Research

The ribosomal protein genes and Minute loci of Drosophila melanogaster

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Abstract

Background: Mutations in genes encoding ribosomal proteins (RPs) have been shown to cause an array of cellular and developmental defects in a variety of organisms. In Drosophila melanogaster, disruption of RP genes can result in the ‘Minute’ syndrome of dominant, haploinsufficient phenotypes, which include prolonged development, short and thin bristles, and poor fertility and viability. While more than 50 Minute loci have been defined genetically, only 15 have so far been characterized molecularly and shown to correspond to RP genes.

Results: We combined bioinformatic and genetic approaches to conduct a systematic analysis of the relationship between RP genes and Minute loci. First, we identified 88 genes encoding 79 different cytoplasmic RPs (CRPs) and 75 genes encoding distinct mitochondrial RPs (MRPs). Interestingly, nine CRP genes are present as duplicates and, while all appear to be functional, one member of each gene pair has relatively limited expression. Next, we defined 65 discrete Minute loci by genetic criteria. Of these, 64 correspond to, or very likely correspond to, CRP genes; the single non-CRP-encoding Minute gene encodes a translation initiation factor subunit. Significantly, MRP genes and more than 20 CRP genes do not correspond to Minute loci.

Conclusion: This work answers a longstanding question about the molecular nature of Minute loci and suggests that Minute phenotypes arise from suboptimal protein synthesis resulting from reduced levels of cytoribosomes. Furthermore, by identifying the majority of haplolethal and haplosterile loci at the molecular level, our data will directly benefit efforts to attain complete deletion coverage of the D. melanogaster genome.
Background

Ribosomes are sophisticated macromolecular machines that catalyze cellular protein synthesis in all cells of all organisms. They have an ancient evolutionary origin and are essential for cell growth, proliferation and viability. Though larger and more complex in higher organisms, both the structure and function of ribosomes have been conserved throughout evolution. Genetic approaches in Drosophila melanogaster have shown that disrupting ribosome function can result in an array of fascinating dominant phenotypes [1,2]. Despite this, there has so far been no comprehensive inventory of genes encoding ribosome components in this organism, nor any systematic effort to determine their mutant phenotypes.

All ribosomes comprise a set of ribosomal proteins (RPs) surrounding a catalytic core of ribosomal RNA (rRNA). Bacteria possess a single type of ribosome composed of three rRNA molecules and typically 54 RPs. All eukaryotic cells, in contrast, contain at least two distinct types of ribosomes: cytoplasmic ribosomes (cytoribosomes) and mitochondrial ribosomes (mitoribosomes). Cytoribosomes are found on the endoplasmic reticulum and in the aqueous cytoplasm. They translate all mRNAs produced from nuclear genes and perform the vast majority of cellular protein synthesis. Each cytoribosome contains four different rRNAs and 78-80 cytoplasmic RPs (CRPs). Mitoribosomes consist of only two rRNA molecules and up to 80 mitochondrial RPs (MRPs). They are located in the mitochondrial matrix and synthesize proteins involved in oxidative phosphorylation encoded by those few genes retained in the mitochondrial genome. A third unique type of eukaryotic ribosome is found within the plastids (for example, chloroplasts) of plant and various algal cells. In all cases, distinct small and large ribosomal subunits exist that join together during the translation initiation process to form mature ribosomes capable of protein synthesis. (See references [3-6] for general reviews of ribosomal structure and function.)

The protein components of ribosomes are interesting from several points of view. First, and most obviously, RPs play critical roles in ribosome assembly and function [7]. Second, several RPs perform important extra-ribosomal functions, including roles in DNA repair, transcriptional regulation and apoptosis [6,8]. Third, misexpression of human CRP and MRP genes has been implicated in a wide spectrum of human syndromes and diseases, including Diamond-Blackfan anemia [9], Turner syndrome [10], hearing loss [11] and cancer [12]. Fourth, mutations in the CRP genes of D. melanogaster are important tools for the study of growth, development and cell competition [2]. Finally, many RPs are conserved from bacteria to humans, so their peptide and nucleotide sequences are useful for studying phylogenetic relationships [13].

The first eukaryotic CRPs characterized in detail were isolated from the rat cytoribosome [3]. Individual proteins were separated by two-dimensional gel electrophoresis and named from their origin in the small (S) or large (L) subunit and their relative electrophoretic migration positions, for example, RPS9 or RPL28. Subsequent studies revealed that some protein spots contained non-ribosomal proteins or chemically modified versions of another CRP, and that some spots contained two co-migrating CRPs [3,5]. Consequently, the nomenclature system used today contains numerical gaps as well as 'A' suffixes for those additional CRPs not resolved by the original electrophoresis (for example, RPL36A). Seventy-nine distinct mammalian CRPs are now acknowledged and their amino acid sequences and biochemical properties have been described [5,14]. With the exception of RPLP1 and RPLP2, each of which forms homodimers in the cytoribosomal large subunit [15], all CRPs are present as single molecules in each cytoribosome [3].

Seventy-eight different mammalian MRPs have been described [6] and their individual amino acid sequences and biochemical properties have been determined [16,17]. Although the nomenclature of MRPs was originally based on electrophoretic properties, the current system reflects homology between mammalian MRPs and their bacterial orthologs [18]. Thus, MRPS1 through MRPS21 are orthologous to Escherichia coli RPs S1-S21, while higher numbers have been assigned to the MRPs not found in bacteria. Gaps also exist in MRP numbering because a gap occurs in the bacterial enumeration or because there is no mammalian ortholog.

The RPs of D. melanogaster were first studied in the 1970s and early 1980s. Up to 78 individual CRPs were observed on two-dimensional gels [19-31] and about 30 were purified and analyzed biochemically [32,33]. A more recent characterization used mass spectrometry to identify 52 D. melanogaster CRPs [34], all of which are orthologous to known mammalian CRPs. The protein composition of Drosophila mitoribosomes has not been characterized biochemically to date.

CRPs and MRPs are encoded by the nuclear genome. Knowledge of the primary sequences of rat CRPs and bovine MRPs has led to the identification and mapping of the RP-encoding genes in many eukaryotic species [14]. Indeed, systematic analyses of whole RP gene sets have been described for several organisms, including Saccharomyces cerevisiae [35], Arabidopsis thaliana [36] and humans [37-40]. However, the complete set of D. melanogaster CRP and MRP genes has not been previously documented or characterized.

Several D. melanogaster RP genes were initially identified by virtue of their dominant 'Minute' mutant phenotypes [2], which include prolonged development, low fertility and viability, altered body size and abnormally short, thin bristles on the adult body. All of these phenotypes may be explained by a cell-autonomous defect in protein biosynthesis: the production of each bristle, for example, requires a very high rate of
protein synthesis in a single cell during a short developmental period. Merriam and colleagues reported the first unequivocal molecular link between a Minute locus and a CRP gene in 1985 [41]. Since then, 14 additional Minute loci have been definitively linked with distinct CRP genes [2,42-53]. However, there are at least 35 genetically validated Minute loci that have not yet been associated with a specific gene and there may be additional Minute genes to be discovered. Several investigators have hypothesized that all Minute loci encode protein components of ribosomes (reviewed in [2]). Whether this is truly the case and whether both CRP and MRP genes are associated with Minute phenotypes are open and intriguing questions.

Many Minute loci were originally identified from the phenotypes of flies heterozygous for a chromosomal deletion [54,55] and all Minute point mutations studied in depth have been found to be loss-of-function alleles [2]. This indicates that Minute phenotypes can be attributed to genetic haploinsufficiency; that is, a single gene copy is not sufficient for normal development. (Note that X-linked mutations that cause Minute phenotypes in heterozygous females are lethal in hemizygous males.) The most popular explanation for the haploinsufficiency of Minute loci is that they correspond to RP genes and that RPs are required in equimolar amounts: halving the copy number of a single RP gene limits the availability of the encoded RP, thereby reducing the number of functional ribosomes that are assembled in the cell and impairing protein synthesis [2]. While this idea is consistent with the available data, there may be other explanations.

The reduced fertility and viability associated with many Minute loci makes the recovery of deletions uncovering them rather difficult - the mutant strains are too weak to maintain as stable heterozygous stocks. In fact, some Minute loci are known only from the phenotypes of transient aneuploids [54,56]. This means that several chromosomal regions containing a Minute locus are not uncovered by current deletion collections [57]. This is frustrating for researchers because deletions are basic tools for mutational analysis and are widely used for mapping new mutations and identifying genetic modifiers. Efforts to maximize deletion coverage of the D. melanogaster genome would benefit from a systematic assessment of the relationship between RP genes and Minute loci. It would allow the isolation of deletions that flank haploinsufficient RP genes as closely as possible, or the design of transgenic constructs or chromosomal duplications to rescue the haploinsufficiency of deletions uncovering Minute genes.

Here, we report the systematic identification, naming and characterization of all the CRP and MRP genes of D. melanogaster. We have used this information, together with phenotypic data obtained from examining mutation and deficiency strains, to assess the correspondence between RP genes and Minute loci. We find that 66 of the 88 CRP genes identified are, or are very likely to be, haploinsufficient and associated with a Minute phenotype, whereas MRP genes and the remaining 22 CRP genes are not. Significantly, we show that all but one of the known Minute loci in the genome correspond to CRP genes - the single exception encodes a subunit of an essential translation initiation factor. Together, these results identify the majority of haploinsufficient loci in the D. melanogaster genome that significantly affect viability, fertility and/or external morphology, and also provide a mechanistic framework for understanding the Minute syndrome and the phenotypic effects of aneuploidy.

