COLD-SENSITIVE MUTANTS OF DROSOPHILA MELANOGASTER DEFECTIVE IN RIBOSOME ASSEMBLY

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ABSTRACT

Thirteen X-linked, cold-sensitive lethal, female-sterile mutants of Drosophila melanogaster located at eight separate loci were screened for their ability to assemble ribosomes at the restrictive temperature of 17°C. Females were labelled with 3H-uridine for either 2 or 20 hours at 17°C. A mitochondria-free extract was prepared and analyzed by means of sucrose gradient centrifugation. Four of the mutants, l(1)TW-ZCS, l(1)HM16C8, l(1)HM23CS, and l(1)HMZOC8, had a lower ratio of cpm in the 40S subunit to cpm in the 60S subunit (40S:60S ratio) than wild type with a 2-hour label. The same was true of a 20-hour label of l(1)TW-ZCS, l(1)HM16C8, and l(1)HM23CS, which are allelic, resulted in a 40S:60S ratio higher than wild type. Four other cs mutants were found to have less drastic effects on ribosome assembly. The ribosomal subunits of mutants l(1)HMlbsc and l(1)HM20c8 sediment at the same rate as their wild-type counterparts. The same is true for the RNA in their ribosomal particles. Sucrose gradient analysis of ribosomes from cold-sensitive lethal, female-sterile mutants appears to be an effective method for finding mutants that affect ribosome assembly.

THE study of ribosome assembly in eukaryotes has barely begun in comparison to the advances that have been made in bacteria (Nomura and Held 1974). However, investigation of this problem in higher organisms is of considerable interest because unlike in bacteria (Nomura 1973), ribosome synthesis in eukaryotes involves a large number of proteins which are a part of the developing ribosomal precursor particle but which are not found in the mature ribosome (Warner 1974). In addition, there is evidence that the complement of ribosomal proteins show both time-specific (Lambertsson 1974) and tissue-specific differences (DeLauany, Mathieu and Schapira 1972) during development. Little is known concerning the loci of the genes involved in eukaryotic ribosome assembly, the genes regulating that assembly, or the genes regulating the appearance of new ribosomal proteins at different times and in different tissues during development. The present study is concerned with the discovery and characterization of such genes in Drosophila melanogaster.

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To date reports on the identity of ribosomal protein genes in Drosophila melanogaster are few and either inconclusive, highly suggestive, or preliminary and incomplete. Steffenson (1973) presented some evidence which he suggested indicates that some ribosomal protein genes are clustered at the proximal end of the X chromosome adjacent to the nucleolar organizer region and the bobbed locus site of the ribosomal RNA genes in Drosophila (Ritossa, Atwood and Spiegelman 1966). Indeed the suppressor of forked gene is the last standard X chromosome gene adjacent to bobbed, and there is good, but not completely conclusive, evidence that it codes for a ribosomal protein (Finnerty et al. 1973; Dudrick, Wright and Brothers 1974). In an abstract Lambertsson and Rasmusson (1971) reported the existence of a dominant streptomycin-resistant mutant, str-R, on the X chromosome that produces streptomycin-resistant ribosomes and that binds approximately one-tenth as much radioactively labelled streptomycin. Finally, Berger and Weber (1974) have surveyed ribosomal proteins from isolated subunits of 18 strains of Drosophila melanogaster by means of one-dimensional electrophoresis and the proteins from the large subunit of six of these strains by two-dimensional electrophoresis. Evidence for an electrophoretic variant was found for only one small subunit protein from only one strain, Minute (3)40130, and the alleles responsible for this difference have yet to be localized.

Prokaryote cold-sensitive (cs) mutations have proven to be a rich source of mutant strains which are defective in ribosome assembly (Guthrie, Nashimoto and Nomura 1969; Tai, Kessler and Ingraham 1969). They have provided insights into the process of ribosome assembly in bacteria (Nomura 1973) and confirmed some features of in vitro ribosome assembly experiments. Studies in the fungi Aspergillus (Waldron and Roberts 1974), Neurospora (Schlitt and Russell 1974), and Saccharomyces (Bayliss and Ingraham 1974) have also shown that some of the eukaryotic cs lethal mutations are defective in ribosome assembly. Thus the existence of thirty-two, partially characterized, X-linked, cs lethal mutants in Drosophila melanogaster (Mayoh and Suzuki 1973; Wright 1973) represented a ready source of mutants which were potentially defective in ribosome assembly. In fact, Mayoh and Suzuki (1973) undertook the two-dimensional gel electrophoretic analysis of ribosomal proteins from five of these mutants in an unsuccessful attempt to establish differences in ribosomal proteins between these mutants and wild type.

