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THE MOLECULAR THROUGH ECOLOGICAL GENETICS OF ABNORMAL ABDOMEN. III. TISSUE-SPECIFIC DIFFERENTIAL REPLICATION OF RIBOSOMAL GENES MODULATES THE ABNORMAL ABDOMEN PHENOTYPE IN *DROSOPHILA MERCATORUM*

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ABSTRACT

The abnormal abdomen (*aa*) syndrome in *Drosophila mercatorum* is controlled by two major *X*-linked genetic elements. We have previously shown that the major *X*-linked element of *aa* is associated with the presence of large inserts in the 28S gene of the ribosomal RNA (rDNA) genes. We show that, in polytene tissue of wild-type *D. mercatorum*, the uninterrupted rDNA repeats are overreplicated relative to interrupted repeats. Uninterrupted rDNA repeats are also overreplicated in polytene tissue of hybrid larval offspring from wild-type and *aa* parents. This overreplication of uninterrupted repeats is not observed in diploid tissues of wild-type hybrids (of wild-type and *aa* parents) and homozygous *aa* larvae or in polytene tissue of *aa* larvae. Furthermore, molecular analysis of an *aa* line that has reverted to the wild type indicates that the reversion phenomenon is associated with the ability to overreplicate uninterrupted rDNA repeats in polytene tissues. The patterns of differential replication of rDNA genes in wild-type hybrids and *aa* larvae of *D. mercatorum* offer a possible mechanism for the tissue-specific control of the *aa* phenotype and suggest that the molecular basis for the second *X*-linked genetic element of *aa* is involved in the control of differential replication in polytene tissues.

VARIATION in gene copy number may provide a means for varying the rate of synthesis of specific RNA and protein products (SCHIMKE *et al.* 1978; ANDERSON and ROTH 1978; ZIMMER *et al.* 1980). A deficiency of rDNA genes or an abundance of interrupted and, therefore, nonfunctional rDNA genes has been implicated as the molecular cause of the bobbed (*bb*) phenotype in *D. melanogaster* (RITOSSA, ATWOOD and SPIEGELMAN 1966; RITOSSA 1982) and *D. hydei* (FRANZ and KUNZ 1982) and the abnormal abdomen (*aa*) syndrome in *D. mercatorum* (DESALLE, SLIGHTON and ZIMMER 1986). A direct correlation of the genetic location of the rDNA genes with the genetic location of the *bb* and *aa* loci exists (RITOSSA 1982; TEMPLETON, CREASE and SHAH 1985; DESALLE, SLIGHTOM and ZIMMER 1986). In particular, in *D. mercatorum* two closely linked genetic elements mapped to the centric region of the *X*

chromosome are the major controlling elements of the *aa* syndrome (TEMPLETON, CREASE and SHAH 1985; DESALLE, SLIGHTOM and ZIMMER 1986).

Presumably, a deficiency of functional rDNA cistrons lowers the production of rRNA and limits ribosome synthesis. The deficiency of ribosomes therefore places a translational control on the production of critical gene products, resulting in the observed phenotypic effects (RITOSSA 1982). In *D. mercatorum* the critical gene product that is involved in the cuticular and reproductive phenotypic changes of the *aa* syndrome appears to be a juvenile hormone-specific esterase (TEMPLETON and RANKIN 1978). In normal larvae a pulse of juvenile hormone esterase from the fat body occurs during a critically short developmental window. The appearance of this esterase in normal larvae causes a derepression of the juvenile hormone in the larval hemolymph. In *aa* larvae the lack of production of the juvenile hormone esterase causes continued high levels of juvenile hormone into the late third larval instar, which can cause the retention of the juvenile cuticle into the adult stage. Another possible pleiotropic effect of a raised juvenile hormone titer is precocious ovarian development and increased ovarian output (BOWNES 1982; WILSON, LANDERS and HAPP 1983). These phenotypes are observed in flies with *aa* genotypes (TEMPLETON and JOHNSTON 1982; TEMPLETON 1983).