**Results**

**Identification of D. melanogaster ribosomal protein genes**

In order to conduct an exhaustive survey of Drosophila CRP and MRP genes, we first performed a series of BLAST searches using human RP sequences as queries, because both CRPs and MRPs have been well-characterized in humans [5,6]. Tables 1 and 2 list the genes we identified together with their cytological locations. Where necessary, D. melanogaster genes were named or renamed according to the standard metazoan RP gene nomenclature proposed by Wool and colleagues [5,58,59] and approved by the HUGO Gene Nomenclature Committee [18], whilst still conforming to FlyBase [60] conventions - that is, CRP genes are given an 'Rp' prefix and MRP genes have an 'mRp' prefix. The seven exceptions to this standard RP nomenclature are mostly genes originally named to reflect a mutant phenotype, for example, the string of pearls (sop) gene encodes RpS2 [61] and bonsai encodes mRpS15 [62,63]. In these cases, the original gene symbol has been preserved, with the apposite RP symbol given as a synonym.

**Cytoplasmic ribosomal protein genes**

We identified 88 genes that encode a total of 79 different CRPs (Table 1). Thus, the D. melanogaster proteome contains orthologs of all 79 mammalian CRPs (32 small subunit and 47 large subunit proteins). While the majority of CRPs are encoded by single genes, nine are encoded by two distinct genes. In addition, we identified another five genes predicted to encode proteins with significantly lower similarity to human CRPs, which we term 'CRP-like' genes. Two fragments of the Rps6 gene were also identified. (The list of 88 CRP genes presented by Cherry et al. [64] originated from an earlier report of our results to FlyBase (MA and SJM, FBrf0178764). These authors also list five CRP-like genes from our original report, but two of these have been eliminated and two additional CRP-like genes have been added in the current analysis.)

The deduced characteristics of D. melanogaster and human CRPs are compared in Additional data file 1. As might be expected, the amino acid identity between the CRPs of the two species is very high (average of 69% with a range of 27-98%, excluding the CRP-like proteins) and the predicted molecular
## Table 1

The CRP genes of *D. melanogaster*

| Human CRP | Symbol\(^a\) | CG number | Location\(^b\) | BLAST E value\(^c\) |
|-----------|--------------|-----------|----------------|---------------------|
| RPSA      | stat/RpSA    | CG14792   | X: 2B1         | 3e-94               |
| RPS2      | sop/RpS2     | CG5920    | 2L: 30E1       | e-118               |
| RPS3      | Rp33         | CG6779    | 3R: 94E13      | e-106               |
| RPS3A     | Rp33A        | CG1268    | 4: 101F1       | e-100               |
| RPS4      | Rp4          | CG11276   | 3L: 69F6       | e-121               |
| RPS5      | Rp5s\(^a\)   | CG8922    | X: 15E5-7      | 1e-98               |
| RPS5b     |              | CG7014    | 3R: 88D6       | 3e-96               |
| RPS6      | Rp6          | CG10944   | X: 7C2         | e-103               |
|           |              | CG11386   | X: 7C2         | 2e-15               |
|           |              | CG33222   | X: 7C2         | 2e-15               |
| RPS7      | Rp7          | CG1883    | 3R: 99E2       | 1e-74               |
| RPS8      | Rp8          | CG7808    | 3R: 99C4       | 1e-82               |
| RPS9      | Rp9          | CG3395    | 3L: 67B11      | 8e-92               |
| RPS10     | RpS10a       | CG12275   | 3R: 98A14      | 4e-43               |
|           | RpS10b       | CG14206   | X: 18D3        | 8e-52               |
| RPS11     | RpS11        | CG8857    | 2R: 48EB-9     | 1e-58               |
| RPS12     | RpS12        | CG11271   | 3L: 69F5       | 2e-43               |
| RPS13     | RpS13        | CG13389   | 2L: 29B2       | 2e-74               |
| RPS14     | RpS14a       | CG1524    | X: 7C6-7       | 3e-71               |
|           | RpS14b       | CG1527    | X: 7C8         | 3e-71               |
| RPS15     | RpS15        | CG8332    | 2R: 53C8       | 6e-62               |
| RPS15A    | RpS15Aa      | CG2033    | X: 11E11-12    | 2e-65               |
|           | RpS15Ab      | CG12324   | 2R: 47C1       | 6e-65               |
| RPS16     | RpS16        | CG4046    | 2R: 58F1       | 2e-69               |
| RPS17     | RpS17        | CG3922    | 3L: 67B5       | 5e-52               |
| RPS18     | RpS18        | CG8900    | 2R: 56F11      | 2e-69               |
| RPS19     | RpS19a       | CG4464    | X: 14F4        | 5e-48               |
|           | RpS19b       | CG5338    | 3R: 95C13      | 1e-43               |
| RPS20     | RpS20        | CG15693   | 3R: 93A1       | 7e-50               |
| RPS21     | oho23B/RpS21 | CG2986    | 2L: 23B6       | 3e-30               |
| RPS23     | RpS23        | CG8415    | 2R: 50E4       | 8e-70               |
| RPS24     | RpS24        | CG3751    | 2R: 58F3       | 7e-55               |
| RPS25     | RpS25        | CG6684    | 3R: 86D8       | 1e-38               |
| RPS26     | RpS26        | CG10305   | 2L: 36F4       | 3e-47               |
| RPS27     | RpS27        | CG10423   | 3R: 96C8       | 2e-39               |
| RPS27A    | RpS27A       | CG5271    | 2L: 31E1       | 8e-80               |
| RPS28     | RpS28\(^a\) | CG15527   | 3R: 99D2       | 1e-21               |
|           | RpS28b       | CG2998    | X: 8E7         | 2e-23               |
|           | RpS28-like   | CG34182   | 2L: 30B3       | 1e-07               |
| RPS29     | RpS29        | CG8495    | 3R: 85EB       | 5e-23               |
| RPS30     | RpS30        | CG15697   | 3R: 93A2       | 6e-32               |
| RPLP0     | RplP0        | CG7490    | 3L: 79B2       | 1e-122              |
### Table 1 (Continued)

**The CRP genes of D. melanogaster**

| Gene    | Accession | Location | Score |
|---------|-----------|----------|-------|
| RpLP0-like | CG1381 | 2R: 46E5-6 | 3e-10 |
| RPLP1     | CG4087   | 2L: 21C2  | 3e-34 |
| RPLP2     | CG4918   | 2R: 53C9  | 5e-32 |
| RPL3      | CG4863   | 3R: 86D8  | 0.0   |
| RPL4      | CG5502   | 3R: 98B6  | e-141 |
| RPL5      | CG17489  | 2L: h35/40B | e-120 |
| RPL6      | CG11522  | 3R: 100C7 | 8e-58 |
| RPL7      | CG4897   | 2L: 31B1  | 2e-75 |
| RpL7-like | CG5317   | 2L: 33C1  | 3e-32 |
| RPL7A     | CG3114   | X: 6B1    | e-102 |
| RPL8      | CG1263   | 3L: 62E7  | e-119 |
| RPL9      | CG6141   | 2L: 32C1  | 1e-66 |
| RPL10     | CG17521  | 3L: h47/80A | 7e-98 |
| RPL10A    | CG3843   | 3R: 88D10 | 3e-65 |
| RPL10Ab   | CG7283   | 3L: 68E1  | 3e-95 |
| RPL11     | CG7226   | 2R: 56D7  | 6e-83 |
| RPL12     | CG3195   | 2R: 60B7  | 7e-75 |
| RPL13     | CG4651   | 2L: 30F3  | 1e-68 |
| RPL13A    | CG1475   | 3R: 83B6-7 | 3e-68 |
| RPL14     | CG6253   | 3L: 66D8  | 2e-30 |
| RPL15     | CG17420  | 3L: h50-52/80F | 5e-90 |
| RPL17     | CG3203   | X: 6C10   | 5e-72 |
| RPL18     | CG8615   | 3L: 65E9  | 3e-71 |
| RPL18A    | CG6510   | 2R: 54C3  | 3e-68 |
| RPL19     | CG2746   | 2R: 60E11 | 4e-83 |
| RPL21     | CG12775  | 2L: 40A-B | 4e-66 |
| RPL22     | CG7434   | X: 1C4    | 4e-40 |
| RpL22-like| CG9871   | 2R: 59D3  | 2e-24 |
| RPL23     | CG3661   | 2R: 59B3  | 1e-68 |
| RPL23A    | CG7977   | 3L: 62A10 | 7e-52 |
| RPL24     | CG9282   | 2L: 34B10 | 7e-55 |
| RpL24-like| CG6764   | 3R: 86E5  | 8e-14 |
| RPL26     | CG6846   | 3L: 75E4  | 1e-59 |
| RPL27     | CG4759   | 3R: 96E9-10 | 1e-43 |
| RPL27A    | CG15442  | 2L: 24F3  | 5e-60 |
| RPL28     | CG12740  | 3L: 63B14 | 3e-31 |
| RPL29     | CG10071  | 2R: 57D8  | 8e-14 |
| RPL30     | CG10652  | 2L: 37B9  | 3e-46 |
| RPL31     | CG1821   | 2R: 45F5  | 5e-47 |
| RPL32     | CG7939   | 3R: 99D3  | 6e-58 |
| RPL34     | CG6090   | 3R: 96F10 | 2e-29 |
| RpL34a    | CG9354   | 3R: 85D15 | 1e-29 |
| RPL35     | CG4111   | X: 5A11   | 2e-38 |
| RPL35A    | CG2099   | 3R: 83A4  | 1e-35 |
| RPL36     | CG7622   | X: 1B12   | 3e-33 |
The CRP genes of D. melanogaster

| CRP | Fly Gene | Fly Gene ID | X-Location | Y-Location |
|-----|----------|-------------|------------|------------|
| RPL36A | RpL36A | CG7424 | 2L: 28D3 | 3e-43 |
| RPL37 | RpL37a | CG9091 | X: 13B1 | 2e-39 |
|       | RpL37b | CG9873 | 2R: 59C4 | 1e-31 |
| RPL37A | RpL37A | CG5827 | 2L: 25C4 | 2e-38 |
| RPL38 | RpL38 | CG18001 | 2R: h46/41C-E | 2e-25 |
| RPL39 | RpL39 | CG3997 | 2R: 60B7 | 3e-18 |
| RPL40 | RpL40 | CG2960 | 2L: 24E1 | 2e-69 |
| RPL41 | RpL41 | CG30425 | 2R: 60E5 | 8e-08 |

