Mol Gen Genet (1988) 213:354–358

MGG

Identification and germline transformation of the ribosomal protein *rp21* gene of *Drosophila*: Complementation analysis with the *Minute* QIII locus reveals nonidentity

Mark A. Kay*, J.-Y. Zhang, and Marcelo Jacobs-Lorena**

Department of Developmental Genetics and Anatomy, School of Medicine, Case Western Reserve University, 2119 Abington Rd, Cleveland, OH 44106, USA

Summary. *Minute* loci represent a class of about 50 different *Drosophila* genes that appear to be functionally related. These genes may code for components of the protein synthetic apparatus. While one *Minute* locus has been recently shown to code for a ribosomal protein, it is not yet known whether any of the other *Minute* loci also code for ribosomal proteins. We have addressed this question by a combined molecular and genetic approach. In this report, a cloned DNA encoding the ribosomal protein *rp21* is partially characterized. The *rp21* gene maps to the same region (region 80 of chromosome 3L) as the temperature-sensitive *Minute* QIII gene. Using P-element mediated transformation, the *rp21* gene was transformed into the germline of *Drosophila*. RNA blot experiments revealed that the transformed gene is expressed in transgenic flies. However, genetic complementation analysis indicated that the QIII locus and the *rp21* gene are not identical. Implications of these findings for the relationship between *Minutes* and ribosomal protein genes are discussed.

Key words: *Drosophila* – *Minute* – Ribosomal protein – Transformation

Introduction

The genes encoding the more than 70 proteins of the eucaryotic ribosome may be regulated at various levels including RNA processing, mRNA translation, mRNA turnover, and protein turnover (Al-Atia et al. 1985; Bozzoni et al. 1984; Faliks and Meyuhas 1982; Fried et al. 1985; Geyer et al. 1982; Ballinger and Pardue 1983; Kay and Jacobs-Lorena 1985; Pearson et al. 1982; Pierandrei-Amaldi et al. 1982; Warner et al. 1985; Jacobs-Lorena and Fried 1987). *Drosophila* offers a number of advantages for elucidating the molecular and developmental processes used in ribosome synthesis. The ribosomal proteins (r-proteins) and some of the corresponding genes were recently characterized (Chooi et al. 1980; Chooi 1980; Qian et al. 1987; Vaslet et al. 1980; Burns et al. 1984). The large fluctuations in the level of expression of these genes that occur during early development (Al-Atia et al. 1985; Kay and Jacobs-Lorena 1985) and the availability of large amounts of synchronized embryos greatly facilitate the study of gene regulation. Finally, cloned genes can be easily introduced into the germline (Rubin and Spradling 1982).

Recently, we have determined that most r-protein genes of *Drosophila* are regulated at the translational level during development (Al-Atia et al. 1985; Kay and Jacobs-Lorena 1985; reviewed in Jacobs-Lorena and Fried 1987). Although the abundance of r-protein mRNAs throughout early development does not change, the translation of these mRNAs is selectively repressed during early embryogenesis. Moreover, little or no rRNA is synthesized during this time (Anderson and Lengyel 1979; Edgar and Schubiger 1986). Apparently, this form of regulation allows for the coordinate synthesis of rRNA and r-proteins.

The *Minute* loci of *Drosophila* represent a class of about 50 phenotypically similar, dispersed, haplo-insufficient, cell autonomous mutations that are believed to affect protein synthesis (Lindsley et al. 1972; Morata and Ripoll 1975; Sinclair et al. 1981; reviewed in Kay and Jacobs-Lorena 1987). The *Minute* phenotype includes several or all of the following traits: prolonged larval and pupal development (but no developmental delay during embryogenesis), 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 (Brehme 1939; Brehme 1941; Schultz 1929). In the homozygous state, the *Minute* condition causes lethality in late embryogenesis or early first-instar larvae (Farnsworth 1957a, b). In the triplo state (M/+/+) *Minutes* act as recessive genes since these flies have a wild-type phenotype (Sinclair et al. 1981). Recently, Kongsuwan et al. (1985), have demonstrated by germline transformation experiments that the r-protein *rp49* gene of *Drosophila* is able to complement the phenotype of a *Minute* locus on the X chromosome.

