Ribosomal Protein Insufficiency and the Minute Syndrome in Drosophila: A Dose-Response Relationship

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ABSTRACT

Minutes comprise >50 phenotypically similar mutations scattered throughout the genome of Drosophila, many of which are identified as mutations in ribosomal protein (rp) genes. Common traits of the Minute phenotype are short and thin bristles, slow development, and recessive lethality. By mobilizing a P element inserted in the 5′ UTR of M(3)95A, the gene encoding ribosomal protein S3 (RPS3), we have generated two homozygous viable heteroalleles that are partial revertants with respect to the Minute phenotype. Molecular characterization revealed both alleles to be imprecise excisions, leaving 40 and 110 bp, respectively, at the P-element insertion site. The weaker allele (40 bp insert) is associated with a ~15% decrease in RPS3 mRNA abundance and displays a moderate Minute phenotype. In the stronger allele (110 bp insert) RPS3 mRNA levels are reduced by ~60%, resulting in an extreme Minute phenotype that includes many morphological abnormalities as well as sterility in both males and females due to disruption of early gametogenesis. The results show that there is a correlation between reduced RPS3 mRNA levels and the severity of the Minute phenotype, in which faulty differentiation of somatic tissues and arrest of gametogenesis represent the extreme case. That heteroalleles in M(3)95A can mimic the phenotypic variations that exist between different Minute rp-gene mutations strongly suggests that all phenotypes primarily are caused by reductions in maximum protein synthesis rates, but that the sensitivity for reduced levels of the individual rp-gene products is different.

The intriguing phenotypic syndromes of the Minute mutations in Drosophila have been studied in detail for more than 70 years and several hypotheses as to their origin have been postulated (Sinclair et al. 1981, and references therein). However, except for the suggestion that these mutations affect various components required for protein synthesis, none of these ideas has survived experimental scrutiny. Dividing cells require the normal complement of household genes and, therefore, should be particularly sensitive to a reduced rate of protein synthesis. In Drosophila, the imaginal discs are engaged in rapid growth during the second and third larval instar, with cell division occurring every 6–15 hr (Nöthiger 1972). In pupae, the abdominal histoblasts, which are mitotically dormant during the larval stages, undergo rapid cell division (Robertson 1936; Garcia-Bellido and Merriam 1971). Bristle formation during the pupal period (Howells 1972; Mitchell et al. 1977), and normal gametogenesis in both sexes, depends on rapid and flawless protein synthesis. Clearly, then, the panorama of striking phenotypes observed in Minutes (e.g., prolonged development, short and thin bristles, missing and deformed antennae, notched or otherwise malformed wings, small body, rough eyes, reduced fertility and viability, and recessive lethality) is compatible with faulty protein synthesis.

Accumulating data now support the notion that the phenotypic characteristics of M minute mutants are attributable to mutations in ribosomal protein (rp) genes. This correlation has been confirmed for nine rp genes, including those encoding the r-proteins 49 (Kongsuwan et al. 1985), S2 (Cramton and Laski 1994), S3 (Andersson et al. 1994), S5 (McKim et al. 1996), S6 (Watson et al. 1992; K. Watson, personal communication), S13 (Sæbøe-Larssen and Lambertsson 1996), L9 (Schmidt et al. 1996), L14 (Sæbøe-Larssen et al. 1997), and L19 (Hart et al. 1993). In addition, a haploinsufficiency for the r-protein p40 gene (sta) results in the stubarista phenotype, which has Minute-like characteristics including shortened antennae, irregular aristae, short and sparse bristles, and female sterility (Melnick et al. 1993). All phenotypes, except the female sterility, could be rescued by transformation with a 4.4-kb genomic fragment harbouring the p40 wild-type gene. Whether or not the sta mutant is developmentally delayed was not mentioned.

While all characterized single-gene Minute mutants are mutations in rp genes, a reverse correlation is apparently not true. This is emphasized by studies of a chromosomal...
mosomal deletion that removes the two closely linked Rp514 genes (Dorer et al. 1991). The mutation is recessive lethal but heterozygotes do not display any visible phenotype. The fact that there are two functional Rp514 genes present per haploid genome (Brown et al. 1988) may explain the lack of phenotype in this haploinsufficient mutant. There is also the possibility that mutations in genes other than ribosomal protein genes may lead to a Minute phenotype. Complete or partial inactivation of genes involved in protein synthesis such as aminoacyl-tRNA synthetases or protein synthesis factors and mutations that affect ribosome synthesis and transport may lead to a Minute phenotype or a phenotype similar to Minute. The bobbed (ribosomal RNA genes; Ritossa 1976) and mini (5S RNA genes; Procuiner and Dunn 1978) genes are two examples.