Translational control of a critical gene product implemented by control of ribosome number might be expected to be more global in its pleiotropic effects. In fact, a deficiency of functional rDNA repeats might be expected to affect all tissues which produce large amounts of a particular gene product. Tissue-specific control of transcription of the ribosomal RNA or tissue-specific control of the number of functional rDNA cistrons could accomplish the narrower range of pleiotropic effects observed in the *bb* and *aa* phenotypes.

Polytene tissues have previously been shown to undergo the differential replication phenomenon (ENDOW and GLOVER 1979; ENDOW 1980, 1983) that causes the overreplication of a particular set of rDNA repeats or nucleolus organizer regions over another. ENDOW (1980, 1982, 1983) has established a replication dominance hierarchy for several nucleolus organizers in *D. melanogaster*. Differential replication of rDNA repeats does not occur in diploid tissues, such as larval brain or imaginal disc tissue. The rDNA content of diploid tissue is controlled by another somatic cell replication phenomenon called compensation (TARTOF 1973; PROCUNIER and TARTOF 1978), and in many cases the rDNA content in a diploid cell is the result of a simple additive or Mendelian genetic mechanism.

The fat body, which produces the juvenile hormone-specific esterase, is a polytene tissue. The polytene nature of the fat body in Drosophila and the differential replication of rDNA repeats in such tissues may afford a mechanism by which the control of the number of functional rDNA repeats could be varied in different tissues. Therefore, we have examined the replicational properties of the rDNA sequences in *aa* and wild-type *D. mercatorum* lines to determine if tissue-specific phenomena are involved in the replication of rDNA. Previously, we have shown that distinct restriction fragment polymorphisms exist in the rDNA of *aa* and wild-type *D. mercatorum*. The molecular basis for

this polymorphism is caused by the presence of long inserts in the 28S genes of a majority of the rDNA cistrons of *aa* flies, whereas wild-type flies have few of their 28S genes interrupted (DESALLE, SLIGHTOM and ZIMMER 1986). This large insert carries new *Eco*RI restriction sites which produce the variant restriction fragment polymorphisms that we have used as markers for the comparison of *aa* and wild-type rDNA structure (DESALLE, SLIGHTOM and ZIMMER 1986). Our approach in the present study is to compare the types and quantity of rDNA repeats that are present in diploid brain and polytene fat body tissues using the restriction fragment polymorphisms discussed above and in DESALLE, SLIGHTOM and ZIMMER (1986). We have examined the patterns of differential replication in wild-type and *aa* flies and hybrids of the two genotypes. We have also examined a revertant stock that has been maintained for about 60 generations. We were interested in ascertaining whether reversion could be explained by simple magnification of the rDNA present in the genome or whether a more complex mechanism was involved.

MATERIALS AND METHODS

Flies and fly stocks: Two abnormal abdomen *D. mercatorum* stocks were used in this study. In the first stock, both males and females express the *aa* cuticle phenotype because females are homozygous for the *aa X* (X^{aa}) and males carry a *Y* chromosome that enhances *aa* (Y^{aa}). The second *aa* stock, however, is maintained with phenotypically normal males because such males carry a *Y* chromosome that suppresses the *aa* phenotype (Y^{+}). SBr-16 and Oahu-1 were used as wild-type *D. mercatorum* standards. An abnormal abdomen revertant strain (rev59) was also examined. Abnormal abdomen is naturally selected against under standard rearing conditions, and artificial selection is needed to maintain the phenotype. The rev59 stock was established from the *aa* stock simply by eliminating the artificial selection for the abnormal cuticle phenotype for 59 generations. Nearly complete reversion of the phenotype occurs rapidly (five to ten generations), and after 59 generations, all flies in this stock had normal cuticle. We also used two parthenogenetic strains, K28-0-Im and K28-*ger*. K28-0-Im does not express the *aa* cuticular phenotype. A daughter strain from the K28-0-Im strain was established from a single female with a mutant *geranium* eye color (K28-*ger*). Subsequent examination of this K28-*ger* daughter stock revealed that the cuticular *aa* phenotype was being expressed (DESALLE, SLIGHTOM and ZIMMER 1986).

