitivity of both phenomena to rDNA content is indicative of a shared step in what are otherwise different pathways leading to an active NO region.

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LITERATURE CITED


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