The purpose of the work reported here (preliminary report by Falke and Wright 1974) was to screen some of these thirty-two, X-linked, cold-sensitive mutants in Drosophila melanogaster for their ability to assemble ribosomes properly, and thereby identify genes whose products function in ribosome assembly. The fact that a number of these thirty-two cs mutants have visible phenotypes, such as short, thin bristles or abnormal abdomens (Mayoh and Suzuki 1973; Wright 1973) that resembles the phenotype of bobbed flies known to be deficient in ribosomal DNA, suggested that some cs mutants might be defective in their ability to assemble ribosomes. Sucrose density gradient centrifugation analysis of ribosomes and ribosomal subunits isolated from females
injected with radioactive uridine at the restrictive temperature was used to screen the cs mutants for their ability to assemble ribosomes. It was thought that the use of this technique could expose not only mutants that cause the abnormal accumulation of ribosome precursor particles but could also reveal altered rates of subunit assembly. This technique was used by Guthrie, Nashimoto and Nomura (1969) in their successful screen of cs mutants in E. coli for mutants affecting ribosome assembly. In a further attempt to maximize the probability of identifying ribosome-assembly-defective mutants, the screen was limited to those cold-sensitive lethal mutants which produced female sterility at the restrictive temperature. It was reasoned that any female that could not assemble competent ribosomes at the restrictive temperature would be unable to produce eggs capable of supporting embryogenesis and therefore would be sterile when placed at the restrictive temperature. The fact that in the work reported here eight of the thirteen X-linked, cold-sensitive lethal, female-sterile mutants screened were found to affect ribosome assembly indicates that this is a highly effective screening method for identifying mutants that affect ribosomes in Drosophila melanogaster.

MATERIALS AND METHODS

Drosophila melanogaster strains: The wild-type stock used was an Oregon-R strain. All visible marker mutants and balancers used except FM7,yrid sc^u^s^n^c^s v B (Merriam and Duffy 1972) are described in Lindsley and Grell (1968). Cold-sensitive strains carrying a TW designation are from Wright (1973) and an HM designation from Mayoh and Suzuki (1973). For the sake of brevity all TW stocks will be referred to as TW-1cs, TW-2cs, etc. and all HM stocks as HM1cs, HM2cs, etc. The TW mutant loci reported in Table 1 are from Wright (1973), and the HM loci are from Mayoh and Suzuki (1973). In cases where a TW mutant was found to be an allele of an HM mutant the TW locus is given.

The female sterility at 17° and 25° reported in Table 1 was determined by crossing homozygous virgin cs/cs females raised at 25° to wild-type males at 25° and 17°. If progeny larvae appeared after two weeks at 17°, the parents were transferred to a fresh vial at 17°. A strain was considered fertile at 17° only if the first vial produced a large number of adult progeny and the second vial exhibited a great deal of larval activity, and sterile if no larvae developed at the restrictive temperature.

All fertile mutants were kept at 25° as homozygous stocks, but strains which were female-sterile at 25° (Table 1) were kept over the FM7 balancer chromosome. The cs/cs females arising from the cross of FM7/cs females to cs/Y males were used for injections.

Replacement of ethyl methanesulfonate (EMS)-treated chromosomes: Mutants TW-1cs, HM16cs, and HM20cs were crossed to multiply marked X chromosome stocks to replace most of the mutagen-treated chromosome around the mutant locus. The mutant HM16cs mutagen-treated chromosome has been replaced with an untreated X chromosome with the exception of some of the region from v (33.0) to g (44.4), HM20cs with the exception of some of the region from g (44.4) to car (62.5), and TW-1cs, except for the region from ptg (23.2) to v (33.0).

After replacement all three cs strains were still female sterile at 17°, and mutants HM20cs and TW-1cs were still completely lethal at 17°. Mutant HM20cs, previously a complete lethal at 17°, became incompletely penetrant permitting occasional escaper progeny to eclose, all of which had a few thin, short bristles. Mutant HM20cs, previously semi-sterile at 25° became completely fertile at the permissive temperature.

Complementation tests for female-sterility: Heterozygous cs a/cs b females were generated by crossing either cs a/cs a females (cs strain fertile at 25°) or cs a/FM4 females (cs strain female-sterile at 25°) to cs b/Y males at 25°. The progeny cs a/cs b females were collected as virgins
### TABLE 1

Genetic and biological properties of cs mutations

<table>
<thead>
<tr>
<th>Strain</th>
<th>Locus*</th>
<th>Leaky lethal at 17°</th>
<th>Fertility‡ at 17° &amp; 25°</th>
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* Units in centimorgans from the distal tip of the X chromosome.
† A strain was considered to be incompletely penetrant (leaky) for lethality if more than an occasional escaper cs male progeny was seen at 17°. See Results for details.
‡ S=sterile; F=fertile; sS=semi-sterile, only a few progeny emerged (criteria for sterility are in MATERIALS AND METHODS).
§ After Wright (1973) and Mayoh and Suzuki (1973), except for l(1)HM20cs.
¶ After Wright (1973).

which were immediately crossed to wild-type males at 17°. Since HM10cs/HM10cs females are semisterile when crossed to wild-type males at 17°, but completely sterile when crossed to HM10cs males, cs a/HM10cs females were crossed to HM10cs/Y males and the fertility of the heterozygous cs a/cs b females at 17° determined by the criteria already presented above.