DNA isolation and manipulation: DNA from larval brain, fat body and salivary gland tissues was isolated by the method outlined in COEN, THODAY and DOVER (1982), except that 3 M NaOAc, pH 6.5, was used instead of 8 M KOAc. Brain and fat body tissues were dissected on ice from the same single individuals. DNA from the different tissues of a single individual were usually run on the same gel in adjacent lanes. Restriction digests were performed as directed by the supplier (New England Biolabs). Electrophoresis, Southern transfer to nitrocellulose, nick translation, hybridization and autoradiography were done by the methods outlined in MANIATIS, FRITSCH and SAMBROOK (1982). The determinations of the amount of rDNA as a percentage of the total genome were done by the methods outlined in DESALLE, SLIGHTOM and ZIMMER (1986).

RNA isolation and manipulation: RNA was isolated from larval brain tissue or larval fat body tissue which had been dissected and placed at −80° before nucleic acid isolation. The tissue was placed in a 1.5 ml Eppendorf tube, and 0.2 ml of a 1:1 solution of phenol and 0.1 M Tris HCl, pH 7.4, with diethyl pyrocarbonate (10% final concentration) was added. The tissue was then dounced gently using a pestle fashioned from a 0.5-ml Eppendorf. The solution was centrifuged for 5 min to separate the phases, and the aqueous phase was removed to a new tube on ice. The phenol layer was back extracted by adding 0.1 ml of 0.1 M Tris HCl, and the aqueous phase of the back

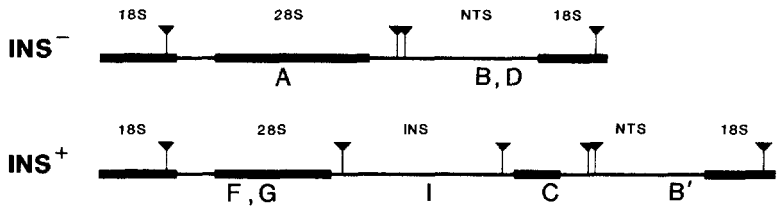

FIGURE 1.—Diagrammatic representation of the *Eco*RI (▼) digestion patterns observed from uninterrupted (*ins*$^-$) and interrupted (*ins*$^+$) rDNA repeats from *D. mercatorum*. Fragment sizes are as follows: A = 6.1 kb, B = 5.2 kb, C = 2.1 kb, D = 4.1 kb, F = 4.4 kb, G = 4.3 kb and I = 4.0 kb. For a full description of the fragment length polymorphisms, see DESALLE, SLIGHTOM and ZIMMER (1986).

extraction was added to the first aqueous phase. The combined aqueous phases were then extracted once with phenol and once with chloroform and were then ethanol precipitated after the addition of a 0.1 volume of 20% KOAc. RNA was electrophoresed on methyl mercury and formaldehyde gels and was transferred to nitrocellulose as described in MANIATIS, FRITSCH and SAMBROOK (1982).

Probes used: The *D. melanogaster Y* chromosomal uninterrupted rDNA repeat pDmrY22, kindly supplied by IGOR DAWID, was used in the Southern blotting experiments. The probes used in Northern blotting experiments can be divided into four classes: (1) coding region 5′ to the *ins* sequence (A5), (2) the 5′ end of the *ins* sequence (INS1), (3) the 3′ end of the *ins* sequence (INS3) and (4) the coding region 3′ to the *ins* sequence (A6). The exact regions that these subclones cover are given in DESALLE, SLIGHTOM and ZIMMER (1986).