*Additional gene synonyms exist in most cases [60]. Bold font indicates CRP-like genes, putative pseudogenic fragments (CG11386 and CG33222) or the member of a duplicate gene pair that is expressed in a small number of tissues and/or at relatively low levels. Computed cytological position is given for euchromatic genes (Genome Release 5 [60]). The cytological and h-band locations for heterochromatic genes are based on data in reference [151] or estimated from images of in situ hybridizations of BACs to polytene chromosomes (RPL5 and RPL21) [152]. The h-band location of RPL15 was provided by B Honda (personal communication). Expect (E) value obtained from a BLASTp search of the D. melanogaster annotated proteome (Genome Release 5.1) with human RefSeq CRP sequences. (E values corresponding to RPL15 and RPL5 were obtained from a BLAST search using Release 5.3.) Where multiple protein isoforms exist, the highest scoring hit is given.

weights and isoelectric points of the homologous proteins are very similar. However, several D. melanogaster proteins (RpL14, RpL22, RpL23A, RpL29, RpL34A, RpL34B, RpL35A) have significantly lower overall identity and different molecular weights owing to terminal deletions or extensions (data not shown; also see [65]). (If these seven proteins are discounted, the average identity of fly and human CRPs increases to 72% with a range of 43-98%.) Similar to humans and other species, there are very few acidic CRPs in other animals, there are very few acidic CRPs in Drosophila melanogaster: only six proteins (RpSA, RpS12, RpS21, RpLP0, RpLP1 and RpLP2) have isoelectric points less than pH 7. (Note that RpS21 is an acidic protein, whereas its human counterpart is basic.) As in other eukaryotes, RpS27A and RpL40 are carboxyl extensions of ubiquitin [66-69], and, as in other animals, RpS30 is fused to a ubiquitin-like sequence. From these gross characterizations of component proteins, it appears that the fly cytoribosome differs only slightly from its human counterpart and is essentially the same as other eukaryotic cytoribosomes.

Previous biochemical analyses estimated that the D. melanogaster cytoribosome contains up to 78 CRPs [29]. This figure compares very well to the 79 different CRPs predicted by our orthology analysis (Table 1). Unfortunately, very few of the CRPs identified in the 1970s and 1980s were characterized to the level of amino acid sequence, so their correspondences to CRP genes are generally unknown, though there are a few exceptions (see references [70-73]). We have been unable, therefore, to correlate the CRPs identified in these earlier studies with those encoded by the CRP genes identified in this study. In contrast, our CRP inventory certainly does contain all 52 CRPs identified by the recent biochemical analysis of D. melanogaster cytoribosomes by Alonso and Santarén [34].

Mitochondrial ribosomal protein genes

We identified 75 D. melanogaster genes encoding proteins of the mitoribosome (28 in the small subunit and 47 in the large subunit) by orthology to human MRPs (Table 2). These data complement and extend previous analyses of homology between human and D. melanogaster MRPs [16,17]. As in these previous studies, genes encoding orthologs of three human MRPs (MRPS27, MRPS36 and LACTB/MRPL56) were not found.

The MRPs of humans and D. melanogaster are much more divergent than are their CRPs: MRPs have an average identity of only 34% (with a range of 15-57%) and several homologous pairs differ markedly in their sizes and isoelectric points (Additional data file 2). Indeed, it is known that the mitoribosome is a rapidly evolving structure whose composition varies among eukaryotic organisms [6]. It is quite possible that there are proteins in Drosophila mitoribosomes that are not found in their human counterparts and these will have been missed by our orthology analysis - a definitive inventory will require biochemical characterization of the fly mitoribosome. As in mammals, three distinct genes encode three different isoforms of MRPS18 (Table 2); it is thought that each mitoribosome contains a single MRPS18 protein and that mitoribosomes may, therefore, be heterogeneous in composition [6].

Duplicate cytoplasmic ribosomal protein genes

Of the 79 different CRPs of D. melanogaster, 9 are encoded by two distinct genes (Table 1). These are distinguished by a lowercase 'a' or 'b' suffix to the gene symbol. (The lowercase 'a' should not be confused with the uppercase 'A' suffix used in the standard CRP nomenclature; for example, RpL37a and RpL37A are different genes that encode different proteins.) Six of these gene pairs encode proteins of the small ribosomal subunit and the other three encode large subunit proteins. In humans, each CRP is typically encoded by a single, functional gene [37,74], but thousands of nonfunctional CRP pseudogenes are known to exist [75]. We therefore investigated the evolutionary origin, sequence conservation and expression
### Table 2

The MRP genes of *D. melanogaster*

| Human MRP | Symbol* | CG number | Location† | BLAST E value‡ |
|-----------|---------|-----------|-----------|----------------|
| MRPS2     | mRpS2   | CG2937    | 2L: 25B1  | 1e-69          |
| MRPS5     | mRpS5   | CG40049   | 3L: h47/80A-B | 4e-63 |
| MRPS6     | mRpS6   | CG15016   | 3L: 64B2  | 2e-20          |
| MRPS7     | mRpS7   | CG5108    | 2L: 31D11 | 6e-38          |
| MRPS9     | mRpS9   | CG2957    | 3R: 84E4  | 8e-81          |
| MRPS10    | mRpS10  | CG4247    | 3R: 88E3  | 1e-33          |
| MRPS11    | mRpS11  | CG5184    | 3R: 89E11 | 8e-26          |
| MRPS12    | tkolmRpS12 | CG7925  | X: 3A3   | 1e-33          |
| MRPS14    | mRpS14  | CG32531   | X: 18C7  | 1e-35          |
| MRPS15    | bonsai/mRpS15 | CG4207 | 2R: 58F3 | 1e-15          |
| MRPS16    | mRpS16  | CG8338    | 2R: 50E1  | 2e-24          |
| MRPS17    | mRpS17  | CG4326    | 2R: 60C1  | 2e-14          |
| MRPS18A   | mRpS18A | CG31450   | 3R: 85A3 | 8e-13          |
| MRPS18B   | mRpS18B | CG10757   | 2L: 38B6 | 4e-33          |
| MRPS18C   | mRpS18C | CG9688    | 3R: 99F4 | 7e-23          |
| MRPS21    | mRpS21  | CG32854   | 3R: 87E8  | 3e-22          |
| MRPS22    | mRpS22  | CG12261   | 3R: 98B6  | 3e-38          |
| MRPS23    | mRpS23  | CG1842    | 2L: 34D6  | 2e-20          |
| MRPS24    | mRpS24  | CG13608   | 3R: 95E6  | 2e-31          |
| MRPS25    | mRpS25  | CG14413   | X: 12F1  | 1e-49          |
| MRPS26    | mRpS26  | CG7354    | 3L: 75B9  | 3e-11          |
| MRPS27    | NA      | NA        | NA        | No hit         |
| MRPS28    | mRpS28  | CG5497    | 2R: 55E2  | 1e-27          |
| DAP3/MRPS29| mRpS29  | CG3633    | 2R: 58E1  | 7e-72          |
| MRPS30    | mRpS30  | CG8470    | X: 13E18  | 3e-21          |
| MRPS31    | mRpS31  | CG5904    | 3L: 72C2  | 5e-35          |
| MRPS33    | mRpS33  | CG10406   | 3R: 89B16 | 3e-30          |
| MRPS34    | mRpS34  | CG13037   | 3L: 72E1-2| 8e-06          |
| MRPS35    | mRpS35  | CG2101    | 3L: 62F4  | 2e-72          |
| MRPS36    | NA      | NA        | NA        | No hit         |
| MRPL1     | mRpL1   | CG7494    | 3R: 84F9-10| 8e-25         |
| MRPL2     | mRpL2   | CG7636    | 3L: 68A7  | 2e-56          |
| MRPL3     | mRpL3   | CG8288    | X: 13E14  | 5e-52          |
| MRPL4     | mRpL4   | CG5818    | 2L: 35F1  | 6e-70          |
| MRPL9     | mRpL9   | CG31478   | 3R: 88F1  | 6e-17          |
| MRPL10    | mRpL10  | CG11488   | 2L: 21B4  | 8e-20          |
| MRPL11    | mRpL11  | CG3351    | 3R: 88C3  | 4e-29          |
| MRPL12    | mRpL12  | CG5012    | 3L: 66E5  | 2e-13          |
| MRPL13    | mRpL13  | CG10603   | 2L: 37B1  | 2e-47          |
| MRPL14    | mRpL14  | CG14048   | X: 3A1   | 3e-31          |
| MRPL15    | mRpL15  | CG5219    | 3L: 77C3  | 4e-74          |
| MRPL16    | mRpL16  | CG3109    | X: 2B14  | 3e-58          |
| MRPL17    | mRpL17  | CG13880   | 3L: 61B3  | 8e-21          |
| MRPL18    | mRpL18  | CG12373   | 2R: 49C2  | 3e-22          |
| MRPL19    | mRpL19  | CG8039    | 3R: 85A5  | 1e-56          |
| MRPL20    | mRpL20  | CG11258   | 3L: 69F5  | 8e-21          |
profile of the duplicate *D. melanogaster* CRP genes in order to assess whether both members of each pair are likely to be functional (Table 3 and Figure 1).