Temperature-shift experiments: Eggs were collected over two-hour laying periods at 25° as described in SPARROW and WRIGHT (1974). Fifty eggs were placed in each 3/4-oz. creamer and 4 were creamers prepared for each shift. Creamers used in shift-down experiments were kept at 25° until the time of shift, when they were moved to a 17° incubator (16° for TW-fcs). Creamers used in the shift-up experiments were placed at 17° (16° for TW-fcs) immediately after the eggs were picked and at the appropriate time intervals were moved to a 25° incubator.
For each shift-point three creamers were shifted, and one was used to determine the developmental stage of the flies at the time of the shift. Creamers were scored for the number of white eggs (mostly unfertilized eggs but possibly including some very early lethal embryos), number of brown eggs (mortality during embryonic development), and the number of eggs which hatched (WRIGHT 1973). Creamers shifted up were scored at intervals until 21 days after the shift for the number of pupal cases and the number of adults hatched. Similarly, creamers shifted down were scored at varying intervals until 60 days after the shift. The calculations for percent mortality are from WRIGHT (1973).

The HMI6cs and HM20cs strains used in the shift experiments contained the X chromosomes in which most of the original EMS-treated X chromosome had been replaced with an untreated X chromosome. The TW-lcs strain had the original EMS-treated X chromosome.

Radioisotopes: The (5,6-3H)-uridine with a specific activity of 43 Curies/mmole and the (U-14C)-uridine with a specific activity of 512 mCi/mmole were purchased from Amersham/Searle.

Injections: All injected flies were raised in 1/2-pint culture bottles at 25° and were aged for 6 days before injection at 25°. Before injection at 17° or 29° flies were aged at 25° for 4 days and shifted to the respective temperature and aged for another 3 days.

Radioisotopes were desiccated by vacuum in a container with CaCl₂ and redissolved in Drosophila Ringers solution (EPRHUS and BEADLE 1936) to a concentration of 1µCi/µl for 3H-uridine and 0.2 µCi/µl for 14C-uridine. All females, injected in the abdomen with 0.2 µl of radioisotope (0.2 µCi/female with 3H-uridine and 0.04 µCi/female with 14C-uridine), were placed at the appropriate temperature for the times indicated in the text when they were collected and frozen at —70°.

When 3H-uridine alone was used in an experiment 25 females were injected and combined with 50 uninjected females of the same genotype. When double label experiments involving the centrifugation of ribosomal particles were performed, 13 wild-type females were injected with 14C-uridine and 25 females of the appropriate genotype were injected with 3H-uridine. The above plus 37 uninjected females of the latter genotype were combined and frozen at —70° after either 2 or 20 hours at 17°. In double label experiments involving RNA extraction, 25 wild-type females were injected with 14C-uridine and 75 females of the appropriate genotype were injected with 3H-uridine and frozen at —70° after either 2 or 20 hours at 17°.

Preparation of crude extracts: Extracts were prepared by a modification of the procedure of TRAVAGLINI and SCHULTZ (1972). Flies were weighed and placed into a mortar with extraction buffer: 50 mM Tris-HCl, 0.35 M sucrose, 25 mM KCl, 5 mM MgCl₂, and 1 mM dithiothreitol, pH 7.6 (10 ml extraction buffer/gm flies). The mixture was ground 100 times with a pestle and centrifuged through four layers of cheesecloth at 1,000 rpm for 1 minute in a SS-34 rotor in a Sorvall RC2-B centrifuge. Four layers of cheesecloth, 1-inch square, were prepared by presoaking them in extraction buffer in order to reduce absorption of the crude extract and were then pushed part way into a 10 mm × 75 mm test tube and centrifuged at 3,000 rpm for 5 minutes to wring them out. The cheesecloth was then pushed into a fresh test tube to form a shallow cup, which was then used to separate out the wings, legs, and assorted parts. The filtrate was then centrifuged at 2,000 rpm for 10 minutes in order to pellet the nuclei. The nucleus-free extract was centrifuged at 10,000 rpm for 20 minutes in order to pellet the mitochondria. The mitochondria-free, nucleus-free (cytoplasmic) extract was then combined with the nuclear pellet to form a mitochondria-free extract which was sonicated on ice with a Bronwill Biosonic IIA sonicator at a setting of 60 for three 15-second bursts with a needle tip with 30-second pauses between sonication bursts. The sonicated mitochondria-free extract was then centrifuged at 10,000 rpm for 20 minutes to remove debris. When a mitochondrial extract was desired, 0.5 ml of extraction buffer was added to the mitochondrial pellet which was then treated in the same manner as the mitochondria-free extract.