RESULTS

Characterization of tissue-specific differential replication of rDNA: Figure 1 shows the *Eco*RI restriction site map for interrupted (*ins*$^+$) and uninterrupted (*ins*$^-$) rDNA repeats in *D. mercatorum*. Figure 2 shows the result of Southern blotting *Eco*RI-digested DNA from the fat body and brain tissue of wild-type and *aa* larvae. Such an experiment reveals the types of rDNA repeats that are present in each stock and in each tissue. In the *aa* tissues, large amounts of the G, F and C bands are present. These bands are unique to interrupted (*ins*$^+$) rDNA repeats (Figure 1) and indicate that in both diploid and polytene tissue there are relatively large numbers of *ins*$^+$ rDNA repeats. In the wild-type brain tissue there are low numbers of *ins*$^+$-specific bands, as shown by the weak G, F and C band signal. In wild-type fat body tissues, however, it appears that *ins*$^+$ sequences may be slightly underreplicated. One of the fat body lanes for the wild-type larvae has been overexposed, and only trace amounts of bands G, F and C appear to be present.

The tissue-specific profiles for the spontaneous K28-*ger* (*aa*) mutation and its parent stock are shown in Figure 3. The difference in restriction fragment patterns in their diploid tissue has previously been discussed (DESALLE, SLIGHTOM and ZIMMER 1986). The observation for the tissue-specific comparisons of K28-0-Im and K28-*ger* are identical to the comparison of wild type and the bisexual *aa* stocks. That is, in both types of tissue of the K28-0-Im larvae, very few *ins*$^+$ rDNA cistrons are present, whereas in both types of tissue of the K28-*ger* larvae the rDNA cistrons are mostly *ins*$^+$.

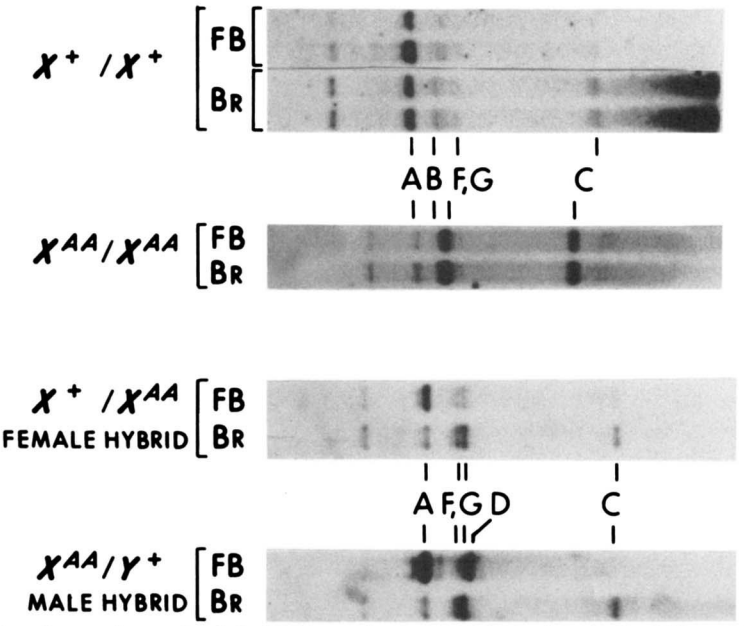

FIGURE 2.—Autoradiograph of *Eco*RI-digested genomic DNA of fat body (FB) and brain (BR) tissue from larvae of the indicated genotypes hybridized to nick-translated pDmrY22. X^+/X^+ is a homozygous wild-type female. X^{AA}/X^{AA} is a homozygous *aa* female. X^+/X^{AA} is a female hybrid, and X^{AA}/Y^+ is a male hybrid. Neither of these types of hybrids express the *aa* cuticle phenotype. Band designations and sizes are given in Figure 1.