Unlike mutations generated by chemical mutagens or radiation, single P-element insertions allow new alleles of the gene to be generated rapidly by imprecisely excising the original element. Studying a range of mutant alleles that includes true nulls and partial revertants is frequently important for understanding gene function and regulation. Imprecise excisions can be selected that delete the gene's promoter and coding sequences or leave small insertions, revealing the true phenotype. P(lac92)M(3)95A is a recessive lethal P-element insertion in the 5′ untranslated region (UTR) of the gene encoding ribosomal protein S3 (RPS3) and produces a strong Minute phenotype in heterozygous mutants (Andersson et al. 1994). The present paper describes two P-element excision alleles of P(lac92)M(3)95A. The new alleles are homozygous viable and partial revertants with respect to the Minute phenotype and exhibit an additive phenotypic effect when combined with each other or with the original mutation. Molecular characterization revealed both alleles to be insertional mutations at the original P-element insertion site and to be associated with reduced RPS3 mRNA levels and distinct phenotypes. Strikingly, a reduction of RPS3 mRNA levels to ~40% of wild type is shown to cause a more severe Minute phenotype compared to P(lac92)M(3)95A, including serious morphological abnormalities and sterility due to arrest of early gametogenesis.

MATERIALS AND METHODS

Fly stocks and generation of P(lac92)M(3)95A excision alleles: Fly stocks were maintained on standard potatomash, yeast and agar substrate at 21°; all crosses were done at 25°. The original P(lac92)M(3)95A mutant was recovered from a mutagenesis screen and has been described earlier (Andersson et al. 1994). Partial revertants were obtained by crossing P(lac92)M(3)95A/ry ry506 Sb P[y′ -2-3](99B) males to Df(3R)ry81 TM6B,Ubx females and rosy ebony females, respectively, and the non-Stubble rosy progeny were selected and scored for the mutant, a dysgenic cross was set up between P(lac92)M(3)95A/ry Sb P[y′ -2-3](99B) TM6B,Ubx males, and P(lac92)M(3)95A/ry Sb P[y′ -2-3](99B) males were collected from the progeny. These

RESULTS

Generation of P(lac92)M(3)95A partial revertants: To mobilize the P element inserted in the P(lac92)M(3)95A mutant, a dysgenic cross was set up between P(lac92)M(3)95A/ry Sb P[y′ -2-3](99B) TM6B,Ubx males, and P(lac92)M(3)95A/ry Sb P[y′ -2-3](99B) males were collected from the progeny. These
males were crossed to Df(3R)ry42/M KRS, rySb females, and non-Stubble rosy males and females were selected and classified with respect to their bristle phenotype. While most of the progeny appeared to be either wild type (precise excision) or M (3)95A large insertion or deletion, two partial revertants were found that display intermediate phenotypes. In heterozygous flies the two alleles, termed P(lac92)M(3)95A prv9 (prv9) and P(lac92)M (3) 95A prv11 (prv11), have a moderate and weak/wild-type Minute bristle phenotype, respectively.

**Genomic organization of partial revertant alleles:** To determine the nature of the mutations generated in the excision events, a genomic fragment covering the P-element insertion site was used to probe a Southern blot containing BamHI + BglII digested genomic DNA from wild type, P(lac92)M (3)95A/TM2, and partial revertant stocks (results not shown). The results showed that the P element had excised imprecisely and left a small insertion in both revertant alleles. To characterize these insertions at the nucleotide level, PCR products were generated from genomic DNA with biotinylated primers flanking the insertion site and sequenced using a direct approach on streptavidin-sequence-specific primers flanking the insertion site and sequences from the promoter region were generated from genomic DNA with biotinylated to reside both upstream and downstream of the transduction event. A genomic fragment covering the excision events, a genomic fragment covering the P-element insertion site in the RPS3 gene was sequenced using a direct approach on streptavidin-sequence-specific primers flanking the insertion site and sequences from the promoter region were generated from genomic DNA with biotinylated to reside both upstream and downstream of the transduction event. The top part shows a schematic diagram of the wild-type M (3)95A gene. Black boxes represent protein coding regions while shaded boxes represent non-coding parts of the exons. The P-element insertion site in the P(lac92)M (3)95A mutant is indicated by a vertical line. The bottom part shows a sequence alignment of 5' UTRs from wild-type (wt), prv9 and prv11 alleles. Gaps are indicated by dashes, the 8-bp target-site duplications are boxed, and sequences from the P-element inverted repeats are indicated by horizontal arrows.