We have been interested in the conditional temperature-sensitive QIII *Minute* allele (Sinclair et al. 1981). *Minute* QIII has been mapped to chromosome 3L near the centromere (79E–80) (Sinclair et al. 1981). Interestingly, a cloned gene that codes for an abundant RNA and a 26000 dalton protein, maps to the same region (Biessmann et al. 1981). In this communication we demonstrate that this cloned gene codes for the r-protein *rp21*. Using P-element mediated transformation, this gene was transformed into the germline of *Drosophila*. The transformed copy of *rp21* appears to be expressed in different transformed lines, as suggested by increased levels of the corresponding mRNA in the

* *Present address:* Department of Pediatrics, Baylor College of Medicine, Texas Medical Center, Houston, TX 77030, USA

** *Present address:* Department of Biochemistry, Tianjing Medical College, Tianjing, People's Republic of China

Offprint requests to: M. Jacobs-Lorena

transgenic flies. However, even though the QIII locus and the *rp21* gene map to the same region, genetic complementation analysis suggests that the two genes are not identical.

Materials and methods

Fly stocks. Flies were raised on yeast-agar media supplemented with fresh baker's yeast. All chromosome stocks and genetic nomenclature can be found in Lindsley and Grell (1968) unless otherwise stated. The temperature-sensitive *Minute* allele QIII (79E5,6–80) (Sinclair et al. 1981) was kept over balancer chromosomes at 25° C (TM3 *Sb Ser* or TM1 *Me*). When reared at 25° C, the eclosion of the QIII heterozygotes is delayed by about 48 h relative to wild type. QIII homozygotes are lethal at 25° C, viable but infertile at 18° C. The *rosy* (*ry*) stock, ry^{506} was used for transformation.

Cloning methods. The pKc1G plasmid is a pBR322 derivative containing a 4.4 kb *Eco*RI genomic fragment of *Drosophila* DNA derived from polytene region 80C (Biessmann et al. 1981). The *Eco*RI fragment was gel purified using glass beads (Vogelstein and Gillespie 1979). The ends of this fragment were filled-in with DNA polymerase I (Maniatis et al. 1982). *Sal*I linkers were added and the 4.4 kb fragment was ligated into the *Sal*I site of the Carnegie 20 P-element transformation vector containing the xanthine dehydrogenase (*rosy*) gene (Rubin and Spradling 1983). This construct was transformed into HB101 *Escherichia coli* cells (Hanahan 1983). Plasmids were isolated from several clones and restriction analyses were performed to verify the identify of the constructs.

Fly transformation procedure. The Carnegie 20 construct was coinjected with p 25.7wc helper P element DNA (Rubin and Spradling 1983) at a concentration of 300 and 100 μg/ml respectively, into the posterior tip of ry^{506} embryos (Rubin and Spradling 1982). The surviving GO adults were mated to *ry* flies. Five independent transformant lines were obtained and analyzed.

Other procedures. Nucleic acid and protein methods have all been described previously: poly(A)-containing RNA isolation, hybrid-selection and in vitro translation, ribosomal protein isolation and purification, two-dimensional gel electrophoresis, RNA blot hybridization (Kay and Jacobs-Lorena 1985), restriction endonuclease mapping, and in situ hybridization to polytene chromosomes (Qian et al. 1987). The cloned *rp49* (Vaslet et al. 1980) and actin (Ruddell and Jacobs-Lorena 1984) probes were also used for RNA blot experiments.