If the subsequent sucrose gradient was to be collected onto glass fiber filters the mitochondria-free extract was diluted 1:1 with extraction buffer before sucrose gradient centrifugation, but if collection was to be into a Triton X-100-based scintillation fluid, the mitochondria-free supernatant was passed through a 0.8 cm × 8 cm Sephadex G-25 column, and the sample eluted with extraction buffer minus the sucrose. This removed radioactive uridine which had not been incorporated into RNA.
RNA extraction: Bulk Drosophila RNA (126 μg) was added to each ribosomal sample and enough 10% sodium dodecyl sulfate (SDS) added to bring the SDS concentration to 1%. The sample cannot be left in this stage since extensive RNA degradation takes place even in the presence of SDS. Two and one-half volumes of a 53:50:1 mixture of phenol: chloroform: isoamyl alcohol were added to the sample, which was then extracted for 10 minutes on ice (Penman 1966). The sample was then centrifuged in a SS-34 rotor in a Sorvall RC2-B centrifuge, the aqueous phase drawn from the phenol, chloroform, isoamyl alcohol mixture and the RNA precipitated with 3 volumes of 0.1 M NaAcetate in 80% ethanol. The RNA was placed at −20° overnight and then centrifuged at 8,000 rpm for 10 minutes. The supernatant was discarded, the centrifuge tube drained for 30 minutes, and the RNA pellet dissolved in 0.3 ml of a solution of 0.05 M NaAcetate, 0.1 mM MgCl₂, and 0.1 M NaCl, pH 5.1.

Sucrose gradient centrifugation: Cell extracts (0.2 ml) were centrifuged on 15% to 30% (w/v) 11.2 ml linear sucrose gradients containing either 50 mM Tris-HCl, 50 mM KCl, 5 mM MgCl₂, and 1 mM dithiothreitol, pH 7.4 (Mg⁺⁺-sucrose gradients) or 10 mM Tris-HCl, 100 mM KCl, 1 mM dithiothreitol, pH 7.3 (Mg⁺⁺⁺-free sucrose gradients) (Berger 1972). In the former monosomes are stable, and in the latter they are unstable, falling apart into 40S and 60S subunit components. Centrifugation was carried out at 5°, 40,000 rpm in a Beckman SW-41 swinging bucket rotor usually for 220 minutes for Mg⁺⁺-gradients and 330 minutes for Mg⁺⁺⁺-free gradients in either a Beckman L2-65B or L3-40 ultracentrifuge.

RNA samples, 0.2 ml, were centrifuged for 410 minutes as above on 5% to 20% (w/v) linear sucrose gradients containing 50 mM NaAcetate, 10 mM NaCl, and 0.1 mM MgCl₂, pH 5.1.

Sucrose gradient fractionation: All sucrose gradients were passed through a flow cell in a Gilford recording spectrophotometer, recorded at 260 nm, and then fractionated with an ISCO Model 182 density gradient fractionator. The gradient was pushed through the spectrophotometer with a 40% (w/v) sucrose solution containing 0.025%, Blue Dextran 2000 which absorbs strongly at 260 nm and gives the peak found at the bottom of the sucrose gradient optical density traces.

When the mitochondria-free extract had been passed through a Sephadex G-25 column the sucrose gradient was fractionated into 30 0.4-ml samples to each of which 8 ml of the Triton X-100 scintillation fluid was added and after stirring poured into a scintillation vial and counted. Four liters of Triton X-100 scintillation fluid contained 320 ml H₂O, 1,200 ml Triton X-100, 400 mg 1,4-bis-(2-((4-methyl-5-phenyloxazolyl)) benzene (diMePOPOP), 16 gm 2,5-diphenyloxazole (PPO), and enough toluene to bring the volume to 4,000 ml.

When extracts had not been passed through Sephadex G-25, the sucrose gradient was collected directly onto Gelman Type E glass fiber filters which were numbered with India ink before use. After collection the filters were dried for 30 minutes at 70° and then treated by a modification of the method of Bollum (1968); ten 1-minute washes in cold 5% trichloroacetic acid (TCA) followed by ten 15-minute washes in cold water with stirring at 5-minute intervals. They were then dried and counted in a scintillation fluid containing 4 gm PPO, 0.05 gm diMePOPOP, and 1 liter of toluene.