Figure 1.—Genomic organization of the M (3)95A gene in wild-type, P(lac92)M (3)95A, and partial revertant prv9 and prv11 alleles. The top part shows a schematic diagram of the wild-type M (3)95A gene. Black boxes represent protein coding regions while shaded boxes represent non-coding parts of the exons. The P-element insertion site in the P(lac92)M (3)95A mutant is indicated by a vertical line. The bottom part shows a sequence alignment of 5' UTRs from wild-type (wt), prv9 and prv11 alleles. Gaps are indicated by dashes, the 8-bp target-site duplications are boxed, ATG start codons have black background, in-frame stop codons are underlined, and sequences from the P-element inverted repeats are indicated by horizontal arrows.

Quantitative Northern analysis: To examine RPS3 mRNA levels (transcriptional efficiency and/or mRNA stability) in the partial revertants, three Northern blots with separate poly(A)+ mRNA extractions from wild type, P(lac92)M (3)95A/ry/TM6B, prv9/prv9, and prv11/prv11 adult females were hybridized with single-stranded RPS3 and RPL14 cDNA probes; one of the Northern blots is shown in Figure 2. Quantitation was carried out by phosphor imaging with the binding of eIF-4B to the cap structure. It has been shown that a hairpin structure with a $\Delta G = -14$ kcal/mol located six nt downstream of the cap site abolishes this binding, whereas a more extensive structure located 37 nt downstream of the cap site does not (Pelletier and Sonenberg 1985b). Located within the 5' UTR these inserts also contain four and five upstream ATG start-codons (uATG). Two of these are in a suboptimal context and are in both alleles located in the most terminal ends of the insert [5' end: CATGATG; 3' end: CATCATG; Drosophila consensus: (C,A,G,T)$_{46}$, (A,G,T)$_{27}$, (T,C)$_{14}$, G$_{3}$] (A$_{G13}$, A$_{70}$, G$_{19}$, C$_{6}$, T$_{5}$) (A$_{G19}$, C$_{21}$, T$_{16}$, G$_{12}$) (A$_{G12}$, C$_{30}$, G$_{36}$, T$_{9}$) ATG; Brown et al. 1994). Translational initiation at the most upstream uATG would produce a three amino acid non-sense product, while at the most downstream uATG which is in frame with the wild type ORF, it would produce a RPS3 protein with a N-terminal extension of ten amino acids. Whether or not these initiations occur in vivo has not been investigated. The promoter region of rp genes in higher eukaryotes are in general known to reside both upstream and downstream of the transcription start site (Hariharan et al. 1989; Atchison et al. 1989). Thus, the insertion present in the 5' UTR of prv9 and prv11 is likely to impair translational initiation. Since the insert in prv9 is considerably longer than in prv11, this effect should be most manifest in prv9, consistent with the differences observed in mRNA levels (see below).
while the picture in Figure 2 is a Polaroid of exposed X-ray film. First, the Northern analysis showed that the prv9 and prv11 alleles produce RPS3 mRNA that is increased in size by approximately the number of nucleotides inserted in their 5' UTR. Thus, transcription is initiated at or near the wild-type initiation-site and is fixed with respect to the upstream region. In prv11, this choice of initiation site has been verified by primer extension analysis (results not shown). Second, quantitative analysis showed that RPS3 mRNA abundance in P{lac92}M(3)95A_Tm6B flies is reduced by 40±7%. This is close to what would be expected for a haploinsufficient mutant and corroborates the data reported by Andersson et al. (1994). In homozygous prv9 and prv11 flies, the RPS3 mRNA levels are 40±5% and 85±2% of wild type, respectively. Thus, an approximate correlation exists between the size of the fragment inserted in the M(3)95A promoter region and reduced mRNA levels. It is also possible that the difference in RPS3 mRNA levels between prv9 and prv11 may be ascribed to the nature of the inserted sequence.

