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Developmental genetics of ribosome synthesis in *Drosophila*

Mark A. Kay and Marcelo Jacobs-Lorena

The coordination of expression of more than 70 genes that code for the ribosome represents a complex problem for the cell and for the developing organism, in terms of gene expression and regulation. The rapid advances made in the fields of genetics and molecular biology of Drosophila *make this organism a valuable model system for studying the regulatory phenomena involved in ribosome synthesis during development.*

The eukaryotic ribosome is a complex structure consisting of at least 70 ribosomal proteins (r-proteins) and four rRNA molecules. The 60S subunit contains about two-thirds of the r-proteins and 28S, 5.8S and 5S RNA molecules, while the 40S subunit contains the 18S RNA molecule and remaining r-proteins. The 18S, 28S and 5.8S RNA molecules (collectively referred to as rRNA) are derived from a single precursor which is synthesized by RNA polymerase I in the nucleolus. In contrast, the 5S rRNA is transcribed elsewhere in the nucleus by RNA polymerase III. The genes coding for r-proteins are transcribed by a third enzyme, RNA polymerase II. *Drosophila* has about 200 rRNA and 5S genes. Unlike in other eukaryotes (e.g. yeast, frog, mouse), the r-protein genes of *Drosophila* are for the most part single copy and distributed over the entire genome (Refs 1–3; S. Qian, J-Y. Zhang and M. Jacobs-Lorena, unpublished; Table 1).

The demand for new ribosomes varies dramatically during different developmental periods[4,5]. Thus, the rate of accumulation of the individual components must be regulated accordingly. Very little information is available on the mechanisms involved in this regulation. However, mutations have been described that appear to affect ribosome synthesis and/or assembly. These and other properties confer upon *Drosophila* important advantages for dissecting the molecular and genetic aspects of ribosome synthesis during development.

Gene regulation during development

The rate of ribosome synthesis in *Drosophila* ovaries[4] is probably the highest of any tissue at any developmental time, but no ribosome synthesis can be detected in early embryos[5]. However, the large stock of maternal r-protein mRNAs is preserved in these embryos[6–9]. Translation of r-protein mRNAs during *Drosophila* development closely parallels rRNA transcription (reviewed in Ref. 6). Ribosomal protein mRNAs are most actively translated during oogenesis[7,8], while a major portion of these mRNAs is stored as translationally inactive postpolysomal messenger ribonucleoprotein particles (mRNPs) during early embryogenesis (embryos aged 0–5 h). In contrast, most other abundant mRNAs remain associated with polysomes during this period. As

Table 1. *Cloned* Drosophila *ribosomal protein genes and relationship to* Minute *mutations.*

Ribosomal protein	Chromosomal location	*Minutes* at this location[a]	Ref.
rp49	99D	M(3)99D	29
rpA1	53CD	M(2)S7[b], M(2)40c	3, 24
7/8	5D	M(1)30	2
S18	15B	M(1)0	2
L12	63E	M(3)LS2	2
rp21	80C	M(3)QIII[c]	30
rp40	53[d]	M(2)S7, M(2)40c	[e]

[a]Number in parentheses indicates the chromosome number.
[b]In P-element transformation experiments, the rpA1 gene did not rescue the M(2)S7 mutation (S. Qian and M. Jacobs-Lorena, unpublished).
[c]In P-element transformation experiments, the rp21 gene did not rescue the M(3)QIII mutation[30].
[d]This location of rp40 is tentative.
[e]S. Qian, J-Y. Zhang and M. Jacobs-Lorena, unpublished.

embryogenesis proceeds, r-protein mRNAs again associate with polysomes[7–9], concomitant with an increased rate of rRNA synthesis[5]. Thus, a temporal relationship exists between the synthesis of rRNA and the translation of r-protein mRNAs. An additional finding was that the degree of translational repression in early embryos varies for different r-protein mRNAs[8,9]. A similar finding was made for *Xenopus*[10]. The reasons for these differences are unclear at present.

Interestingly, *Drosophila* and *Xenopus* use different strategies to regulate r-protein gene expression. In both organisms, rRNA synthesis is undetectable and r-protein synthesis is selectively repressed in early embryos. However, while r-protein mRNAs are translationally regulated in *Drosophila*, r-protein mRNAs are selectively degraded in *Xenopus*[10]. Thus, while in both organisms r-protein synthesis is down-regulated, the mechanisms involved are very different.