In five cases, one member of the gene pair lacks introns (*RpS10a*, *RpS15Ab*, *RpS28a*, *RpL10Aa* and *RpL37b*) while the other member does not. These five intronless genes are likely to have arisen by retrotransposition; that is, generated via reverse transcription of mRNA from the precursor gene followed by insertion into a new genomic location. In contrast, the *RpS5*, *RpS19* and *RpL34* duplicates arose through gene transposition events as both members of each pair retain introns. The *RpL34* duplication occurred through an intrachromosomal transposition on chromosome arm 3R, and *RpL34a* and *RpL34b* have retained almost identical gene structures. In contrast, the *RpS5* and *RpS19* duplications involved interchromosomal transposition events that must have been followed by extensive gene remodeling as the intron-exon structures differ within each pair. Finally, *RpS14a* and *RpS14b* probably arose via unequal exchange: these paralogs are situated adjacent to each other as a tandem duplication on the *X* chromosome, share identical intron-exon structures and encode identical proteins [76]. All nine duplicate genes appear to have arisen within the *Drosophilidae* (Figure 1).

Neither member of these 9 CRP gene pairs contains a nonsense mutation in the protein-coding region (data not shown), indicating that all 18 genes are potentially functional.
Moreover, the low ratio of nonsynonymous to synonymous substitutions ($K_a/K_s$) between the members of each gene pair suggests that there are selective constraints on their protein-coding regions (Table 3; a $K_a/K_s$ ratio significantly lower than 0.5 indicates functional constraints on both genes). Branch-specific $K_a/K_s$ values further indicate that the putatively retrotransposed genes have been under overall purifying selection since their formation. Together, these data argue that...
none of these duplicate genes are nonfunctional pseudogenes, which is consistent with a previous analysis [77]. Indeed, the recovery of multiple cDNA clones for the majority (15/18) of these duplicate genes supports their expression in vivo (Table 3).

Although none of these CRP gene duplicates appear to be pseudogenes, it is evident that one member of each pair - the one with higher similarity to its human ortholog, where this difference exists (Table 1 and Additional data file 1) - is expressed at a significantly higher level and, in some cases, in a wider array of tissues than the other. This suggests that one gene of the pair produces the majority of each CRP in most cells, while the other gene has a more restricted expression pattern and, perhaps, a specialized function (indicated by bold font in Tables 1 and 3). In eight of the nine duplication events, the 'younger' gene copy has adopted the lower expression level or more restricted expression pattern; the \( \text{RpL}34 \) gene pair is exceptional in this regard (Figure 1 and Table 3).

The expression of \( \text{RpS}5b, \text{RpS}19b, \text{RpL}10Aa \) and \( \text{RpL}37b \) appears enriched in the adult testis, suggesting the existence of testis-specific CRPs and a testis-specific cytoribosome (Table 3). Significantly, three of these genes (\( \text{RpS}5b, \text{RpS}19b \) and \( \text{RpL}37b \)), together with \( \text{RpS}10a, \text{RpS}15Ab \) and \( \text{RpS}28a \), are autosomal copies of X-linked genes. These duplication events are consistent with previous studies reporting that genes with male-biased expression are predominantly autosomal [78], and that retrotransposed genes in \( \text{D. melanogaster} \) have preferentially retrotransposed from the X chromosome onto autosomes [79]. It is possible that these autosomal duplicates enable CRP expression in male germ-line cells, where it is hypothesized that X chromosome inactivation occurs during spermatogenesis [80]. Similarly, in humans, \( \text{RPS}4\gamma \) is a Y-linked duplicate of the X-linked \( \text{RPS}4 \) gene [10] and \( \text{RPL}10L, \text{RPL}36AL \) and \( \text{RPL}39L \) are autosomal retrogene copies of X-linked progenitors [74]. It is worth noting that expression of \( \text{D. melanogaster} \text{RpS}5b, \text{RpS}10a \) and \( \text{RpS}19b \) is also enriched in the germline cells of embryonic gonads [81] and/or stem cells of adult ovaries [82]. These findings suggest a germine-specific role, rather than a testis-specific role, for these CRP gene duplicates.

To conclude, the ‘principal’ CRPs of \( \text{D. melanogaster} \) - those that are expressed at high levels in most cells - are each encoded by single genes.
Cytoplasmic ribosomal protein-like genes

We identified five *D. melanogaster* ‘CRP-like’ genes that encode proteins with significantly lower identity to human CRPs than those described above. These are RpS28-like, RpLPo-like, RpL7-like, RpL22-like and RpL24-like (shown in bold font in Tables 1 and 3). Of these, RpLP0-like and RpL24-like show the most divergence from their cognate proteins, RpLP0 and RpL24. Consistent with this, RpLP0-like and RpL24-like have ancient evolutionary origins, while RpL7-like, RpL22-like and RpS28-like arose more recently within the Diptera (Figure 1).

cDNA evidence indicates that all five of these CRP-like genes are expressed *in vivo*, albeit at far lower levels than their cognate genes (Table 3). The evolutionary conservation of RpLP0-like and RpL24-like suggests they have important cellular functions. Indeed, the yeast ortholog of RpL24-like is found in pre-ribosomal complexes where it is thought to function in large subunit biogenesis [83]. It remains to be seen whether the other CRP-like proteins have similar functions. Interestingly, the RpL22 gene is X-linked and expressed ubiquitously, whereas RpL22-like is an autosomal gene that is expressed predominantly in germline cells [81,82,84,85]. This suggests that RpL22-like may have a specialized role in the germline, and perhaps within germline-specific cytoryosomes, as proposed above for some of the CRP duplicates.

*CG11386 and CG33222 are 99% identical in DNA sequence and are tandem repeats of the third exon and flanking regions of the *RpS6* gene. They likely arose via two sequential unequal crossover events [86]; the first occurring after the evolutionary split of the melanogaster subgroup, and the second being specific to *D. melanogaster* (Figure 1). Gene prediction algorithms suggest that *CG11386* and *CG33222* are distinct genes encoding identical amino-terminally truncated versions of RpS6 [87]; however, such proteins would lack critical functional domains and would probably be nonfunctional. In a different scenario, *CG11386* and/or *CG33222* could serve as alternative third exons of the *RpS6* gene: the proteins produced would be full-length, but would differ substantially in their carboxy-terminal two-thirds from the *RpS6* generated by using the conventional third exon [86]. There is, however, no direct evidence that such alternative transcripts are made. Indeed, only three cDNA clones suggest that *CG11386* or *CG33222* are expressed at all (Table 3). We have tentatively classified *CG11386* and *CG33222* as nonfunctional pseudogenic fragments.

Chromosomal distribution of ribosomal protein genes

As has been found for other eukaryotes [35,36,38,39], the RP genes of *D. melanogaster* are distributed across the entire genome (Figure 2). Some RP genes are tightly linked to other RP genes and, while this posed challenges for determining the phenotypes associated with individual genes (see below), we have no evidence that this distribution has functional consequences or that closely linked RP genes are transcriptionally co-regulated. Five RP genes (*RpL5, Qm/RpL10, RpL15, RpL38*, and *mRpS5*) are located within heterochromatic regions, as are certain human MRP genes [38] and some *Arabidopsis thaliana* CRP genes [36]. As heterochromatin is generally associated with the silencing of gene expression [88], the regulation of these genes must have adapted to the heterochromatic environment in order for the encoded proteins to be expressed at sufficiently high levels to meet the demand for ribosome synthesis in the cell [89].

Ribosomal protein gene haploinsufficiency and the Minute syndrome

Classical genetic studies have defined more than fifty regions of the *D. melanogaster* genome that are haploinsufficient and associated with the dominant phenotypes of prolonged development and short, thin bristles - the *Minute* loci [2] (Figure 3). To date, only fifteen *Minute* loci have been tied unequivocally to molecularly defined genes and all of these encoded RPs (reviewed in reference [2]; also see references [48-53]). It has not been clear, however, if all *Minute* loci correspond to RP genes, or whether *Minute* loci may correspond to both CRP and MRP genes. We have conducted a new survey of *Minute* loci in the *D. melanogaster* genome which, combined with our RP gene inventory, has now allowed us to assess these relationships systematically.

Recent large-scale projects have provided a wealth of new genetic reagents that enable the mapping of *Minute* loci with a precision unavailable only a few years ago. Hundreds of new deletions with molecularly defined breakpoints have been provided by the efforts of the DrosDel consortium [90,91], Exelixis, Inc. [92], and the Bloomington *Drosophila* Stock Center [92]. When combined with older deletions characterized primarily through polytene chromosome cytology, these deletions have increased euchromatic genome coverage to 96-97%. In addition, transposable element insertions now exist within 0.5 kb of 57% of all genes (R Levis, personal communication), largely through the efforts of the *Drosophila* Gene Disruption Project [93] and Exelixis, Inc. [94]. We used...
Figure 2 (see legend on previous page)
these resources to conduct a genome-wide search for Minute loci. In so doing, we considered the characteristic Minute bristle phenotype (Figure 3) to be diagnostic of the Minute syndrome; we did not methodically evaluate more subtle Minute traits, such as slower development, or traits observed in only a subset of Minute mutants, such as impaired fecundity, reduced viability or altered body size. By combining our observations with information gleaned from published studies, we have identified 61 distinct Minute loci. Many of these correlate with Minute loci described previously (Additional data file 3), though our work has often refined their map positions. Significantly, six Minute loci (M(2)31E, M(2)34BC, M(2)45F, M(2)50E, M(3)93A and M(3)98B) are reported here for the first time. We also found four instances (M(2)31A, M(2)53, M(2)58F and M(3)67C) where a single Minute locus characterized by previous aneuploidy analyses actually comprises two separable, closely linked Minute genes. As we have inferred the existence of four additional Minute loci from patterns of deletion coverage (described below), we conclude that there are 65 distinct Minute loci in the D. melanogaster genome.