Results

The pKc1G DNA contains the gene coding for ribosomal protein rp21

The pKc1G clone is complementary to an abundant small mRNA that codes for a low molecular weight protein (Biessman et al. 1981). Ribosomal protein mRNAs from *Drosophila* embryos share similar properties to those of pKc1G mRNA (Al-Atia et al. 1985; Kay and Jacobs-Lorena 1985). In order to determine whether the pKc1G DNA codes for a r-protein, embryonic poly(A)-containing RNA was used to hybrid-select mRNA corresponding to the plasmid insert. This RNA was translated in a cell-free system containing ^{35}S-methionine. The radioactive translation products were mixed with purified *Drosophila* r-proteins and the mixture was analyzed by 2-dimensional gel electrophoresis (Fig. 1). By overlapping the autoradiogram with

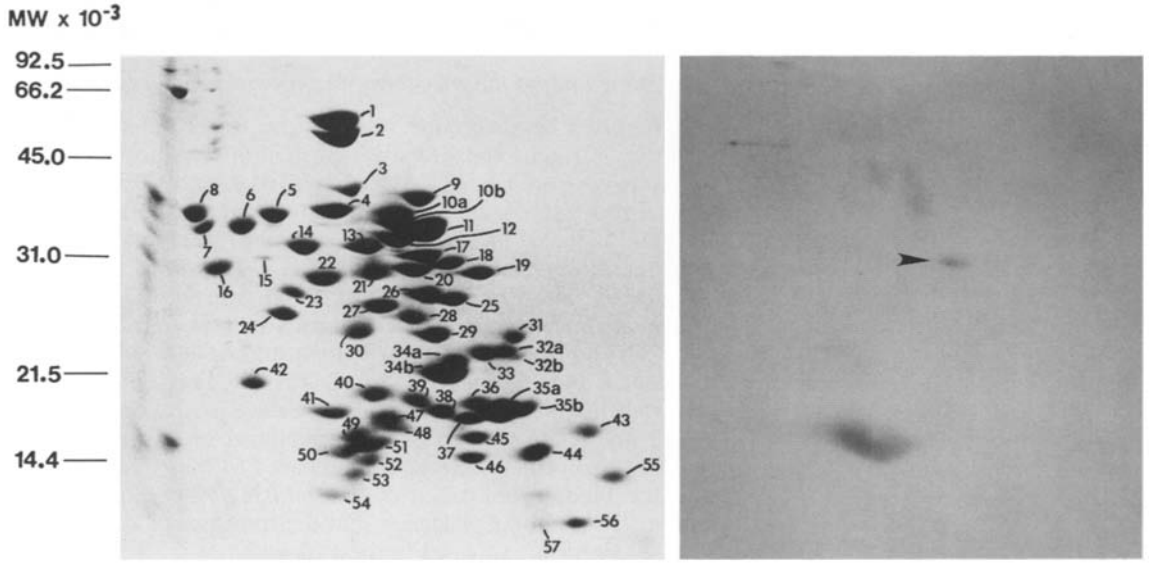

Fig. 1. The pKc1G insert codes for the ribosomal protein 21 gene. Polyadenylated RNA from early embryos was hybridized to pKc1G DNA immobilized on a nitrocellulose filter. The hybridized RNA was eluted from the filter and translated in a rabbit reticulocyte cell-free system in the presence of ^{35}S-methionine. The radioactive products were mixed with about 300 μg of purified r-proteins and analyzed on 2D-gels. The gels were stained with Coomassie Blue and then subjected to fluorography. Left: representative staining profile of *Drosophila* ribosomal proteins. Right: fluorogram of the translation products of mRNA selected with the pKc1G DNA. The radioactive signal depicted by the *arrowhead* comigrates with r-protein 21 (see Left). The large radioactive spot near the bottom of the gel represents endogenous globin synthesized in the rabbit reticulocyte cell-free system. The streak at the upper left is an artifact of the system. Direction of electrophoresis is left to right and top to bottom

the stained gel, it was determined that the pKc1G DNA selected a mRNA whose translation product comigrated exactly with r-protein 21 suggesting that pKc1G codes for the *rp21* gene. In support of this assertion we note that the gel electrophoresis protocol is designed to fractionate small basic proteins and that very few cellular proteins other than r-proteins migrate in this region of the gel. In conclusion, our results indicate that the pKc1G DNA codes for the *rp21* gene.