Genetic mutations affecting ribosome production, including the *bobbed* locus

A number of genetic loci that affect ribosome synthesis in *Drosophila* have been described. For instance, the suppressor of forked [*suf(f)*] allele has been described as a putative r-protein mutant[11]; however, this now seems unlikely. Falke and Wright[12] have described eight separate X-linked cold-sensitive female-sterile mutants that appear to be defective in ribosome assembly, but no further molecular characterization of these mutants has been reported.

Drosophila has about 200 5S RNA genes per haploid genome. A mutation called *mini* (*min*), that deletes 47% of the 5S genes has been isolated and characterized[13]. Not surprisingly, the phenotype of *min* was found to be very similar to that of mutants that affect genes coding for other ribosomal components (*bobbed* and *Minute*, see below).

The best studied genetic alterations of ribosome synthesis in *Drosophila* are mutations in the rRNA structural genes known as the *bobbed* (*bb*) locus; *bobbed* flies have short thoracic bristles, delayed development and etched abdominal tergites[14]. The X and Y chromosomes of *Drosophila* each have a cluster of about 200 tandemly arranged rRNA genes. The total number of genes may vary somewhat from strain to strain and the distribution of genes between the two sex chromosomes may not be identical[14]. It has been well established that *bb* mutations are partial deletions of the rRNA genes[14]. The severity of the phenotype is dependent to a first approximation (see below) on the number of rRNA genes present. Generally, a 50% reduction in the number of rRNA genes results in a *bb* phenotype while a 90% reduction is lethal[14].

Early studies could not always correlate the exact gene number (as measured by saturation hybridization experiments) with the severity of the phenotype[14–16]. More recently, it was shown that as many as two-thirds of the rRNA genes contain insertion sequences and that these interrupted genes are not transcribed and therefore not functional[17,18]. Thus, rather than the absolute number of rRNA genes, it is the number of *functional* genes that is important. Indeed, a direct correlation exists between the severity of the *bb* phenotype as determined by bristle length, and the rate of rRNA accumulation in the fly[15,16].

The intriguing observation has been made that the total RNA content of developing and mature oocytes is the same in *bb* as in wild-type flies[19,20], despite the deficiency of rRNA genes in the mutant. However, oogenesis in *bb* flies progresses at a reduced rate (i.e. the time that developing *bb* oocytes spend at each stage of oogenesis is significantly increased)[20]; thus it takes considerably longer to produce an egg in a *bb* than in a wild-type fly. One may speculate that in the mutants, accumulation of rRNA becomes the rate-limiting process in development.

Protein synthesis must also proceed at a decreased rate in *bb* oocytes. However, when the proportion of the ribosomes that is engaged in protein synthesis (polysome-associated) in ovaries of *bb* and wild-type flies was measured, no difference was found. Moreover, the average size of polysomes and the distribution of several mRNAs between polysomal and postpolysomal fractions were also the same[9]. These observations led to the hypothesis that the reduction in the rate of protein synthesis occurs by a concerted decrease in the rates of initiation, elongation and termination of translation[9]. Precedents exist in *Drosophila* for such a mechanism of coordinate reduction of protein synthesis. For instance, during the final stages of oogenesis and during early embryogenesis the total polysome content per egg chamber or embryo is very high, even though no detectable protein accumulation occurs. This and other observations suggested that at the end of oogenesis the efficiency of translation drops by about 20-fold[21]. A similar decrease in the rates of protein synthesis also occurs when *Drosophila* tissue culture cells are subjected to heat shock[22].

The body plan of *bb* mutants is relatively normal, despite the limitation in the capacity for protein synthesis and the fact that development is delayed. This suggests that there is a built-in developmental program that provides for an orderly progression after each body component is synthesized, as opposed to a pre-set 'clock' type mechanism. The slowed oogenesis and the constancy of the ribosome number in eggs of even severe *bb* flies is an example of this principle. However, the developmental program of *bb* flies is not without some imperfections. For instance, during pupal development, progression through the developmental program may not be sufficiently delayed to allow components of bristles and of the cuticle to be synthesized in sufficient quantities; hence the short bristle and etched cuticle phenotype characteristic of *bb* mutants.

The *Minute* loci

The *Minute* (*M*) loci of *Drosophila* comprise a class of about 50 phenotypically similar, unlinked mutations that are believed to affect protein synthesis[23,24]. *Minutes* are cell-autonomous, recessive lethals[25,26]. The dominant phenotype is very similar to that of *bb* mutants. The *Minute* phenotype includes any or all of the following: prolonged larval development (the pupal but not the embryonic development may also be somewhat delayed), short and narrow thoracic bristles, etching of the abdominal tergites, delay in the rate of cell division, small cell size leading to reduced body size, and lowered fertility (Fig. 1).