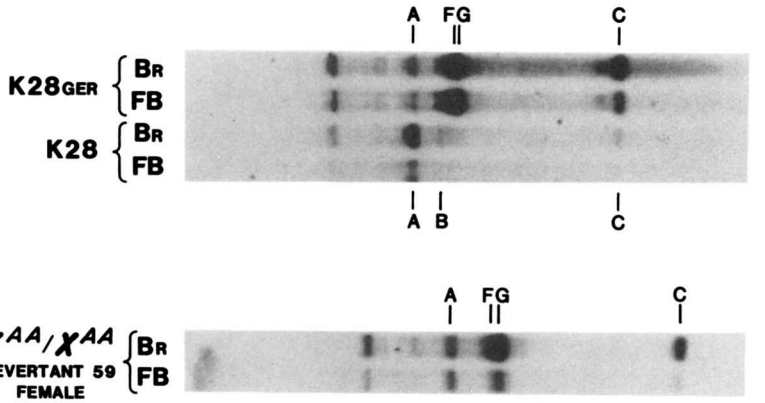

FIGURE 3.—Autoradiograph of *Eco*RI-digested genomic DNA of fat body (FB) and brain (BR) tissue from larvae of the indicated stocks hybridized to nick-translated pDmrY22. K28 is a parthenogenic strain that does not express the *aa* cuticle phenotype. K28-*ger* is a parthenogenic strain established from K28 (DESALLE, SLIGHTOM and ZIMMER 1986) that expresses the *aa* cuticle phenotype with high penetrance. X^{AA}/X^{AA} revertant 59 female is from the revertant stock maintained for 59 generations. X^{AA}/X^{AA} revertant 59 females do not express the *aa* cuticular phenotype. Band designations and sizes are given in Figure 1.

Characterization of tissue-specific rDNA differential replication in *aa*/wild-type hybrids: Examination of the diploid rDNA restriction patterns of hybrid females (X^+/X^{aa}) showed the expected additive patterns (Figure 2). Ap-

proximately equal amounts of the A band (specific to uninterrupted rDNA cistrons) and the G band (ins^+ specific) were observed. Polytene tissues, on the other hand, showed a drastic reduction of ins^+ rDNA sequences in the hybrid females, as shown by the almost complete lack of bands G and F in polytene tissues. When males with a suppressor of *aa* *Y* chromosome (Y^+) and an *aa* *X* chromosome (X^{aa}) are examined, we observe the expected additive rDNA restriction fragment patterns for diploid tissue. In the fat body tissue, however, the ins^- repeats of the *Y* chromosome are overreplicated. We have previously characterized the rDNA repeats on the Y^+ as having a shorter nontranscribed spacer (NTS) region (DESALLE, SLIGHTOM and ZIMMER 1986; WILLIAMS, DESALLE and STROBECK 1985). This shorter NTS region, when cleaved with *Eco*RI, produces the D band shown in Figure 1. This observation (Figure 2) implies a massive overreplication of the *Y* chromosomal repeats containing the D band in fat body tissues.

Abnormal abdomen revertants also undergo differential replication: A revertant to the wild type was established as a separate stock by not selecting for the *aa* phenotype for 59 generations. We first examined rDNA as a percentage of the total genome in diploid tissue to see if a simple magnification phenomenon could be responsible for the reversion. On the basis of at least six measurements for each sex, we determined that the rDNA percentage of the genome for revertant males and females was 2.18 ± 0.37 and 2.28 ± 0.41, respectively. When these values are compared to similar measures for *aa* males and females (2.10 ± 0.60 and 2.01 ± 0.46, respectively, based on at least eight measurements for each sex), no significant difference is seen between any of the measurements. We conclude that little or no increase in percentage of rDNA per total genome has occurred from the original *aa* stock to the revertant stock. We therefore examined the rDNA restriction fragment patterns in diploid and adult tissue to see if there had been a turnover of rDNA repeats from ins^+ to ins^- in the revertant stock. Figure 4 shows that no turnover of rDNA repeats has occurred in the revertant stock diploid tissue. Furthermore, Figure 4 shows that the reversion phenomenon in males is not caused by the turnover of rDNA repeats specific to the *Y* chromosome, because no D band (which is diagnostic of the shortened NTS of ins^- *Y* chromosomal rDNA repeats) appears in the lanes with male DNA digests.