We were able to demonstrate definitively that a particular Minute locus corresponds to a specific RP gene when a Minute bristle phenotype was observed in one or more of the following situations: flies heterozygous for a molecularly characterized mutation in a RP gene (for example, M(2)36F/RpS26); flies heterozygous for a chromosomal deletion when the Minute phenotype could be mapped unambiguously to a single RP gene with deletion breakpoints (for example, M(2)25C/RpL37A); or flies heterozygous for a chromosomal deletion when the Minute phenotype could be rescued by a specific RP transgene (for example, M(3)99D/RpL32). We found that there are 26 unequivocally Minute CRP genes by these criteria (Additional data file 4; summarized in Table 4). In contrast, no MRP or CRP-like genes were definitively demonstrated to be Minute genes.

These 26 cases of proven CRP gene-Minute locus correspondences provide a strong precedent for expecting that other CRP genes are also Minute genes. Although existing reagents do not allow us to demonstrate the correspondences definitively, we judged that a CRP gene very likely corresponds to a genetically defined Minute locus when one or more of the following criteria are fulfilled: a Minute phenotype is seen for a heterozygous multi-gene deletion that uncovers a single CRP gene (for example, M(3)63B/RpL28); a CRP gene lies in a gap in deletion coverage and a molecularly uncharacterized Minute mutation maps to the same region (for example, M(1)80F/RpS28B); or a CRP gene lies in a gap in deletion coverage and previous studies of transient aneuploids document the presence of a Minute locus in the same region (for example, M(3)99E/RpS7). In this way, we identified an additional 36 CRP genes that likely correspond to 34 genetically defined Minute loci (Additional data file 4; summarized in Table 4). Closely linked pairs of CRP genes map to the same regions as M(2)60B and M(3)93A and, as it was impossible to determine whether one or both genes of each pair are haploinsufficient, we have classified all four CRP genes as likely Minute genes. No CRP-like genes mapped to the regions of proven Minute loci. Although five MRP genes map to regions containing Minute loci, it is unlikely that any of them are haploinsufficient: MRP genes are not associated with Minute phenotypes in any other situation, and each of these five MRP genes is closely linked to a CRP gene (Additional data file 4).

We concluded that a further four CRP genes (RpL17, RpL18A, RpL34b and RpL35A) are likely to be Minute genes despite no Minute phenotype having been associated with the genomic region in which they reside. In each of these cases, the CRP gene lies in a gap in deletion coverage (Table 4, Additional data file 4), suggesting that it is a Minute associated with strongly reduced fertility and/or viability, which prevents the establishment of stable deletion stocks (in the absence of a corresponding duplication). Supporting this view, such severe haploinsufficiency also appears to be associated with 15 other CRP genes - all these CRP genes lie in gaps in deletion coverage and they are only considered Minute genes here because they have point or transposon insertion (likely hypomorphic) mutations that cause Minute phenotypes, or because they lie in regions known to harbour Minute loci from the phenotypes of transient aneuploids (Table 4, Additional data file 4).

For all of the 40 CRP genes classified as 'likely Minute genes' (through correlation with genetically proven Minute loci or gaps in deletion coverage), we determined the maximum number of candidate genes that could possibly account for the haploinsufficiency. We used deletions to define the smallest chromosomal interval containing the Minute and then eliminated genes known not to be associated with a Minute phenotype from previous studies or from our own examinations of mutant fly strains. (This task benefited greatly from the recent work of the Bloomington Drosophila Stock Center which, in its efforts to maximize genomic deletion coverage, has systematically generated deletions flanking haploinsufficient loci.) The number of candidate genes defined in this way was always small, ranging from 2 to 33 genes with a median of 8.5 candidate genes per Minute locus (Table 4, Additional data file 4). These data increase our confidence in the likely correspondences between these Minute loci and CRP genes.

The results presented above indicate that 66 CRP genes are, or are likely to be, Minute genes, whereas the remaining 22 CRP genes are not (Table 4 and Additional data files 4 and 5; summarized in Figure 4). CRPs of the large and small ribosomal subunit are encoded by both Minute and non-Minute genes, with no apparent bias. Notably, none of the nine duplicate CRP genes with relatively restricted expression is a Minute, whereas seven of the more highly and widely expressed gene pair members are Minute genes. This is consistent with the idea that only one member of each of these
gene pairs contributes significantly to cytoribosomal function in the majority of cells, while the one with restricted expression encodes a component of qualitatively distinct cytoribosomes in certain cell types. As the 'principal' copy of \textit{RpS14} or \textit{RpL10A} is not a \textit{Minute}, it is unsurprising that the simultaneous heterozygous deletion of both \textit{RpS14} genes \cite{95} or both \textit{RpL10A} genes (in flies with genotypes \textit{Df(3L)ED4475/Df(3R)ED10556} or \textit{Df(3L)ED4475/Df(3R)ED5660}; our observations) does not produce flies with \textit{Minute} phenotypes.

Other possible reasons for different dosage sensitivities among CRP genes are discussed below.

The one verified \textit{Minute} locus that does not correspond to a CRP gene is \textit{M(1)14C}. We mapped this \textit{Minute} gene to region 14C6 by showing that the deletions \textit{Df(1)ED7364 (14A8;14C6)} and \textit{Df(1)FDD-0230908 (14C6;14E1)} are each associated with a Minute phenotype. Moreover, we could rescue these phenotypes, as well as the Minute phenotype associated with
## Table 4

### CRP gene haploinsufficiency

| Symbol      | Location | CRP gene mutations<sup>c, d</sup> | Deletions removing a single CRP gene<sup>e, f</sup> | Other evidence                                                                 | Is the CRP gene a Minute<sup>g</sup> | No. of candidate Minute genes<sup>h</sup> | Minute synonym<sup>i</sup> | Reference<sup>j</sup> |
|-------------|----------|-----------------------------------|----------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------|---------------------------------|-----------------|-----------------|
| **X chromosome** |          |                                   |                                                    |                                                                                  |                                       |                                 |                 |                 |
| Rpl36       | 1B12     | M                                 |                                                   |                                                                                  | Yes                                   | M(1)1B                         | [53]            |                 |
| Rpl22       | 1C4      | +                                 | +                                                 |                                                                                  | No                                    |                                 |                 |                 |
| sta/RpSA    | 2B1      | +                                 | +                                                 |                                                                                  | No                                    |                                 |                 |                 |
| Rpl3S       | 5A11     |                                   |                                                   |                                                                                  | Likely                                | 33                              | M(1)5A         |                 |
|             |          |                                   | Lies in a gap in deletion coverage. 5A6-13 aneuploids were Minute (Merriam et al. [55]) |                                                                                  |                                       |                                 |                 |                 |
| Rpl7A       | 6B1      | M                                 |                                                   |                                                                                  | Likely                                | 2                               | M(1)5D6A       |                 |
| Rpl17       | 6C10     |                                   | Lies in a gap in deletion coverage                |                                                                                  | Likely                                | 12                              | New?            |                 |
| RpS6        | 7C2      | M                                 | M                                                 |                                                                                  | Yes                                   | M(1)7BC and M(1)7C           | [2]             |                 |
| RpS14a      | 7C6-7    |                                   |                                                   |                                                                                  | No                                    |                                 |                 |                 |
| **RpS14b**  | 7C8      |                                   |                                                   |                                                                                  | No                                    |                                 |                 |                 |
| RpS28b      | 8E7      |                                   | Lies in a gap in deletion coverage. A Minute mutation was mapped to 8D8-9A2 (153) |                                                                                  | Likely                                | 17                              | M(1)8F          |                 |
| RpS15Aa     | 11E11-12 | M                                 |                                                   |                                                                                  | Likely                                | 8                               | M(1)11F        |                 |
| RpL37a      | 13B1     |                                   | Lies in a gap in deletion coverage                |                                                                                  | Likely                                | 2                               | M(1)13A        |                 |
| RpS19a      | 14F4     | M                                 |                                                   |                                                                                  | Likely                                | 14                              | M(1)14E        |                 |
| RpS5a       | 15E5-7   | M                                 | Lies in a gap in deletion coverage                |                                                                                  | Yes                                   | M(1)15D        | [44]            |                 |
| RpS10b      | 18D3     | M                                 | M                                                 |                                                                                  | Yes                                   | M(1)18C         |                 |                 |
| **Chromosome arm 2L** |        |                                   |                                                    |                                                                                  |                                       |                                 |                 |                 |
| RplP1       | 21C2     | M                                 | M                                                 |                                                                                  | Yes                                   | M(2)21C         | [50]            |                 |
| oha23B/RpS21| 23B6     | M                                 | M                                                 |                                                                                  | Yes                                   | M(2)23B         | [49]            |                 |
| Rpl40       | 24E1     | M                                 |                                                   |                                                                                  | Likely                                | 4                               | M(2)24D        |                 |
| Rpl27A      | 24F3     | M                                 | M                                                 |                                                                                  | Yes                                   | M(2)24F         |                 |                 |
| Rpl37A      | 25C4     | M                                 |                                                   |                                                                                  | Likely                                | 5                               | M(2)28DE*      |                 |
| RpL36A      | 28D3     | M                                 |                                                   |                                                                                  | Likely                                | 5                               | M(2)29B*       |                 |
| RpS13       | 29B2     | M                                 | M                                                 |                                                                                  | Yes                                   | M(2)31A         |                 |                 |
| sop/RpS2    | 30E1     | +                                 | +                                                 |                                                                                  | No                                    |                                 |                 |                 |
| Rpl13       | 30F3     | M                                 | The interval between flanking non-Minute deletions contains only Rpl37A |                                                                                  | Yes                                   | M(2)31A         |                 |                 |
| Rpl7        | 31B1     | M                                 |                                                   |                                                                                  | Likely                                | 2                               | M(2)31A        |                 |
| RpS27A      | 31E1     | M                                 |                                                   |                                                                                  | Likely                                | 6                               | M(2)31E*       |                 |