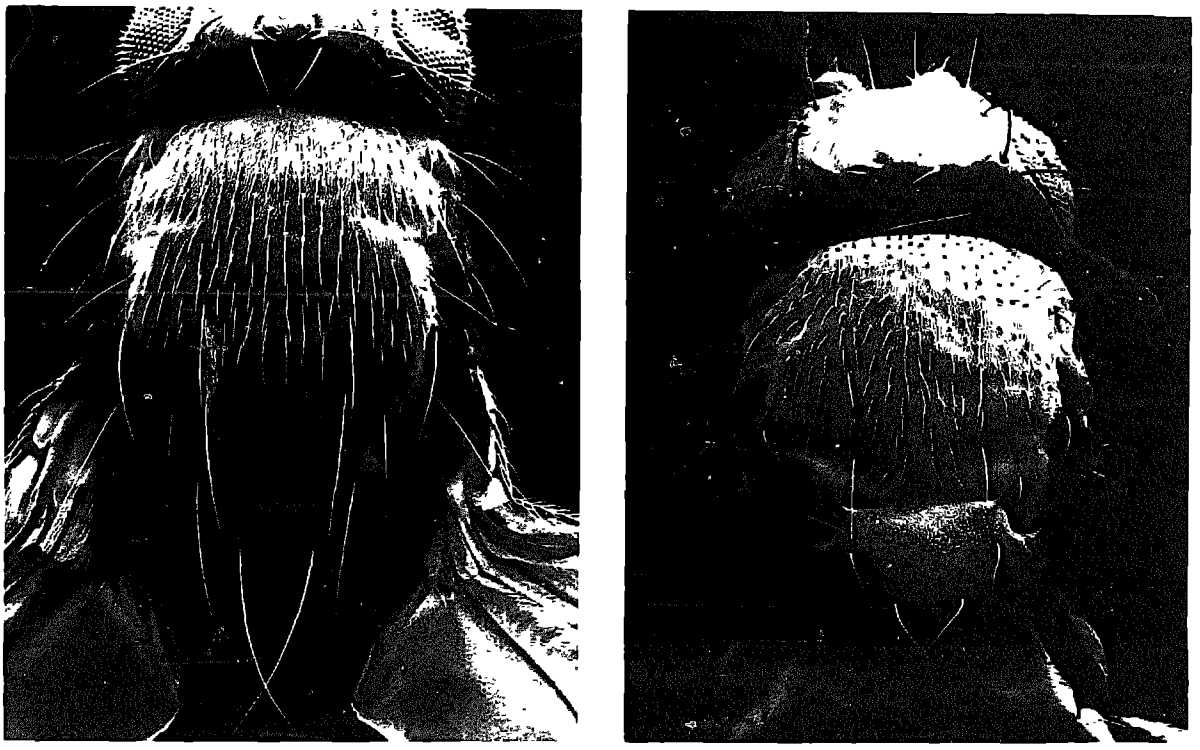

Fig. 1. *Scanning electron micrographs of* Minute *and wild-type* Drosophila. *The wild-type fly on the left has bristles of normal size while the* Minute M(2)S7 *fly on the right has much shorter bristles. Note the reduced body size of the* Minute *fly, which is probably due to a general reduction of cell size. The magnification of the two micrographs is the same. Mutations that affect ribosome synthesis, such as* bobbed *and* mini, *have similar phenotypes to* Minute.

In the course of over half a century of study, *M* mutations have been proposed to affect genes of very diverse functions (see Ref. 23 for references). More recently, based mostly on indirect evidence, the proposal has been made that *M* loci specify component(s) required for protein synthesis. Evidence consistent with the hypothesis that r-protein genes are affected by *M* mutations comes from the finding that all cloned r-protein genes map cytologically on polytene chromosomes to regions near *M* loci (Table 1).

Homozygous *Minutes* die as late embryos or early first-instar larvae[27], indicating that the products of the wild-type loci are required at least during the later portion of embryogenesis. Most *M* loci appear to represent small chromosomal deletions, indicating that the genes are haploinsufficient[24]. This conclusion is corroborated by the observation that in the triplo state *M* acts as a recessive gene (M/+/+ flies have a wild-type phenotype[28]). Interestingly, the effect of *M* mutants is not additive; combinations of two or three different *M* loci do not increase the severity of the phenotype[28]. This was the first evidence to support the idea that these loci code for genes of similar function. This finding is also consistent with the hypothesis that *Minutes* are mutations in r-protein genes, since failure of ribosome assembly due to lack of one or of two r-proteins is expected to be equivalent.