Finally, we examined the patterns of differential replication in diploid brain and polytene fat body tissue. The expected abnormal abdomen *Eco*RI pattern is observed in the diploid tissue DNA (Figure 3), but a change in the ratio of ins^+ to ins^- repeats is observed in the fat body of revertant larvae. In particular, it appears that the ratio of ins^+ to ins^- repeats is lowered, creating a situation in which about one-half of the rDNA repeats in the fat body tissues are ins^-.

The *ins* sequences produce little or no detectable RNA: Northern hybridization of 5′ *ins* sequences (INS1) to total cellular RNA made from fat body and diploid brain tissue from *aa* and wild-type larvae showed little hybridization to the RNA. Identical results are obtained when we probe the same Northern blots with the middle *ins* probe (INS2) and the 3′ *ins* probe (INS3)

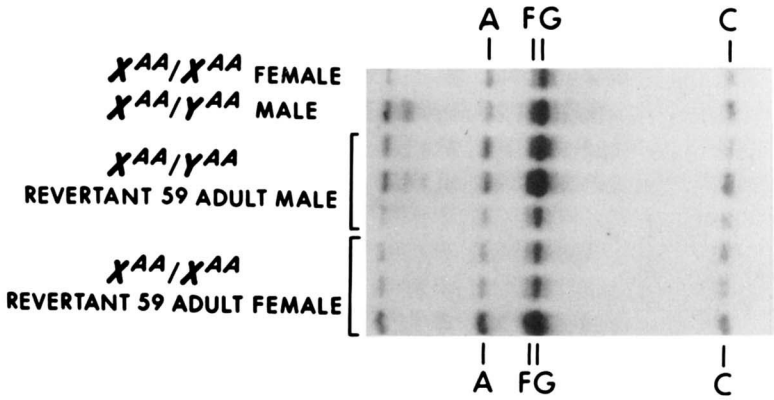

FIGURE 4.—Autoradiograph of *Eco*RI-digested genomic DNA of adult tissue of the indicated stocks hybridized to nick-translated pDmrY22. X^{AA}/X^{AA} is a female and X^{AA}/Y^{AA} is a male from the *aa* stock that has a high penetrance for expression of the *aa* cuticular phenotype. Three revertant-59 adult males and females are included in this autoradiograph. All revertant-59 flies do not express the *aa* cuticular phenotype. Band designations and sizes are given in Figure 1.

(data not shown). We conclude from this experiment that *ins* sequences are either not transcribed or are rapidly processed and degraded.

DISCUSSION

Differential replication controls the amount of ins^+ rDNA cistrons in fat body tissue, but not in diploid tissue: Examination of differential replication patterns in wild-type, *aa*, spontaneous parthenogenetic *aa* mutants and revertants indicates that dominance of rDNA clusters exists in *D. mercatorum*. This result is similar to the observations made for *D. melanogaster* by ENDOW (1980; 1983). In *D. mercatorum* the wild-type *X* and *Y* rDNA cistrons are dominant to the *aa X* rDNA repeats with respect to replication in the polytene tissues. This dominance ensures that, in polytene cells of wild-type (X^+/X^+) and hybrid (X^+/X^{aa} or X^{aa}/Y^+) larvae, the majority of rDNA repeats in polytene tissues will be ins^-. On the other hand, when an X^{aa} is found in the homozygous state or with a Y^{aa}, the majority (but not all) of the rDNA repeats in the polytene tissue will be ins^+.