RESULTS

Genetic and biological properties of cs mutations

Lethality, female fertility, and visible phenotypes: Because most of the cs strains were incompletely penetrant (leaky) for lethality at 17° the cs stocks were selected for lethality at 17° for up to 6 generations in some cases. Single non-virgin FM7/cs females were placed with FM7 males in vials at 17° and the resultant progeny scored. Ten vials were set per cs strain, and progeny from the vial producing the lowest frequency of escaper cs male progeny were used to set up 10 vials for the succeeding generation. A strain was categorized as leaky when more than a quarter of the vials at 17° had escaper males and also when the escaper males represented more than 4% of the total progeny in a
vial (25% if they were completely viable at 17°C) (Table 1). The results for individual leaky mutants were often variable. In strain \textit{HM22} for example, over a period of 5 generations the number of vials with cs escaper male progeny relative to the total number of vials which had progeny was 0 of 2, 6 of 6, 3 of 4, 3 of 6, and 3 of 10. Among those vials producing cs male progeny the average percent of escaper males was 0%, 29%, 22%, 7% and 5% respectively. Of 30 cs strains only 7 were completely penetrant (non-leaky), allowing virtually no escaper males to develop and eclose at 17°C (Table 1).

In this study only the effects of \textit{TW-2}, \textit{HM16} and \textit{HM20} were examined at 29°C, and all were viable, fertile, and exhibited mutant morphological phenotypes similar to those shown at 25°C.

Fourteen cs strains are either female-sterile or female-semi-sterile at 17°C (Table 1). The \textit{HM10} strain, however, is different since at both 17°C and 25°C \textit{HM10}/\textit{HM10} females mated to wild-type males produce \textit{HM10}/+ female progeny but no \textit{HM10}/Y hemizygous male progeny; but if \textit{HM10}/\textit{HM10} females are crossed to \textit{HM10}/Y males, no progeny are produced at all. On the other hand, at 17°C heterozygous \textit{HM10}/FM7 females mated to wild-type males produce significant numbers of \textit{HM10}/Y males. Our results above are different from the original observations of Mayoh and Suzuki (1973) and indicate that the mutant effects of \textit{HM10} have moderated so that now the presence of the wild-type allele during oogenesis in the female or during development in the zygote can alleviate the mutant effects.

Visible morphological phenotypes of the cs strains are given in Table 1. Four of the female-sterile strains have an abnormal abdomen phenotype and three of them have a short, fine bristle phenotype also seen in bobbed flies deficient for ribosomal DNA.

\textbf{Allelism of cs strains which are female-sterile at the restrictive temperature:} A determination of the allelism of the cs strains is important before one can discuss the number of cs loci which might be involved in ribosome synthesis. More of the cs strains are completely penetrant for female sterility at 17°C (13) than for lethality (7) (Table 1). Thus female sterility would appear to be a valid criterion to use in complementation tests. Since the approximate genetic locus of most of the cs mutants had been determined (Wright 1973; Mayoh and Suzuki 1973), only those strains that had been mapped relatively close to each other between the same mapping markers were tested against each other. However, since the locus of \textit{HM10} was unknown, it was tested against almost all of the cs female-sterile mutants.

On the basis of female sterility, mutants \textit{TW-2}, \textit{HM16}, and \textit{HM23} were found to be allelic at 41.8. Mutant \textit{TW-2} males at 17°C and \textit{HM16} flies at 25°C have short, thin bristles. When \textit{FM4}/\textit{16} females were crossed to \textit{TW-2} males at 17°C, \textit{HM16}/\textit{TW-2} female progeny were obtained in large numbers, all of which had short, thin bristles. Since \textit{TW-2} is a leaky lethal at 17°, the survival of \textit{HM16}/\textit{TW-2} heterozygotes is not surprising and the fact that these females had bobbed-like bristles is further evidence that \textit{HM16} and \textit{TW-2} are alleles. The \textit{HM16}/\textit{HM23} heterozygote is inviable at 17°C.
F. V. Falke and T. R. F. Wright

**Figure 1a.** Shift up of *l(1)TW-fcs*. Mortality expressed as percent of "fertilized" eggs (see Materials and Methods). Predominant stage of development at time of shift: E = embryonic. Percent mortality: T = total; E = embryonic; L = larval; P = pupal. Abscissa is the time that samples of creamers were shifted up to 25°C. Data for the ordinate were collected at intervals until 21 days after the shift.