Recent evidence from Kongsuwan *et al.*[29] has shown that a cloned DNA containing sequences coding for the r-protein rp49 rescues a previously undescribed *M* mutation on the X chromosome, establishing the identity between the r-protein rp49 gene and this *M* mutant. In contrast, the gene coding for r-protein rp21, which is located in region 80 of chromosome 3L, has been recently shown not to be identical to the temperature-sensitive QIII *M* allele that genetically maps to the same location[30].

The best criterion to establish the identity of a r-protein gene and a *M* mutant would be the rescue of this *Minute* by the cloned r-protein gene via P element-mediated transformation. However, there are difficulties with this approach. There may not be a known *M* mutation corresponding to a given cloned r-protein gene. Moreover, *M* deletions may be too large and encompass more than one r-protein gene, in which case rescue by a cloned gene becomes impossible. To overcome some of these limitations, our laboratory has recently attempted to inactivate a r-protein gene by constructing the corresponding antisense gene. A cloned DNA coding for r-protein rpA1 Ref. 3 was placed in reverse orientation in front of the heat shock promoter and introduced into flies via P element-mediated transformation. Surprisingly, no noticeable effect on the rate of development or bristle morphology was observed. However, oogenesis was severely affected in the transgenic (but not in control) flies, and then only when expression of the antisense sequence was induced by a brief heat pulse (S. Qian, S. Hongo and M. Jacobs-Lorena, unpublished). Although a more complete analysis is needed, these preliminary results indicate that the antisense approach may prove to be useful in the elucidation of r-protein gene function in *Drosophila*.

Most homozygous *Minute* mutants develop until

hatching and die as early first-instar larvae[27], while heterozygotes develop normally during this period of development. The first noticeable phenotypic change in homozygous *Minutes* occurs during mid-embryogenesis (10–12 h after fertilization) and is characterized by a slower development of the midgut, with yolk frequently remaining in its lumen[31]. Hatched larvae are considerably smaller than controls[31]. This phenotype suggests that the synthesis of new *M* transcripts is required for normal late embryonic development. Moreover, if we assume that *M* loci encode r-proteins, the above observations imply that newly transcribed r-protein mRNAs are at least in part translated in late embryos and that the increased association of r-protein mRNAs with polysomes in late embryos[7–9] is not entirely due to maternal mRNA transcripts.

An unanswered question remains as to whether there is a perfect correspondence between *Minutes* and r-protein genes. While it has been estimated that 40–50 *M* loci exist in the *Drosophila* genome, it is clearly established that at least 70 proteins are present in the ribosome. One obvious interpretation of these observations is that not all *M* loci have yet been discovered. Alternatively, it is possible that deletion of one dose of some r-protein genes does not result in a *M* phenotype. Such r-proteins may represent a set of r-protein genes that are normally overproduced in wild-type flies. A possible example is the set of r-protein genes whose translation is not effectively repressed during early embryogenesis[9]. The more efficient translation of these r-protein mRNAs would increase the protein output per mRNA molecule, thus overcoming the haploinsufficiency that is characteristic of the *M* mutation.

The possibility that *M* mutations affect genes other than those coding for r-proteins should be considered. *Minutes* appear not to be mutations of structural tRNA genes, but genes coding for tRNA synthetases, initiation and/or elongation factors are possible candidates for being targets of *M* mutations. However, these alternatives are less attractive since genes coding for enzymes (which are needed in relatively small amounts) are not expected to be haploinsufficient.

Comparison of ribosomal protein genes in eukaryotes and prokaryotes

If *Minutes* do in fact code for r-proteins, a fundamental difference may exist between eukaryotes and prokaryotes. Thirteen different *E. coli* mutants lacking one or two of the 52 r-proteins have been isolated by Dabbs and coworkers[32]. Immunological tests indicated that these were null mutants. Surprisingly, all of these mutants are viable, although the growth rate of some is affected. This is despite the fact that several of the mutated genes code for proteins that had been determined from *in vitro* studies to have important roles in ribosomal assembly or function. For instance, protein L24 was thought to be an essential assembly-initiator protein. A mutant lacking protein L24 (confirmed by sequencing the mutant gene) still makes viable ribosomes, although a temperature-sensitive growth phenotype was observed[33]. Apparently other r-protein(s) can take over the assembly-initiator function at lower temperatures.

At present it is unclear which of the remaining 39 *E. coli* r-protein genes, if any, are essential. In contrast, all of the large number of *M* loci of *Drosophila* and several r-protein genes of yeast[34,35] are known to be essential for viability. This difference between eukaryotes and prokaryotes in the requirement for r-proteins may result from differences in ribosome assembly between these two classes of organism. Since the eukaryotic ribosome is more complex than the prokaryotic, one may expect that the deficiency of a given r-protein leads to complete interruption of ribosomal assembly rather than to the synthesis of partially functional ribosomes, as appears to be the case in *E. coli*.