<sup>a</sup> Genetica analysis; <sup>b</sup> Genetic analysis; <sup>c</sup> CRP gene mutations; <sup>d</sup> Deletions removing a single CRP gene; <sup>e</sup> Other evidence; <sup>f</sup> Lies in a gap in deletion coverage. 5A6-13 aneuploids were Minute (Merriam et al. [55]); <sup>g</sup> Is the CRP gene a Minute; <sup>h</sup> No. of candidate Minute genes; <sup>i</sup> Minute synonym; <sup>j</sup> Reference.
Table 4 (Continued)

| CRP gene haploinsufficiency | Chromosome arm 2R | | Chromosome arm 3L |
|-----------------------------|-------------------|------------------|------------------|
| RpL9                         | 32C1              | M                | RpL23            | 62A10             | M                | RpL23A           | 62A10             | M                |
| RpL24                        | 34B10             | M                | RpL29            | 57D8             | +                | RpL29            | 57D8             | +                |
| RpS26                        | 36F4              | M                | RpS16            | 5BF1             | M                | RpS24            | 5BF3             | M                |
| RpL30                        | 37B9              | +                | RpS24            | 5BF3             | M                | RpS24            | 5BF3             | M                |
| RpL21                        | 40A-B             | M                | RpS24            | 5BF3             | M                | RpS24            | 5BF3             | M                |
| RpL5                         | 40B               | M                | RpL19            | 60E11            | M                | RpL19            | 60E11            | M                |
| RpL38                        | 41C-E             | M                | RpL24            | 34B10             | M                | RpL23            | 62A10             | M                |
| RpL31                        | 45F5              | M                | RpL18A           | 54C3             | M                | RpL29            | 57D8             | +                |
| RpS15Ab                      | 47C1              | +                | RpL11            | 56D7             | M                | RpS16            | 5BF1             | +                |
| RpS11                        | 48EB8-9           | M                | RpS16            | 5BF1             | M                | RpS24            | 5BF3             | M                |
| RpS23                        | 50E4              | M                | RpS24            | 5BF3             | M                | RpS24            | 5BF3             | M                |
| RpS15                        | 53C8              | M                | RpS24            | 5BF3             | M                | RpS24            | 5BF3             | M                |
| RpLP2                        | 53C9              | M                | RpS18            | 56F11            | M                | RpL24            | 5BF3             | M                |
| RpL18A                       | 54C3              | M                | RpS16            | 5BF1             | M                | RpS24            | 5BF3             | M                |
| RpL11                        | 56D7              | M                | RpS24            | 5BF3             | M                | RpS24            | 5BF3             | M                |
| RpS18                        | 56F11             | M                | RpS24            | 5BF3             | M                | RpS24            | 5BF3             | M                |
| RpS16                        | 57D8              | +                | RpS24            | 5BF3             | M                | RpS24            | 5BF3             | M                |
| RpS24                        | 5BF3              | M                | RpS24            | 5BF3             | M                | RpS24            | 5BF3             | M                |
| RpL23                        | 59B3              | +                | RpL19            | 60E11            | M                | RpL19            | 60E11            | M                |
| RpL37b                       | 59C4              | +                | RpL19            | 60E11            | M                | RpL19            | 60E11            | M                |
| RpL12                        | 60B7              | RpL12 and RpL39   | RpL12 and RpL39  | Lie in the same | Lie in the same | RpL12 and RpL39  | Lie in the same | Lie in the same |
| RpL39                        | 60B7              | RpL12 and RpL39   | RpL12 and RpL39  | Lie in the same | RpL12 and RpL39  | RpL12 and RpL39  | RpL12 and RpL39  | RpL12 and RpL39  |
| RpL41                        | 60E5              | +                | RpL12 and RpL39  | Lie in the same | Lie in the same | RpL12 and RpL39  | Lie in the same | Lie in the same |
| RpL19                        | 60E11             | M                | RpL19 and RpL41  | is Minute        | RpL19 and RpL41  | RpL19 and RpL41  | RpL19 and RpL41  | RpL19 and RpL41  |

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Table 4 (Continued)

**CRP gene haploinsufficiency**

| Gene   | Chromosome arm | Type | Description                                                                 | Likely | M(3)XX |
|--------|----------------|------|-----------------------------------------------------------------------------|--------|--------|
| RpL8   | 62E7           |      | Lies in a gap in deletion coverage containing only RpL8. 62E-63A aneuploids were Minute [54] | Yes    |        |
| RpL28  | 63B14          | M    |                                                                 | Likely | 10     |
| RpL18  | 65E9           | M    |                                                                 | Likely | 8      |
| RpL14  | 66D8           | M    | Lies in a gap in deletion coverage                                           | Yes    |        |
| RpS17  | 67B5           |      | A deletion removing both RpS17 and RpS9 is Minute. The sequenced Minute mutations RpS17o and RpS17h complement the Minute mutation RpS9EP3299, suggesting RpS17 is a Minute gene | Likely | 13     |
| RpS9   | 67B11          | M    | A deletion removing both RpS17 and RpS9 is Minute                             | Yes    |        |
| RpL10Ab| 68E1           |      |                                                                 | No     |        |
| RpS12  | 69F5           |      | A deletion that removes RpS12 and RpS4 is Minute                             | No     |        |
| RpS4   | 69F6           |      | A deletion that removes RpS12 and RpS4 is Minute                             | Likely | 2      |
| RpL26  | 75E4           |      |                                                                 | No     |        |
| RpL0   | 79B2           |      |                                                                 | No     |        |
| Qm/RpL10| 80A            | M    |                                                                 | Likely | 23     |
| RpL15  | 80F            | M    |                                                                 | Likely | = 11   |

**Chromosome arm 3R**

| Gene   | Chromosome arm | Type | Description                                                                 | Likely | M(3)XX |
|--------|----------------|------|-----------------------------------------------------------------------------|--------|--------|
| RpL35A | 83A4           |      | Lies in a gap in deletion coverage                                          | Likely | 3      |
| RpL13A | 83B6-7         | M    | Lies in a gap in deletion coverage                                          | Yes    | M(3)83B* [52] |
| RpL34b | 85D15          |      | Lies in a gap in deletion coverage                                          | Likely | 3      |
| RpS29  | 85E8           | M    | A deletion that removes RpS25 and RpL3 is Minute                            | Yes    | M(3)B5E |
| RpS25  | 86D8           | M    | A deletion that removes RpS25 and RpL3 is Minute                            | Likely | 14     |
| RpL3   | 86D8           | +    |                                                                 | No     |        |
| RpS5b  | 88D6           | +    |                                                                 | No     |        |
| RpL10Aa| 88D10          |      |                                                                 | No     |        |
| RpS20  | 93A1           |      | A deletion that removes RpS20 and RpS30 is Minute                           | Likely | 21     |
| RpS30  | 93A2           |      | A deletion that removes RpS20 and RpS30 is Minute                           | Likely | 21     |
| RpS3   | 94E13          | M    |                                                                 | Yes    | M(3)95A [43] |
| RpS19b | 95C13          |      |                                                                 | No     |        |
| RpS27  | 96C8           | M    |                                                                 | Likely | 5      |
| RpL27  | 96E9-10        | M    |                                                                 | Likely | 6      |
| RpL34a | 96F10          | +    |                                                                 | No     |        |
| RpS10a | 98A14          | +    |                                                                 | No     |        |
| RpL4   | 98B6           | M    |                                                                 | Likely | 6      |
| RpS8   | 99C4           |      | Lies in a gap in deletion coverage. 99B aneuploids were Minute [157]        | Likely | 11     |
the M(1)14C305-29 point mutation [44], using the small tandem duplication Dp(1;1)FDDP-0024486 (14C4;14D1). The Minute region defined by these experiments contains only two genes: CG4420 and eIF-2α. Significantly, flies heterozygous for P[RS5]eIF-2α 5'HA-1790, an insertion in the 5' untranslated region (UTR) of eIF-2α that creates a likely hypomorphic allele, show a discernable, albeit weak Minute phenotype (our observations). This identifies eIF-2α as M(1)14C. Consistent with this conclusion, flies expressing a dominant-negative eIF-2α protein grow slowly and attain a small body size [96], phenotypes that are typical of the Minute syndrome. eIF-2α is one of the three subunits that constitute eIF2, a key translation initiation factor that delivers the methionine-loaded initiator tRNA to the ribosome by transiently associating with the small cytoribosomal subunit [97]. Although eIF-2α is not a component of cytoribosomal complexes isolated by standard biochemical preparations, a reduction in eIF-2α gene dosage might still be expected to adversely affect cytoribosomal function and decrease overall rates of protein synthesis by specifically impairing translation initiation.