Revertants to wild type have also been characterized, and it is clear that the reversion phenomenon is not caused by simple magnification of ins^- rDNA repeats. Revertant diploid tissue contains large amounts of ins^+ rDNA. Fat body tissue, however, appears to overreplicate the ins^- rDNA repeats that are present in these cells. It appears that ins^- rDNA genes can be overreplicated relative to the ins^+ rDNA genes within the same chromosome or nucleolus organizer. The differential replication phenomenon in *D. mercatorum*, in effect, produces a tissue-specific control of the ratio of ins^- to ins^+ rDNA repeats.

rRNA transcripts in wild-type, *aa*, revertant and hybrid *D. mercatorum*: We next attempted to determine if the presence of *ins* sequences in the 28S rDNA gene affected transcription of the ins^+ cistrons. Several studies have previously used Northern blotting analysis with the *ins* sequences as probe for

an assay of *ins*$^+$ rDNA transcription (LONG and DAWID 1979; LONG, REBBERT and DAWID 1980; LONG *et al.* 1981). When total cellular RNA from diploid and polytene tissue are challenged with the *ins* sequence probes, little or no hybridization is observed.

This type of result has previously been taken to indicate one of the following: (1) The *ins*$^+$ repeats are not transcribed, (2) the transcription of *ins*$^+$ rDNA repeats is perturbed by the presence of the *ins* sequence, causing proper transcription to terminate at the *ins*, and (3) the repeats are transcribed completely, but the *ins* sequences are processed out and are rapidly degraded. LONG and DAWID (1979), LONG, REBBERT and DAWID (1980) and LONG *et al.* (1981) have previously shown that *ins* sequences are present in very small amounts in the RNA of *D. melanogaster* by Northern techniques. Furthermore, JAMRICH and MILLER (1984) using electron microscopy techniques have presented evidence to support the idea that the majority of the *ins*$^+$ rDNA repeats are not transcribed and that, in those *ins*$^+$ repeats that are transcribed, the *ins* sequences are processed and degraded rapidly during synthesis. These results suggest that *ins*$^+$ rDNA repeats are effectively transcriptionally inactive.

Possible molecular mechanism for the tissue-specific control of *aa*: The original hypothesis that a deficiency of functional rDNA genes is the molecular basis for the *bb* phenotype in *D. melanogaster* and *D. hydei* and for the *aa* syndrome in *D. mercatorum* is based on the idea that the functional rDNA deficiency causes a perturbation in the production of ribosomal RNA and, hence, of ribosomes. This perturbation then imposes a translational control on the synthesis of critical gene products involved in the *bb* and *aa* phenotypes. That the phenotypic effects of *bb* and *aa* appear to be localized or tissue specific suggests that the control of the quantity and transcription of functional and nonfunctional rDNA cistrons might also be tissue specific.

In the *aa* syndrome of *D. mercatorum*, one critical gene product appears to be a juvenile hormone-specific esterase (TEMPLETON and RANKIN 1978; TEMPLETON 1977, 1983) that is produced during an extremely brief developmental window in the fat body of the late third larval instar. We have shown in this study that differential replication in hybrid and wild-type larvae effectively eliminates *ins*$^+$ rDNA repeats in the fat body cells, whereas the lack of differential replication in *aa* fat body cells maintains a large number of *ins*$^+$ repeats. In diploid brain tissue, differential replication has no effect on the ratio of *ins*$^+$ to *ins*$^-$ repeats. We propose that, because the developmental window requiring the production of large amounts of juvenile hormone esterase in the fat body is brief, the fat body cells cannot tolerate even small amounts of *ins*$^+$ repeats. On the other hand, in tissues where there is no requirement for the production of large amounts of a critical gene product in a narrow developmental window, a relatively high number of *ins*$^+$ rDNA repeats can be tolerated. This hypothesis requires that at least two molecular elements be present for the expression of the *aa* syndrome in *D. mercatorum*. First, a large proportion of the rDNA genes must be interrupted by *ins* sequences, and second, the differential replication phenomenon must maintain a large number of these *ins*$^+$ rDNA repeats in the fat body cells of affected larvae. These two molecular

components correlate well with the genetic analysis of TEMPLETON, CREASE and SHAH (1985), in which two genetic loci controlling the *aa* syndrome have been mapped to the centric end of the *X* chromosome.

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