Transformation of the ribosomal protein 21 *gene into the* Drosophila *germline*

Using P-element mediated transformation, *ry* embryos were injected with the Carnegie 20 construct containing the *rp21* gene. Five independent transformant lines were obtained. Two methods were used to confirm that the transformed flies carried the *rp21* gene. The first involved genomic Southern blot analysis, an example of which is shown in Fig. 2. Restriction enzyme *Pst*I was used because it was known not to cut within the pKc1G clone (Biessmann et al. 1981). The additional 9.6 kb band is detected only in DNA from the transformed flies (Fig.2) and represents the exogenous copy of the *rp21* gene. Since the host and transformed flies are genetically identical except for the transformed DNA, this 9.6 kb band cannot be due to a polymorphism between the two fly stocks.

The transformant lines were also subjected to analysis by in situ hybridization of ^{3}H-labeled pKc1G DNA to polytene chromosomes. An example is shown in Fig. 3 for transformed line 1. Two hybridization bands are visualized. One represents hybridization to the endogenous *rp21* gene in region 80 of the left arm of the 3rd chromosome (3L-80)

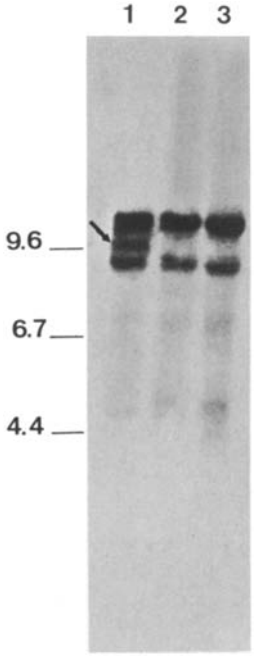

Fig. 2. Genomic Southern blot analysis of transformed flies. Genomic DNA isolated from transformant line 1 (lane 1), *ry*/*ry* (lane 2), or QIII red/TM3 (lane 3) adult flies was digested with *Pst*I and electrophoresed on a 0.6% agarose gel. The DNA was blotted onto nitrocellulose and probed with ^{32}P-labeled pKc1G DNA. The 9.6 kb band depicted by an *arrow* represents the transformed copy of the gene. An autoradiogram is shown