Concluding remarks

Mutations that affect the development of *Drosophila* have been known for decades. With the advent of molecular approaches, it was determined that several of these mutations affect structural ribosomal genes. Only recently have the molecular events leading to the control of ribosome synthesis during development begun to be dissected. However, progress in understanding the actual control mechanisms has been slow. A promising approach for the future may be the use of a combined genetic and molecular approach to characterize genes that do not themselves code for ribosomal components but rather for proteins that play a regulatory function in their synthesis.

Acknowledgements

We thank Ms K. Maier and Dr A. Mahowald for the electron micrographs. The work from the authors' laboratory was supported by a grant from the National Institutes of Health (USA).

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Mammalian spermatogenic gene expression

Keith Willison and Alan Ashworth

Spermatogenic genes are those genes that are newly expressed or whose expression is augmented in spermatogonia, spermatocytes or spermatids. They include: (1) genes expressed exclusively during spermatogenesis, (2) testis-specific isozymes, isotypes and variants and (3) somatic genes showing increased levels of expression in testis. Molecular-genetic analysis is beginning to provide information about the control and expression of spermatogenic genes.

Spermatogenesis is a general term used to describe the whole collection of processes involved in the development of spermatozoa from spermatogonia. Spermiogenesis is a more specific term for the morphogenetic period during which the round spermatid is sculptured into the shape of the mature spermatozoon.

The testis differentiates from the genital ridges of the embryo which, at 11–12 days' gestation in mice, become populated by migrating primordial germ cells which themselves appeared four days earlier in the endodermal yolk sac. A few days after birth, type A spermatogonia become apparent and primordial germ cells disappear. Type A spermatogonia may cycle and remain as stem cells or differentiate into type B spermatogonia and become committed to the spermatogenic pathway. In mice, spermatogenesis takes 35 days of which approximately 12 are spent in meiosis; in humans these periods are 63 and 24 days respectively. Table 1 shows a list of the different cell types in the mammalian testis and some of their characteristics.

Some features of the spatial distribution of germ-cell types in the seminiferous epithelium are a reflection of the mechanisms of initiation of spermatogenesis. When one examines the distribution of cell types at cross-sections along the length of the seminiferous tubule, at any place there are particular combinations of cell types at different points in the spermatogenic pathway. These combinations are called cell associations and they have been classified into 14 stages histochemically[1]; different stages can even be visualized in live isolated tubules by their light absorption pattern[2]. Cell associations are a consequence of the fact that initiation of spermatogenesis occurs every 12 days but the process lasts 35 days; this is shown schematically in Fig. 1 and the mixtures of cell types corresponding to the 14 stages are shown in Fig. 2.

A further aspect of the initiation of rodent spermatogenesis is the fact that initiation occurs around the circumference in segments of the tubule, so that along the length of an entire tubule are different segments of particular associations of cells – this is the spermatogenic wave[1]. The spermatogenic wave is like the wave favoured by Mexican crowds in the 1986 World Cup football competition: apparent horizontal motion is imitated by successive vertical movements. The spermatogenic wave of the rat has been studied in beautiful detail by Clermont's laboratory[1]. In humans, patches of cell associations are found throughout the epithelium, thus there are species differences in the propagation of the initiation signals for spermatogonia to enter spermatogenesis.

Genetics of spermatogenesis

The examination of specific gene expression in rodent spermatogenesis is aided by the synchronicity of the process and by the availability of mutants and cell separation techniques. The two major non-germ cell types in the testis are the Leydig cell and the Sertoli cell, and proliferating cell lines of both have been isolated. The Leydig cells produce steroid hormones and lie outside the seminiferous tubules. The Sertoli cells are supporting cells within the tubules and probably provide nourishment to the developing germ cells in addition to aiding the morphogenesis of the spermatozoa. The development of sedimentation separation techniques has permitted the purification of spermatogenic cells – spermatids, spermatocytes and spermatogonia – in quantities large enough for biochemical analysis.

A useful staging method is to make use of the first round of spermatogenesis and prepare samples from prepubertal mice of different ages – 1 week-, 2 week- and 3 week-old testes permit the rough analysis of events occurring in spermatogonia, meiosis and the early spermatid stage respectively. However, there are two potential problems with this technique since the ratios of cell types will vary, Sertoli and Leydig cells constituting a greater percentage of total cell number at earlier stages. Also, gene expression in the non-germ cells may vary with the different cell associations[3].

Finally, the use of mutants which are blocked at