Interestingly, the gene encoding eIF-2γ, another subunit of the eIF2 translation initiation factor, is also haploinsufficient. Transcripts from the Su(var)3-9 gene are alternatively spliced to produce two different proteins with distinct functions: one protein is the eIF-2γ translation factor, the other is responsible for suppression of position effect variegation [98]. Mutations that specifically eliminate the suppressor protein are homozygous viable and are not associated with Minute phenotypes [99], but deletions of the entire gene are haplolethal in the absence of P(iry), 11.5kb, a transgenic construct carrying the complete Su(var)3-9 genomic region (our observations; Additional data file 6). These data indicate that the regions of the Su(var)3-9 transcription unit encoding eIF-2γ are haplolethal. Moreover, it is possible that this haplolethality actually represents an extreme Minute phenotype associated with the eIF-2γ-coding regions; hypomorphic eIF-2γ mutants, if isolated, may show less severe Minute phenotypes.

To assess the possibility that other translation factor genes might also be haploinsufficient/Minute, we examined the haploinsufficient loss-of-function phenotypes of 68 translation factor genes we identified from BLAST searches and/or Gene Ontology classification (Additional data file 6). We identified no other cases of haploinsufficiency, though five genes could not be assessed with existing deletion and mutation strains. In contrast to the genes encoding the other two subunits of eIF2, the eIF-2β gene is not haploinsufficient.

As mentioned above, we have compared our inventory of Minute genes with the Minute loci defined and named from previous genetic analyses (Additional data file 3). In so doing, we failed to validate the existence of several Minute loci described in the past, namely M(1)3E [55], M(1)4BC [55], M(2)21AB [100-102], M(2)44C [55], M(3)76A [55], M(3)82BC [55] and M(3)96A [103]. The existence of some of

### Table 4 (Continued)

| CRP gene haploinsufficiency | RpS28a | RpL32 | RpS7 | RpL6 |
|-----------------------------|--------|-------|------|------|
| 99D2                        |        |       |      |      |
| The Minute phenotype of a deletion removing RpS28a and RpL32 is rescued by a RpL32 transgene [41] |        |       |      |      |
| No                          |        |       |      |      |
| RpL32                       | 99D3   |       |      |      |
| The Minute phenotype of a deletion removing RpS28a and RpL32 is rescued by a RpL32 transgene [41] |        |       |      |      |
| Yes                         | M(3)99D | [41]  |      |      |
| RpS7                        | 99E2   |       |      |      |
| Lies in a gap in deletion coverage. 99E-F aneuploids were Minute [157] |        |       |      |      |
| Likely                      | 11     |      |      |      |
| RpL6                        | 100C7  |       |      |      |
| Lies in a gap in deletion coverage. 100C-F aneuploids were Minute [157] |        |       |      |      |
| Likely                      | 16     |      |      |      |

### Chromosome 4

| RpS3A | I01F1 | M | M | Yes | M(4)10l | [48] |
|-------|-------|---|---|-----|---------|-----|

- Bold font indicates the member of a duplicate gene pair that is expressed in a small number of tissues and/or at relatively low levels.
- Complete details are given in Additional data file 4.
- A blank indicates the absence of appropriate mutations or deletions.
- Mutations mapped molecularly to a single CRP gene.
- Deletions removing several genes, but only a single CRP gene.
- Judged according to evidence summarized in previous three columns and presented in detail in Additional data file 4.
- The maximum number of genes that could correspond to the Minute locus and CRP gene. Where no reference is given, the correspondence is shown for the first time in this study.

* Asterisks indicate new synonyms assigned in this study.
* Reference demonstrating definite correspondence between a Minute locus and CRP gene.
Minute loci defined in this study (65)

Genetically proven Minute loci (61)

Minute loci that correspond or are likely to correspond to CRP genes (60)

Minute loci for which a CRP gene is the likely candidate (34)

Minute loci that are not CRP genes (1)

Minute loci proven to correspond to CRP genes (26)

Minute loci that correspond or are likely to correspond to CRP genes (60)

Minute loci for which a CRP gene is the likely candidate (34)

Minute loci inferred from lack of deletion coverage (4)

CRP genes proven to be Minute genes (26)

CRP genes that likely correspond to proven Minute loci (36)

CRP genes in gaps in deletion coverage (4)

CRP genes that are likely to be Minute genes (40)

CRP genes that are or are likely to be Minute genes (66)

CRP genes in the D. melanogaster genome (88)

Figure 4 (see legend on next page)
these loci has been questioned previously and many cases appear to have involved chromosomal aberrations that were unusually complex or point mutations that were mismapped. Our failure to observe a Minute phenotype for deletions of S-adenosylmethionine synthetase (Sam-S), also known as M(2)21AB, is consistent with the phenotypic instability of dominant Sam-S mutations documented previously [100-102]. This suggests that mutations in Sam-S can phenocopy Minute mutations under certain conditions, but that Sam-S is not a typical Minute gene.

In summary, CRP genes are likely to correspond to all but one of the 65 Minute loci defined in this study, with the sole exception encoding a translation initiation factor subunit (Figure 4). No MRP or CRP-like genes are unequivocally associated with a Minute phenotype, indicating that the Minute syndrome is specifically related to the function of the cytoribosomes responsible for the majority of cellular protein translation, rather than the function of specialized cytoribosomal variants. Twenty-five percent of CRP genes are not associated with an obvious haploinsufficient phenotype, clearly reinforcing previous findings that not all CRP genes are Minute genes [61,95,104].

Discussion

CRP gene haploinsufficiency and the cytoribosome

When one examines the phenotypes of flies carrying chromosomal deletions, one is struck by the remarkable tolerance of Drosophila to aneuploidy: flies heterozygous for deletions of hundreds of kilobases of DNA usually have no obvious dominant phenotypes. For this reason, the haploinsufficiency of single genes is all the more remarkable. It is even more striking that the vast majority of these haploinsufficient genes encode proteins of the cytoribosome and that haploinsufficiency is not apparent for genes encoding components of equally elaborate cellular complexes, such as mitoribosomes or spliceosomes. What accounts for the exquisite dosage sensitivity of CRP genes?

The primary cause of CRP gene haploinsufficiency is reasonably clear: halving the copy number of a CRP gene results in reduced mRNA expression of that CRP [105]. Similarly, depleting CRP mRNAs through antisense- or RNA interference (RNAi)-mediated approaches can also produce Minute phenotypes [106,107] (SUM and SJL, unpublished data). As there appear to be no compensatory increases in transcription [105], reducing dosages of CRP genes must result in reduced CRP protein levels in the absence of dramatic changes in mRNA stability or CRP protein turnover. How then does the reduction in the level of a single CRP result in impaired cytoribosomal function and reduced general protein synthesis, and how is this manifested as the Minute phenotype?

One possibility, termed the 'balance hypothesis' [108,109], is linked to the multisubunit nature of the cytoribosome. It posits that an imbalance in the concentrations of CRPs results in the assembly of incomplete and non-functional ribosomal subunits. Indeed, it is known that depletion of individual CRPs in Saccharomyces cerevisiae causes inefficient ribosomal subunit assembly and/or function [110,111]. Nevertheless, the balance hypothesis also predicts that overexpression of individual CRPs should cause stoichiometric imbalances and phenotypes similar to those produced by underexpression. This prediction is not upheld in either S. cerevisiae [112] or D. melanogaster [113]. Consequently, imbalance per se cannot account for the haploinsufficiency of CRP genes.

A simpler explanation of CRP haploinsufficiency is that a high concentration of cytoribosomes is required for proper cellular functions and that the cytoribosome population decreases sharply when the level of a single CRP is reduced. Cytoribosomes and their components do appear to be required in unusually high quantities: CRP mRNAs are among the most abundant cellular transcripts both in yeast [114] and in flies [60], and can account for 50% of all RNA polymerase II-mediated transcription [89]. What seems critical, however, to the high-level production of fully formed cytoribosomes is that the concentration of each and every CRP never falls below a minimal level. In other words, cytoribosomal assembly is strictly limited by the availability of the least abundant component. This is probably not just a matter of simple self-assembly kinetics as improperly assembled ribosomal subunits and excess CRPs are actively degraded in yeast [111,115]. Similarly, RNAi-mediated depletion of single CRPs leads to the depletion of other CRPs in flies [64], suggesting the existence of similar degradation processes. Consequently, halving the supply of a single, limiting CRP is expected to halve the number of functional cytoribosomes. This may be tolerated by many cellular processes but will have severe effects wherever high protein synthesis is required, such as bristle formation and oocyte production in flies, or growth of S. cerevisiae in rich media [116]. It appears, therefore, that it is the combination of high demand for cytoribosomes and an assembly mechanism that assures that the level of the least abundant CRP determines the final concentration of cytoribosomes that makes CRP genes so exquisitely and specifically dosage sensitive. This perspective also provides a context for understanding the non-additivity of Minute mutations [117], where
Gene expression and dosage sensitivity

variants and extreme CRP deficiency may arise in different ways. Although 21 of the 27 CRP genes are haploinsufficient for suppression of neurocysticercosis in zebrafish [127]; and RPS19 haploinsufficiency is a causative factor of Diamond-Blackfan anemia in humans [128,129]. In fact, our reliance on the obvious bristle phenotype to distinguish Minute from non-Minute loci may present a biased assessment of CRP gene haploinsufficiency in the fly: it is quite possible that the 22 CRP genes classified as non-Minute in this study are associated with more subtle haploinsufficient phenotypes. How reduced CRP expression gives rise to diverse phenotypes is a mystery that, at least in part, reflects our current ignorance of

Regardless of the exact causes and mechanisms of haploinsufficiency, it is pertinent to ask why the majority of CRPs are expressed so close to the level of sufficiency, such that loss of one gene copy is debilitating, rather than being synthesized in excess? One possibility concerns economics: cytoribosomal synthesis is an incredibly costly affair [89] and excessive CRP production would both be wasteful and monopolize the limited resources of the cell. A second possibility is that CRP levels are normally constrained to guard against inappropriate activation of cell growth, proliferation or apoptosis - processes in which CRPs and cytoribosomes have been postulated to play direct roles [125]. A final and intriguing possibility is that the barely sufficient expression levels of some CRP genes may have evolved as a viral defense mechanism. Cherry et al. [64] found that reducing the levels of 64 of the 79 principal CRPs by RNAi inhibits the propagation of Drosophila C virus in Drosophila adults and cultured cells. Because this virus requires high concentrations of cytoribosomes in its host cell to undergo efficient translation, tightly controlled expression of CRP genes at levels just sufficient for normal growth and development may protect against viral infection and provide a selective advantage. Interestingly, we found a modest correlation between a CRP gene being a Minute and it being able to inhibit virus replication in this assay. Clearly, further work will be required to test whether there is truly a relationship between normal CRP gene expression levels and susceptibility to viruses.