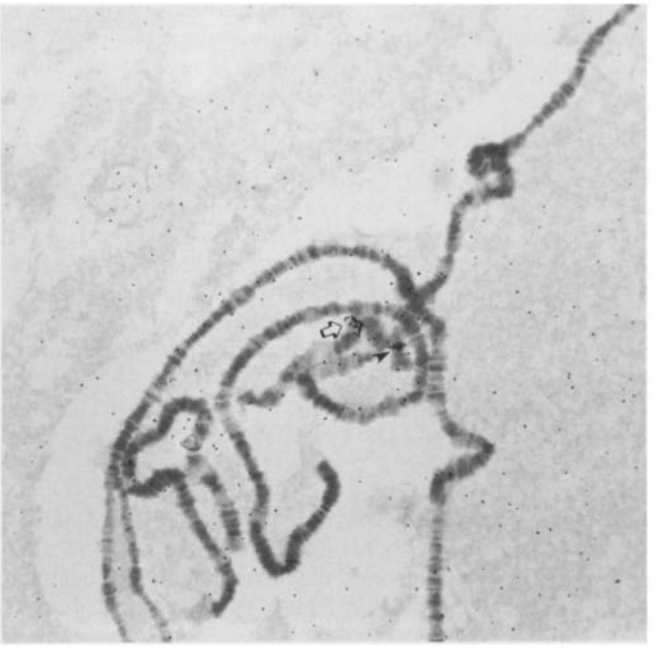

Fig. 3. Chromosomal localization of the transformed copy of the ribosomal protein 21 gene. Salivary gland polytene chromosome squashes of third instar larvae from transformant line 1 were hybridized with ^{3}H-labeled pKc1G DNA. After exposure to autoradiographic emulsion, the chromosomes were stained with Giemsa and photographed. The silver grains depicted by the *filled arrowhead* represent hybridization to the transformed gene on chromosome 4 at band 101F while the *open arrow* represents the silver grains at the location of the endogenous *rp21* gene at 3L–80

while the other represents the transformed copy at the 101F region of the 4th chromosome (4–101F). Other lines had insertions at 3L–89D (line 2), 2L–33DE (line 3), and 2R–47EF (line 4). Line 5 contained one insert in chromosome 3L and possibly another insert at an undetermined location.

Expression of the transformed ribosomal protein rp21 *gene*

In order to determine whether the transformed copy of the *rp21* gene is expressed, r-protein mRNA accumulation was measured by RNA blot analysis (Fig. 4). Equal mass of ovary RNA from *ry* or transformant flies was analyzed using labeled pKc1G DNA as a probe. The blots were also hybridized with labeled actin and *rp49* probes to control for variation in RNA loading and transfer. The relative abundance of the *rp21* transcripts in transformed and *ry* flies can be determined by comparing the autoradiographic signal due to *rp21* mRNA relative to that of actin and *rp49* mRNAs. As shown in Fig. 4, there is an approximately 2-fold relative increase in the abundance of *rp21* transcripts in transformed lines when compared to the *ry* host. Thus, the higher abundance of *rp21* mRNA (but not of *rp49* mRNA) strongly suggests that the transformed copy of the *rp21* gene is expressed in these flies.

Complementation test of the Minute *QIII with the* rp21 *ribosomal protein gene in transgenic flies*

In order to determine whether the *rp21* gene rescues the QIII mutant phenotype, flies heterozygous for the transformed *rp21* gene were mated to QIII heterozygotes in the following scheme:

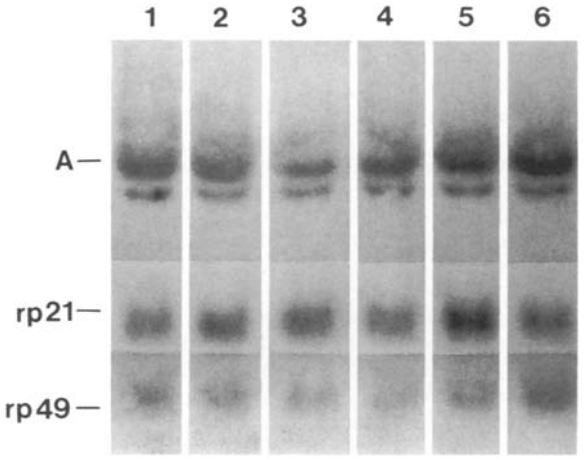

Fig. 4. RNA blot analysis of ribosomal protein 21 transcripts in transgenic flies. Five micrograms of RNA from the ovaries of transgenic flies line 1 (lane 2); line 5 (line 3); line 2 (lane 4); line 4 (lane 5); and *ry/ry* host controls (lanes 1 and 6) were electrophoresed on a denaturing formaldehyde-agarose gel and blotted onto a nylon membrane. The membrane was hybridized with a mixture of 3 ^{32}P-labeled probes: *rp21* (r-protein 21), *rp49* (r-protein 49) and A (actin). An autoradiogram is shown

Table 1. Complementation analysis between the *rp21* gene and the *Minute* QIII mutation

Genotype	QIII/+; [*rp21*$^+$]/+ or QIII/+; +/+		Bal/+; [*rp21*$^+$]/+ or Bal/+; +/+	
Phenotype 1	Normal bristles	Short bristles	Normal bristles	Short bristles
	0	89	246	0
Phenotype 2	Developmental time		Developmental time	
	10–12 days	>12 days	10–12 days	>12 days
	3	86	210	36