**Figure 1b.** Shift down of *l(1)TW-fcs*. Mortality expressed as percent of "fertilized" eggs (see Materials and Methods). Predominant stage of development at the time of the shift: E = embryonic; 1L = 1st instar larvae; 2L = 2nd instar larvae; 3L = 3rd instar larvae; P = pupae prior to disk eversion; P0 = pupae after disk eversion-no eye pigment; Pb = pupae-brown eye pigment; P = pupae-red eye pigment. Percent mortality: T = total; E = embryonic; L = larval; P = pupal. Abscissa is the time that samples of creamers were shifted down to 16°C. Data for the ordinate were collected at intervals until 60 days after the shift.
Mayoh and Suzuki (1973) determined that mutants $HM5^{cs}$, $HM6^{cs}$, and $HM15^{cs}$ were alleles which mapped to the right of carnation (1-62.5). On the basis of female sterility, mutant $HM5^{cs}$ was found to be allelic with $TW-5^{cs}$ which had been mapped to a locus of 63.8 (Wright 1973).

In summary, of the 13 cs strains that are homozygous female-sterile at 17°, 6 ($TW-1^{cs}$, $HM1^{cs}$, $HM10^{cs}$, $HM13^{cs}$, $HM20^{cs}$, and $HM21^{cs}$) appear to be non-allelic mutations at separate loci, three ($TW-2^{cs}$, $HM16^{cs}$, $HM23^{cs}$) are alleles at 41.8, and four ($TW-5^{cs}$, $HM5^{cs}$, $HM6^{cs}$, and $HM15^{cs}$) are alleles at 63.8. This gives a maximum estimate of 8 separate loci for the strains tested. Since the results of intra-allelic complementation can be misinterpreted as being inter-allelic complementation, it is possible that some of the mutations reported here

![Graph 1](image1)

**Figure 2a.**—Shift up of $l(1)HM16^{cs}$. See Figure 1a legend for details.

![Graph 2](image2)

**Figure 2b.**—Shift down of $l(1)HM16^{cs}$. See Figure 1b legend for details.
as being non-allelic may actually be alleles, thus reducing the number of loci tested.

Temperature-sensitive period and lethal phase of 1(1)TW-1\textsuperscript{cs}, 1(1)HM16\textsuperscript{cs} and 1(1)HM20\textsuperscript{cs}: The effective lethal phase (ELP) for a conditional lethal is that time or stage during development when individuals exposed continuously to the restrictive temperature die. The beginning of the temperature-sensitive period (TSP) can be determined by collecting eggs at the permissive temperature, immediately placing them at the restrictive temperature, and then shifting samples of developing individuals back to the permissive temperature at progressively later stages in development. The beginning of the TSP is the initial time during development when a shift from the restrictive to the permissive temperature fails to rescue the organism from death. The end of the TSP is determined by shifting samples of individuals from the permissive to the restrictive temperature at progressively later stages in development. The end of the TSP is that time in development when a shift from the permissive to the restrictive temperature effects the rescue of a significant number of individuals (Suzuki 1970).

The ELP of TW-1\textsuperscript{cs} (Figure 1a, No Shift) is primarily embryonic with some larval mortality. The TSP for TW-1\textsuperscript{cs} extends from embryogenesis through development until after the imaginal discs evert during pupation (Figure 1a and 1b). A shift down during early embryogenesis results in embryonic death. If individuals are shifted down at any stage from late embryogenesis to the second larval instar, death occurs primarily during the larval stages. If third instar larvae are shifted down, they develop into pupae before dying. Pupae shifted down before the deposition of brown eye pigment die also as pupae.

The ELP of HM16\textsuperscript{cs} is embryonic (Figure 2a, No Shift). The beginning and end of the TSP in HM16\textsuperscript{cs} (Figures 2a and 2b) are similar to that found in TW-1\textsuperscript{cs}, but there is not as clear a demarcation of the end of the TSP since HM16\textsuperscript{cs} individuals do not develop synchronously.

The ELP of HM20\textsuperscript{cs} is primarily embryonic, with some larval and pupal mortality (Figure 3a, No Shift). The end of the TSP is late third instar–early pupa (Figure 3b), the beginning is during embryogenesis, and the lethal phases are similar to those for HM16\textsuperscript{cs}, although the results are not as clear-cut.

Sucrose density gradient analysis of ribosome synthesis

Time course of ribosome synthesis in wild type at 17°: Wild-type females maintained at 17° were injected with \textsuperscript{3}H-uridine, and the incorporation of the \textsuperscript{3}H-uridine into wild-type ribosome subunits and ribosomes was monitored from 0 to 20 hours after injection. There is an initial incorporation of label primarily into the ribosomal subunits (Figure 4b), followed by a detectable amount of radioactivity in the monosome peak at one hour. By two hours after injection (Figure 4d), the height of radioactivity in the 60S subunit peak is approximately equal to the height of the radioactivity in the 80S ribosome peak (this point is reached 3/4 of an hour after injection at both 25° and 29°). Increasing the label-
ling times resulted in a relative increase in the amount of radioactivity in the ribosome. By 20 hours the radioactivity sedimentation profile is similar to the optical density sedimentation profile. The slight peak in fraction number 24 of the 20-hour label is in the position of the disome. This is the highest polysome class that can be demonstrated with the techniques used in this paper. Therefore, the label seen in the monosome class comes from both free ribosomes and polysomal ribosomes.