The P{lac92}M(3)95A phenotype: The phenotypic measurements obtained for P{lac92}M(3)95A in these studies supplement those reported by Andersson et al. (1994). All flies involved in these analyses were constructed to have identical genetic and maternal background (wild-type Canton-S) which produces the most accurate results. The P{lac92}M(3)95A phenotype features larval development prolonged by ~51 hr, a ~45% reduction of female fertility (egg production rate) and vitality (fraction of hatched eggs appearing as adults) reduced by ~10%. The scutellar bristles of P{lac92}M(3)95A/+ are reduced in length and thickness by ~40%. These data describe a strong Minute phenotype that is the result of a ~40% reduction in RPS3 mRNA abundance.
Figure 4.—The ovary phenotype of homozygous prv9 female flies. (A–B) Scanning electron micrograph of ovaries from wild-type (A) and homozygous prv9 (B) flies. O, ovary; Ol, ovariole (between arrows); Uo, undeveloped ovary; t, trachea. (C–D) Ovarioles from wild-type (C) and homozygous prv9 (D) ovaries stained with DAPI which binds to DNA. The wild-type ovariole is seen to contain a germarium (left) and egg chambers of approximately stages 2, 3, 4, 5 and 7 in ascending order, while the homozygous prv9 ovary contains scattered germaria with stalled stage 2 egg chambers with no enveloping follicle cells and many small nuclei in what appears to be a disorganized mass of cells. The mutant ovariole is enlarged three times compared to wild type. (E–F) Transmission electron micrograph of cross section from the apical part of wild-type ovary (E) with developing egg chambers and homozygous prv9 ovary (F). The latter contains a germainium and stalled-degenerating stage 1 egg chambers (unmarked arrowheads). bs, basal stalk cell; fc, follicle cell; fn, follicle cell nucleus; nc, nurse cell; ncn, nurse cell nucleus; oc, ovariolar cavity; po, pro-oocyte; tc, tracheal cell. Bar, 5 μm.

The effects on different body parts indicate that development of various imaginal discs is impaired as a secondary consequence of greatly reduced RPS3 mRNA levels and reduced protein synthesis.

Both sexes of homozygous prv9 flies are completely sterile (females lay no eggs and males are unable to fertilize wild type females), and dissection of the animals revealed undeveloped gonads to be the cause of this. A normal ovary consists of a cluster of about 16 parallel ovarioles held together by an enveloping peritoneal sheath which contains a network of anastomosing muscle fibres. In the adult female, each of the tubular ovarioles contains a germarium at its anterior end where the egg chambers are assembled and a vitellarium at its posterior end with seven to eight egg chambers in progressively older stages of oogenesis. Oogenesis starts
during the pupal stage and the oldest egg chambers at eclosion are in stage 7; it then takes more than 24 hr to produce the first mature egg. Scanning electron micrographs of ovaries from wild type (Figure 4A) and homozygous prv9 (Figure 4B) animals clearly reveal the size differences and the absence of ovarioles in the mutant ovary. Ovaries were stained with DAPI, which binds to the DNA, and inspected with a fluorescence microscope. Whereas the different stages of oogenesis in normal ovaries are clearly and distinctly revealed by the nuclei of both nurse and follicle cells (Figure 4C), the undeveloped ovaries of homozygous prv9 females (2-4 day old) are malformed and disorganized, and contain scattered germaria and staged egg chambers that may correspond to stage 2 (Figure 4D). The nuclei of the enveloping follicle cells, which are seen at very early stages in a normal ovariole, are missing in the prv9 ovaries. There are, however, numerous small nuclei present (Figure 4D) but whether these originate from nurse cells or follicle cells is not known at the moment. These findings were further confirmed by transmission electron microscopy studies. Whereas egg chambers with polyploid nurse cells and enveloping follicle cells are easily recognized in wild-type ovaries (Figure 4E), homozygous prv9 ovaries contain occasional germaria with a germarial cyst and one or two egg chambers stalled at approximately stage 2 and lack enveloping follicle cells (Figure 4F). The prv9 germaria are strikingly reminiscent of those present in developing ovaries in 48-hr-old pupae, in which follicle cells are easily observed (King et al. 1968). Since ovaries of newly hatched wild-type females contain ovarioles with stage 6 or stage 7 egg chambers, the results show that oogenesis in homozygous prv9 is arrested at very early stages, which explains why the ovaries remain small.