Progeny from the following cross were examined: QIII/Bal × +/+; [*rp21*$^+$]/+, where QIII represents the temperature-sensitive *Minute* mutation, Bal represents either the TM1 or TM3 balancer chromosomes, [*rp21*$^+$] represents the transformed copy of the r-protein *rp21* gene, and +/+ represents wild-type chromosomes not containing an extra *rp21* gene. Developmental time is the time of development from egg to adult. The number of progeny with a given phenotype is indicated. The results from transformed lines 1, 3, 4, and 5 were similar and were pooled. The increased developmental time of some of the balancer-containing control flies may have been due to a larval crowding effect. If the QIII locus encodes the *rp21* gene, half of the flies carrying the QIII chromosome should have had a wild-type phenotype (normal bristles, short developmental time), an expectation that was not realized

QIII p^p/TM1 *Me* or QIII *red*/TM3 *Sb Ser* × *ry*/*ry*; [*rp21*$^+$/+];

where [*rp21*$^+$] represents the transformed copy of the *rp21* gene and "+" represents a wild-type chromosome not containing an extra *rp21* gene. The eggs were incubated at 25° or 29° C. All flies not containing the balancer chromosome, as determined by the appropriate dominant phenotypic markers, contain the QIII mutant allele. Half of the QIII flies also contain the transformed *rp21* gene. Three phenotypes were examined for rescue of the QIII mutant phenotype. The first was bristle length. If the *rp21* gene corresponds to the QIII locus, half of the QIII flies should have wild-type bristles. In none of the experiments were wild-type bristles found (Table 1).

The second phenotype examined was the time of development. Eggs laid over an 8–12 h interval were incubated at 29° C and checked every 12 h for eclosion into adults. The QIII flies raised at 29° C eclose about 2 days later than sibs carrying a wild-type QIII homologue. As in the previous case, identity between QIII and *rp21* predicts that half of the QIII flies emerge at approximately the same time as flies carrying the TM1 or TM3 chromosome. The results presented in Table 1 are not consistent with this prediction.

As a final test of complementation, the QIII-bearing flies obtained from the above crosses were mated to each other to determine whether the lethality of QIII homozygotes at 25° C would be rescued by the transformed *rp21* gene. The appearance of the recessive markers (*red* or p^p) in mildly *Minute* flies was the expected outcome from such a mating if the QIII allele is complemented by the extra copy of the *rp21* gene (Sinclair et al. 1981). However, no such flies were obtained. Since none of the *Minute* phenotypes are rescued by the *rp21* transformed gene, it was concluded that the *rp21* gene does not correspond to the QIII locus.

Discussion

The above results, together with those previously reported, indicate that the cloned pKc1G gene codes for the ribosomal protein *rp21*, that it is expressed when transformed into flies, and that it maps to the same region as the QIII *Minute* locus. However, since the transformed *rp21* gene did not correct the QIII *Minute* phenotype, it is likely that *rp21* and QIII correspond to different genes. This conclusion is supported by the observation that triploid QIII/+/+ females are viable and have wild-type phenotype (Sinclair et al. 1981), indicating that one extra dose of the gene is sufficient to rescue the *Minute* phenotype. A second *Minute* gene (*M(3)S34*) appears to be located near QIII but unfortunately it has been lost (D. Sinclair and T. Grigliatti, personal communication). It is possible that *rp21* corresponds to *M(3)S34*, explaining the failure of the transformed *rp21* gene to complement QIII. A number of r-protein genes have been cloned and mapped by in situ hybridization to polytene chromosome (Vaslet et al. 1980; Burns et al. 1984; Qian et al. 1987). Interestingly, all these genes are located near *Minute* loci. However, as the present experiments demonstrate, the coincidental localization by genetic criteria and in situ hybridization to polytene chromosomes cannot serve as a strong argument for the identity of *Minutes* and r-protein genes.