For screening the cs mutants the two-hour labelling period was chosen for a short labelling time. This is a transition period and any mutant affecting ribosome assembly or the rate at which ribosomal subunits move into either polysomes or the monosome pool should be detectable by an alteration in the relative amount of radioactivity in the 60S and 80S particles. In addition, the use of a short labelling period might reveal a short-lived intermediate in ribosome biosynthesis. The 20-hour labelling period was chosen for a long labelling period since it appears to be close to a steady state and the accumulation of any precursor in ribosome biosynthesis should be detectable.
To rule out the possibility that a significant proportion of the radioactivity associated with the ribosomal subunits and the ribosomes might not be ribosomal in origin, RNA was extracted from the 40S, 60S, and 80S particles derived from females labelled for both 2 and 20 hours and examined by sucrose gradient centrifugation. The major peak(s) from the 40S subunit was 18S, from the 60S subunit 28S, and from the 80S monosome 18S and 28S for both the 2- (Figure 9d, 10a, and 10d) and 20-hour labelling period.

A mitochondrial extract of females labelled for 2 hours at 17º was examined on Mg++-sucrose gradients. Most of the radioactivity in both sucrose gradients was in the 80S ribosomal fractions. Since insect mitochondrial ribosomes sediment as 60S particles (KLEINOW, NEUPERT and BüCHER 1971), most of the radioactivity seen in the mitochondrial extracts came from cytoplasmic ribosomal contamination, and therefore mitochondrial ribosomes do not contribute significantly to the radioactivity sedimentation profiles seen in Figure 4.

Screen of cold-sensitive strains for mutants affecting ribosome assembly: Of the 14 cs female-sterile strains listed in Table 1, all but TW-6cs (known to induce very high frequencies of meiotic nondisjunction (WRIGHT 1974)) were put through the screen. In addition HM3cs, which is a good cs lethal, and HM24cs,
were also examined. In the screen for mutants affecting ribosome assembly, homozygous females of each strain maintained at 17° were monitored for the incorporation of radioactive uridine into ribosomal subunits and ribosomes 2 and 20 hours after injection at 17°. Mitochondria-free extracts were examined on both Mg++-sucrose gradients and Mg++-free sucrose gradients.

*Mg++-sucrose gradients of 2-hour labelled cs strains fractionated into Triton X-100.* With a 2-hour label of wild type at 17° the height of radioactivity in the 60S subunit peak is approximately equal to the height of radioactivity in the 80S monosome peak (Figure 5a). Of the 15 cs mutants screened, seven exhibited the 2-hour wild-type radioactivity sedimentation profile (Table 2). Four of the mutants were found to exhibit a 2-hour radioactivity profile which differs moderately from the wild-type 2-hour radioactivity profile. Typical of this class of mutants is *HM5cs* (Figure 5b). This sedimentation pattern is repeatable for all 4 cs strains, *TW-Jcs, HM21cs, HM5cs*, and *HM10cs*, grouped in this moderate class of mutants (Table 2). A second class of mutants comprising 4 strains was found in which the height of radioactivity in the 80S monosome peak and the height of radioactivity in the 40S subunit peak were severely depressed relative to the height of radioactivity in the 60S subunit peak (Table 2). A radioactivity

![Figure 5](https://example.com/f5.png)

**Figure 5.** Incorporation of ³H-uridine into wild type, *l(1)HM5cs*, and *l(1)HM20cs* ribosomes at 17°. Mg++-sucrose gradients fractionated into Triton X-100. In Figure 5a the 40S subunit peak is in fraction 8, the 60S subunit peak is in fraction 13, and the 80S monosome peak is in fraction 17. Optical density at 260 nm (---); ³H cpm (----).
TABLE 2

Summary of radioactive sedimentation profiles of cs strains, l(1)su(f)^ts67g, and bb

<table>
<thead>
<tr>
<th>Locus</th>
<th>Depression of the 80S monosome fraction relative to the 60 S subunit fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None* = Wild type*</td>
</tr>
<tr>
<td>0.6</td>
<td>HM1^cs</td>
</tr>
<tr>
<td>1.4</td>
<td>HM15^cs</td>
</tr>
<tr>
<td>26.3</td>
<td>HM3^cs(F)^§</td>
</tr>
<tr>
<td>34§</td>
<td>HM21^cs</td>
</tr>
<tr>
<td>33§</td>
<td></td>
</tr>
<tr>
<td>41.8</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td></td>
</tr>
<tr>
<td>63.8</td>
<td>TW-5^cs, HM6^cs, HM15^cs</td>
</tr>
<tr>
<td>65.9</td>
<td>&quot;(F)&quot;</td>
</tr>
<tr>
<td>66.0</td>
<td>bb</td>
</tr>
<tr>
<td></td>
<td>HM10^cs</td>
</tr>
<tr>
<td></td>
<td>HM24^cs(F)</td>
</tr>
</tbody>
</table>

* Height of 80S radioactive peak approximately equal to height of 60S radioactive peak (height = that fraction with the most cpm).
† Height of 80S radioactive peak depressed to approximately 80% of the height of 60S radioactive peak.
‡ Height of 80S radioactive peak depressed to less than 50% of the height of 60S radioactive peak.
§ Allelism not established.
¶ Female fertile mutants.