Figure 5, A and B shows scanning micrographs of wild type and homozygous prv9 testes from 3-5-day-old males. The prv9 testes (Figure 5B) are considerably smaller than wild type (Figure 5A), and have small bulges spread along their length. Transmission electron microscopy of wild type and mutant testes revealed that, whereas the apical part of wild-type testes is filled with individualized spermatid bundles containing 64 spermatids (Figure 5C), there are neither spermatocytes nor spermatids nor sperm present in homozygous prv9 testes (Figure 5D). There is, however, a disorganized mass of cells that may contain remnants of spermatid cysts. Both sections are in the apical part of the testis but, because the prv9 testes are much smaller than wild type, the sections may not be fully comparable.

The P(lac92)M(3)95A/prv11 phenotype: Homozygous prv11/prv11 flies are characterized as moderate Minutes and feature larval development prolonged by \( \sim 22 \) hr and \( \sim 20\% \) shortening of scutellar bristles. The egg production rate of females is reduced by \( \sim 40\% \), and viability is unaffected. In prv11/+ heterozygotes the only measurable phenotype is a \( \sim 5\% \) reduction of scutellar bristle length, which can be recognized only after close examination of postalarare bristles against the alula (wing flap).

Whether or not the \( \sim 15\% \) reduction in RPS3 mRNA abundance observed in homozygous prv11 is the exclusive cause of the moderate Minute phenotype has not been addressed experimentally. However, the prv11 phenotype is somewhat more severe than that of heterozygous prv9/+ flies, which have a \( \sim 30\% \) reduction of RPS3 mRNA abundance. Thus, it cannot be ruled out that the sequences inserted into the 5' UTR of prv11, containing several uATGs and a putative hairpin structure, may have a negative effect on the translation of this aberrant mRNA and thereby contribute to the phenotype. In principle, the inserts present in prv9 and prv11 have the same basic features, but the prv9 allele contains an additional 66-bp fragment that separates the inserted repeats. This separating fragment may impair the formation of a stem-loop structure, and thus, the two mRNAs may behave differently with respect to translational efficiency.

Additivity of phenotypes: A complementation analysis was carried out at 25° by crossing wild type, P(lac92)M(3)95A, and partial revertants in all possible combinations (Table 1). These tests revealed that (1) prv9 is lethal in combination with P(lac92)M(3)95A, (2) prv9/prv11 heterozygotes have a strong Minute phenotype comparable with that of P(lac92)M(3)95A/+ and, (3) prv11/P(lac92)M(3)95A heterozygotes have an extreme/semi-lethal Minute phenotype with a vitality (fraction of hatched eggs appearing as adults) of only 3–5%. (Most die as pupae or are too weak to break out of the pupal case.) Hatched prv11/P(lac92)M(3)95A flies are also sterile, have severe morphological lesions similar to those of prv9 homozygotes, and usually live for one or two days only. The results show that there is an approximate correlation between reduced RPS3 mRNA levels and the severity of the Minute phenotype, in which disruption of gametogenesis and imaginal disc development represents the extreme consequence prior to lethality.
translational initiation at uAUGs followed by premature termination could result in nonsense-mediated mRNA degradation (Theodorakis and Cleveland, 1996, and references therein). Both prv9 and prv11 have an uAUG in a sub-optimal context close to the 5' terminus that is followed by a stop codon after one triplet. However, since there is a four-fold difference in the reduction of RPS3 mRNA between the two partial revertants this cannot be a likely explanation.

Due to the presence of uATGs and inverted repeats in the 5' UTR of the prv9 and prv11 alleles, some uncertainty exists regarding the efficiency with which the mRNAs are translated to yield functional protein. A tendency of translational initiation at an uAUG would
show no Minute phenotype. One interpretation of these extraribosomal function (Wool 1996, and references therein), and a secondary structure located close to the 5' terminus may lessen initiation by interfering with the binding of eIF-4B to the cap structure (Pelletier and Sonenberg 1985b). In particular this applies to prv11 in which a ~15% reduction in RPS3 mRNA abundance is seen to cause a phenotype more severe than that of prv9/+ (~30% reduction of RPS3). Obviously, the prv11 mRNA is not translated with normal efficiency. The major difference between prv9 and prv11 is that the insert present in the prv9 allele contains an additional 66-bp fragment that separates the inverted repeats. Supposing that this separating fragment has a negative effect on the formation of a stem-loop structure, the prv9 mRNA may be translated with higher efficiency. This interpretation is consistent with the observation that prv9/+ has a Minute phenotype less severe than that of P{lac92}M(3)95A/ + (~40% reduction of RPS3).