It has been estimated that 40–50 *Minute* loci exist in the *Drosophila* genome (Lindsley et al. 1972), but it is clearly established that the *Drosophila* ribosome contains at least 70 proteins (Chooi 1980; Chooi et al. 1980). Thus, some of the r-protein genes may not be encoded by *Minute* loci and mutations in these genes may have different phenotypes or no phenotype at all (Kay and Jacobs-Lorena 1987). Standard mutagenesis methods may be applied to further clarify this point. Alternatively, antisene gene constructs may be

used to determine the phenotype produced by insufficient expression of any r-protein gene. Preliminary results from this laboratory indicate that this is a promising approach.

Acknowledgements. We would like to thank Don Sinclair for discussions concerning the *Minute* QIII gene, J. Hirsch for instruction on the *Drosophila* transformation procedure, and P.J. Harte for critical reading of the manuscript. This work was supported by a grant from the National Institutes of Health.

References

Al-Atia GR, Fruscoloni P, Jacobs-Lorena M (1985) Translational regulation of mRNAs for ribosomal proteins during early *Drosophila* development. Biochemistry 24:5798–5803

Anderson KV, Lengyel JA (1979) Rates of synthesis of major classes of RNA in *Drosophila* embryos. Dev Biol 70:217–231

Ballinger DG, Pardue ML (1983) The control of protein synthesis during heat shock in *Drosophila* cells involves altered polypeptide elongation rates. Cell 33:103–114

Biessmann H, Kuger P, Schropfer C, Spindler E (1981) Molecular cloning and preliminary characterization of a *Drosophila melanogaster* gene from a region adjacent to the centrometric b-heterochromatin. Chromosoma 82:493–503

Bozzoni I, Fragapane P, Annesi F, Pierandrei-Amaldi P, Amaldi F, Beccari E (1984) Expression of two *Xenopus laevis* ribosomal protein genes in injected frog oocytes. J Mol Biol 180:987–1005

Brehme KS (1939) A study of the effect on the development of *Minute* mutations in *Drosophila melanogaster*. Genetics 24:131–159

Brehme KS (1941) Development of the *Minute* phenotype in *Drosophila melanogaster*. A comparative study of the growth of three *Minute* mutants. J Exp Zool 88:135–160

Burns DK, Stark BC, Macklin MD, Chooi WY (1984) Isolation and characterization of cloned DNA sequences containing ribosomal protein genes of *Drosophila melanogaster*. Mol Cell Biol 4:2643–2652

Chooi WY (1980) Purification of *Drosophila* ribosomal proteins. Isolation of proteins S8, S13, S14, S16, S19, S20/L24, S22/L26, S24/, S25/S27, S26, S29, L4, L10/L11, L12, L13, L16, L18, L19, L27, L1, L7/8, L9, and L11. Biochemistry 19:3469–3476

Chooi WY, Sabatini LM, Macklin M, Fraser D (1980) Group fractionation and determination of the number of ribosomal subunit proteins from *Drosophila melanogaster* embryos. Biochemistry 19:1425–1433

Edgar BA, Schubiger G (1986) Parameters controlling transcriptional activation during early *Drosophila* development. Cell 44:871–877

Faliks D, Meyuhas O (1982) Coordinate regulation of ribosomal protein mRNA level in regenerating rat liver. Study with the corresponding mouse cloned cDNAs. Nucleic Acids Res 10:789–800

Farnsworth MW (1957a) Effects of the homozygous *Minute*-IV deficiency on the development of *Drosophila melanogaster*. Genetics 42:7–18

Farnsworth MW (1957b) Effects of homozygous first, second, and third chromosome *Minutes* on the development of *Drosophila melanogaster*. Genetics 42:19–27