Minute mutations attracted the attention of early geneticists because they were isolated so often in D. melanogaster. In fact, Schultz said in 1929, "...so many have been found that this mutant type is one of the most frequent in Drosophila" [117]. As a considerable number of Minute mutants have also been isolated in other Drosophila species [60], one might be justified in thinking Drosophila are unusually sensitive to CRP gene haploinsufficiency. On the other hand, the phenotypic consequences of CRP gene haploinsufficiency may simply be more noticeable in flies because they include conspicuous changes in external morphology. In fact, 'Minute-ness' may be a widespread phenomenon that is under-recognized because CRP gene haploinsufficiency has different and varied phenotypic consequences in other organisms. Recent research suggests this is the case [116,126-132]. For example, RPS5 haploinsufficiency disrupts cell division and causes developmental and growth phenotypes in Arabidopsis [126]; several CRP genes are haploinsufficient for suppression of nerve sheath tumors in zebrafish [127]; and RPS19 haploinsufficiency is a causative factor of Diamond-Blackfan anemia in humans [128,129]. In fact, our reliance on the obvious bristle phenotype to distinguish Minute from non-Minute loci may present a biased assessment of CRP gene haploinsufficiency in the fly: it is quite possible that the 22 CRP genes classified as non-Minute in this study are associated with more subtle haploinsufficient phenotypes. How reduced CRP expression gives rise to diverse phenotypes is a mystery that, at least in part, reflects our current ignorance of

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the regulation and roles of CRPs in different cell types. This is certainly a topic worthy of more research.

Conclusion
We have assessed an idea that has been discussed for more than three decades; namely, that the haploinsufficient Minute loci of Drosophila correspond to the genes encoding protein components of ribosomes [2,133]. Our results confirm this idea and add important details. We have shown that Minute genes encode proteins of cytoplasmic ribosomes and not mitochondrial ribosomes, and we have defined the subset of CRP genes that are haploinsufficient. While duplicate genes encoding tissue-specific CRPs are not associated with Minute CRP genes that are haploinsufficient. While duplicate genes encoding ribosomes, and we have defined the subset of CRP genes that are haploinsufficient. While duplicate genes encoding tissue-specific CRPs are not associated with Minute genes account for the vast majority of the haploinsufficient genes in the D. melanogaster genome with effects on fertility and viability strong enough to prevent the recovery of chromosomal deletions in the absence of corresponding duplications. Indeed, there are very few additional genes (for example, dpp [134], Abd-B [135]) or chromosomal regions (for example, Tpl [136], wupA [137], Fs(1)10A [138]) unequivocally associated with haplolethality or haplosterility. (A few other regions have been reported but not investigated in detail.) The most immediate practical use for our data will be in systematic efforts to maximize genome deletion coverage. Knowing which specific genes are haploinsufficient will make it feasible to flank each one as closely as possible with pairs of deletions, or to delete these genes in the presence of duplications or transgenic rescue constructs. Further improvements in deletion coverage will undoubtedly identify and map the remaining haplolethal or haplosterile loci.

Collectively, our inventories of the RP genes and Minute loci of D. melanogaster provide a solid foundation for further studies of RPs, ribosomes, and the causes and consequences of haploinsufficiency in flies and other organisms.

Materials and methods

Bioinformatics
RefSeq human RP sequences were obtained from the National Center for Biotechnology Information [139]. The FlyBase BLASTp service [140] was used to identify high scoring hits from among the annotated proteins of D. melanogaster; tBLASTn was used when orthologs were not identified by a BLASTp search. The ExPASy proteomics server [141] was used to compute the average pi and molecular weight of the RPs. The percentage identity between human and D. melanogaster RP sequences or between D. melanogaster RP pairs was calculated using the NPS@ ClustalW alignment tool at the Pôle Bioinformatique Lyonnais using default parameters [142,143]. \( K_d/K_u \) values were estimated using the program package PAML [144]. cDNA clone data were obtained from FlyBase [60].

The identification of CRP gene orthologs and the plotting of their evolutionary emergence (Figure 1) were achieved using a combinatorial approach. First, sequences corresponding to the relevant CRP genes from D. melanogaster and Homo sapiens were used as queries in BLASTn, BLASTp, tBLASTn and BLAT searches of the genomes of other Drosophilid and insect species using the FlyBase BLAST server [140] and the UCSC Genome Bioinformatics BLAT server [145,146]. High scoring matches were judged to be potential orthologs and were analyzed further using the FlyBase OrthoView tool [87]. Second, the coding sequences (CDS) of relevant CRP genes from D. melanogaster and H. sapiens were used as queries in BLASTn searches of the GLEAN CDS prediction sets of other Drosophilid species [140]; phylogenetic trees were then generated from the high scoring matches, with the CDS of S. cerevisiae CRPs as roots, using the ClustalW tools at EMBL-EBI [147]. Third, NCBI Homologene [148] was searched for any relevant homology calls: RpLIo-like, RpLPo-like, Rpl24-like were found in HomoloGene clusters 102903, 64526 and 9462, respectively. The results of all these analyses were then compared, with the most parsimonious interpretations being used to annotate the dendrogram shown in Figure 1.

Assessing Minute phenotypes and mapping Minute mutations
In order to compile a list of all genetically defined Minute loci, we first catalogued all the Minute loci described in the fly literature [2,54,55,60]. We then inspected deletions for all genomic regions having deletion coverage to confirm or refute the existence of these Minute loci and to identify any new Minute loci that had previously gone undetected. Minute phenotypes were scored primarily by visual inspection of bristle length, although body size, developmental timing, fertility and viability were considered when information was available. Deletion-bearing flies were outcrossed to Oregon-R or Canton-S wild-type strains whenever we could not unambiguously score Minute phenotypes in stocks. The phenotypic effects of deleting or disrupting X-linked genes were assessed only in heterozygous females. Finally, the cytological locations of all verified Minute loci were correlated with the positions of RP genes to identify candidate genes.

To assess RP gene haploinsufficiency directly, we inspected flies heterozygous for deletions and/or mutations of molecularly identified RP genes. Minute phenotypes were scored as described above. For some deletions that had not been characterized molecularly, it was necessary to refine the mapping of breakpoints with complementation tests against...
molecularly mapped mutations or with polytene chromosome preparations to determine whether RP genes were deleted. (In a few cases, RP genes were classified as lacking deletion coverage when the only existing deletions were not useful in a practical sense owing to their associated chromosomal rearrangements or extremely large size.) A transposable element insertion was judged to disrupt a RP gene if it failed to complement other mutations in the gene, if we saw a Minute phenotype in the insert strain, or if the transposon is inserted in the protein-coding region or 5' UTR of the gene based on FlyBase annotations [87] or our own BLAST analyses. (By these criteria, many nearby insertions, intronic insertions, and insertions in 3' UTRs were not used in our analysis.)

We included molecularly characterized point mutations in our analyses for the few RP genes where they were available. Molecularly uncharacterized Minute point mutations from the Bloomington Stock Center were complementation tested against mutations and deletions known to disrupt or delete specific RP genes.

Fly strains were obtained from the Bloomington, Szeged, Kyoto and Harvard Drosophila Stock Centers. Helene Doerflinger and Daniel St Johnston provided Df(3R)IR16 and Df(3R)MR22 stocks, and Yuri Sedkov and Alexander Mazo provided mRpL16A, mRpL16B and mRpL16C stocks.

Abbreviations
CDS, coding sequence; CRP, cytoplasmic ribosomal protein; MRP, mitochondrial ribosomal protein; RNAi, RNA interference; RP, ribosomal protein; rRNA, ribosomal RNA; snoRNA, small nuclear RNA; UTR, untranslated region.

Authors’ contributions
SJM, JR, GR, AL and KRC conceived, designed and performed the experiments. SJM, MA, PH, ZY and KRC analyzed the data. JR, GR and KRC contributed fly strains. MA, GM and NK helped with gene classification and nomenclature. TCK and SJL provided funds, lab space and general support. SJM and KRC wrote the paper.

Additional data files
The following additional data are available with the online version of this paper. Additional data file 1 is a table comparing the physical characteristics of D. melanogaster and human CRPs, together with their RefSeq accession numbers. Additional data file 2 is a similar table comparing D. melanogaster and human MRPs. Additional data file 3 is a table listing all the Minute loci in a historical context. Additional data file 4 is a table showing our comprehensive genetic analyses of ribosomal protein gene haploinsufficiency. Additional data file 5 is a table listing CRP gene-Minute locus correspondences arranged in alpha-numerical order by RP gene symbol. Additional data file 6 is a table showing our genetic analyses of translation factor gene haploinsufficiency.

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