A rp-gene mutation with a phenotype similar to prv9 has been described previously. string of pearls (sopP) is a P-element insertion in the promoter region of the gene encoding RPS2, and was reported to result in a recessive Minute phenotype (Cramton and Laski 1994). The sopP mutation causes an incomplete inactivation of transcription, and 10-15% of homozygous sopP embryos manage to reach the adult stage. Surviving homozygous sopP/ sopP flies have a 60-70% reduction in RPS2 mRNA levels and display an extreme/semi-lethal Minute phenotype as well as female sterility due to arrest of oogenesis at stage 5. The stage 5 cysts are normal in that they have 15 nurse cells and one oocyte positioned properly at the posterior end. Major differences between prv9 and sopP are the stages reached during oogenesis (2 and 5, respectively). Also, prv9 males are sterile, many prv9 flies also have morphological defects, and sopP/+ flies show no Minute phenotype. One interpretation of these differences could be that the impairing of protein synthesis is more severe in prv9 than in sopP. Alternatively, RPS2 and RPS3 may have specific, but different, bifunctional roles during gametogenesis.

All cells involved in gametogenesis require the normal supplement of household genes to maintain a balance between the levels of soluble proteins, various membranes and ribosomes in order to optimize conditions for this process. The gonad primordium is established during embryogenesis when the migrating germ cells become enfolded by somatic cells of mesodermal origin (Sonnenblick 1941; Campos-Ortega and Hartenstein 1985). During the larval stages these groups of cells divide continuously; they begin differentiation during the pupal stages (King 1970). The absence of developing germ cells and the early stall of gametogenesis in homozygous prv9 may be a secondary effect of a faulty differentiation of the somatic parts of the gonads. It is equally possible, however, that the growth and division of the germ-line stem cells are more sensitive to a reduction in protein synthesis than are the somatic portions of the gonads. The similarity between the defects in both females and males suggests that RPS3 plays a role common to the early stages of gametogenesis in both sexes. A flawless protein synthesis is a prerequisite in both oogenesis and embryogenesis where each cell is dependent on receiving a sufficient supply of proteins and organelles. Thus, a reduction in the maximum protein synthesis rate, caused by strongly reduced RPS3 mRNA abundance, appears to be the most logical explanation for the arrested gametogenesis in homozygous prv9 flies. This reduction appears to be deleterious at critical stages in gametogenesis and development, and different degrees of reduction in r-protein levels or other ribosomal components may arrest gametogenesis at specific stages.

There is evidence that some ribosomal proteins have extraribosomal function (Wool et al. 1996, and references therein), and it has been suggested that the blocking of

<table>
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<th>Genotype</th>
<th>RPS3 mRNA level</th>
<th>Scutellar bristle length</th>
<th>Developmental delay (hr)</th>
<th>Egg production rate</th>
<th>Survival rate to eclosion</th>
<th>Denoted Minute phenotype</th>
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<td>prv9 / M (3)95A</td>
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<td>95</td>
<td>ND</td>
<td>ND</td>
<td>SI</td>
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</table>

NA, not applicable; ND, no detectable deviation from wild type; NI, not investigated.

^ Percent of wild type.

^ Putative values calculated from combination of alleles.

^ Semi-lethal.

^ Both males and females are sterile and have morphological defects.
ogogenesis in homologous sp² females (60–70% reduction in RPS2 mRNA abundance) is the result of such a non-translational role played by this protein (Craigton and Laski 1994). However, the observation that an equivalent percentage-wise reduction in RPS2 and RPS3 mRNA abundance produces similar phenotypes indicates that arrest of oogenesis may be a general effect of strongly reduced protein synthesis. It is also possible that the many morphological defects frequently observed in homoygous prv9 flies are a consequence of strongly reduced protein synthesis through incomplete growth/development of imaginal discs and somatic tissues.

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LITERATURE CITED


Brown, C. M., P. A. Stockwell, M. E. Dalphin and W. A. Tate, 1994 The translational signal database (TransTerm) now also includes initiation context. Nucleic Acids Res. 22: 3620–3621.


Robertson, C. W., 1936 The metamorphosis of Drosophila melanogaster, including an accurately timed account of the principal morpho- logical changes. J. Morph. 59: 351–398.


Watson, K. L., K. D. Konrad, D. F. Woods and P. J. Bryant, 1992 Drosophila homolog of the human S6 ribosomal protein is re-


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