Fried HM, Nam HG, Loecher S, Teem J (1985) Characterization of yeast strains with conditionally expressed variants of ribosomal protein genes tcml and cyh2. Mol Cell Biol 5:99–108

Geyer PK, Meyuhas O, Perry RP, Johnson LF (1982) Regulation of ribosomal protein mRNA content and translation in growth-stimulated mouse fibroblasts. Mol Cell Biol 2:685–693

Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol 166:557–580

Jacobs-Lorena M, Fried H (1987) Translational regulation of ribosomal protein gene expression in eukaryotes. In: Ilan J (ed) Translational regulation of gene expression. Plenum Press, New York, pp 63–85

Kay MA, Jacobs-Lorena M (1985) Selective translational regulation of ribosomal protein gene expression during early development of *Drosophila melanogaster*. Mol Cell Biol 5:3582–3592

Kay MA, Jacobs-Lorena M (1987) Developmental genetics of ribosome synthesis in *Drosophila*. Trends Genet 3:347–351

Kongsuwan K, Qiang Y, Vincent A, Frisardi MC, Rosbash M, Lengyel JA, Merriam J (1985) A *Drosophila Minute* gene encodes a ribosomal protein. Nature 317:555–558

Lindsley DL, Grell EH (1968) Genetic variations of *Drosophila melanogaster*. Carnegie Inst Washington

Lindsley DL, Sandler L, Baker BS, Carpenter ATC, Denell RE, Hall JC, Jacobs PA, Miklos GLG, Davis BK, Gethmann RC, Hardy RW, Hessler A, Miller SM, Nozawa H, Parry DM, Gould-Somero M (1972) Segmental aneuploidy and the genetic gross structure of the *Drosophila* genome. Genetics 71:157–184

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

Morata G, Ripoll P (1975) *Minutes:* mutants of *Drosophila* autonomously affecting cell division rate. Dev Biol 42:211–221

Pearson NJ, Fried HM, Warner JR (1982) Yeast use translational control to compensate for extra copies of a ribosomal protein gene. Cell 29:347–355

Pierandrei-Amaldi P, Campioni N, Beccari E, Bozzoni I, Amaldi F (1982) Expression of ribosomal-protein genes in *Xenopus Laevis* development. Cell 30:163–171

Qian S, Zhang J-Y, Kay MA, Jacobs-Lorena M (1987) Structural analysis of the *Drosophila* rpA1 gene, a member of the eucaryotic "A" type ribosomal protein family. Nucleic Acids Res 15:987–1003

Rubin GM, Spradling AC (1982) Genetic transformation with transposable element vectors. Science 218:348–353

Rubin GM, Spradling AC (1983) Vectors for P-element mediated transfer in *Drosophila*. Nucleic Acids Res 11:6341–6351

Ruddell A, Jacobs-Lorena M (1984) Preferential expression of actin genes during oogenesis of *Drosophila*. Dev Biol 105:115–120

Schultz J (1929) The *Minute* reaction in the development of *Drosophila melanogaster*. Genetics 14:366–419

Sinclair DAR, Suzuki DT, Grigliatti TA (1981) Genetic and developmental analysis of a temperature-sensitive *Minute* mutation of *Drosophila melanogaster*. Genetics 97:581–606

Vaslet CA, O'Connell P, Izquierdo M, Rosbash M (1980) Isolation and mapping of a cloned ribosomal protein gene of *Drosophila melanogaster*. Nature 285:674–676

Vogelstein B, Gillespie D (1979) Preparative and analytical purification of DNA from agarose. Proc Natl Acad Sci USA 76:615–619

Warner JR, Mitra G, Schwindinger WF, Studeny M, Fried HM (1985) *Saccharomyces cerevisiae* coordinates accumulation of yeast ribosomal proteins by modulating mRNA splicing, translational initiation, and protein turnover. Mol Cell Biol 5:1512–1521

Communicated by B.H. Judd

Received May 18, 1987 / March 22, 1988