The results are from Mg++-sucrose gradients fractionated into Triton X-100 of cs strains and bb which had been labelled for 2 hours at 17°. Mutant l(1)su(f)^ts67g was labelled for 3 hours at 29°.

sedimentation profile of one of this class of mutants, HM20^cs, is presented in Figure 5c. It should be emphasized that while the 2-hour radioactivity sedimentation profiles of all 4 strains in this class are the same, mutant HM20^cs differed from the other cs strains in that it was the only one in which the optical density sedimentation profile differed from wild type (Figure 6) where it is readily apparent that there is an excess of free 60S subunits in the HM20^cs strain. The other strains in this class, TW-2^cs, HM16^cs, and HM23^cs, are all allelic to one another and have a wild-type optical density sedimentation profile. Thus the severely affected mutants are found at two different genetic loci and can be easily differentiated from each other on the basis of their optical density sedimentation profiles.

Mg++-sucrose gradients of 20-hour labelled cs strains fractionated into Triton X-100: Fourteen of the fifteen cs strains tested had 20-hour radioactivity sedimentation profiles which were similar to wild type. In Figure 5e the radioactivity sedimentation profile of a 20-hour label of mutant HM5^cs is presented. The profile is typical of the 14 cs mutants in this class, including TW-2^cs, HM16^cs and HM23^cs. The only mutant with a profile different from wild type is mutant HM20^cs (Figure 5f), which had an excess in the relative amount of the free 60S subunit.
Mg\(^{++}\)-free sucrose gradients of 2- and 20-hour labelled cs strains fractionated into Triton X-100: When centrifuged on Mg\(^{++}\)-free sucrose gradients there was no evidence that any of the cs strains accumulated intermediates in ribosome assembly analogous to those found in *E. coli* cs mutants by Guthrie, Nashimoto and Nomura (1969) and in yeast by Bayliss and Vinopal (1971). In some strains there did seem to be an alteration of the amount of radioactivity in the 40S subunit relative to the amount found in the 60S subunit (40S:60S ratio). Unfortunately the high background of radioactivity at the top of the sucrose gradient made it difficult to make accurate measurements of the amount of radioactivity incorporated into the two subunits. Prior to centrifugation the mitochondria-free extracts had been passed through a Sephadex G-25 column in order to remove the \(^3\)H-uridine which had not been incorporated into RNA or high molecular weight material, a procedure designed to permit the rapid collection of sucrose gradient fractions into scintillation vials with a Triton X-100-based scintillation fluid without the need for tedious procedures used in trichloroacetic acid (TCA) precipitation of RNA. While this procedure worked well with the Mg\(^{++}\)-gradients and did not interfere with the interpretation of results (see Figure 5), it left an undesirable amount of background radiation at the top of the
Mg\(^{++}\)-free gradients. Therefore some of those cs mutants which appeared to have altered 40S:60S ratios were examined again and the gradients fractionated onto glass fiber filters which were washed with TCA and water, thus removing background radiation at the top of the gradient. Other than these modifications, all of the cs strains reexamined were treated as before.

*Mg\(^{++}\)-free sucrose gradients fractionated into TCA*: The 40S:60S ratios of wild type and the cs strains are presented in Table 3. With 2- and 20-hour labels, mutant *HM20\(^{cs}\)* has a significantly lower 40S:60S ratio than wild type, as do mutants *TW-2\(^{cs}\)*, *HM16\(^{cs}\)*, and *HM23\(^{cs}\)* with a 2-hour label. However, when the latter three mutant alleles are left at 17\(^{\circ}\) for 20 hours, the 40S:60S ratio for *TW-2\(^{cs}\)* appears to be slightly higher than wild type, while for *HM16\(^{cs}\)* and *HM23\(^{cs}\)* the ratios are clearly greater than for wild type. The 40S:60S ratio for mutant *HM5\(^{cs}\)* is similar to wild type with a 2-hour label and slightly lower with a 20-hour label.

Forty-eight- and 72-hour labels of wild type, *HM16\(^{cs}\)*, and *HM20\(^{cs}\)* (Table 3) demonstrated that the high 40S:60S ratio seen in *HM20\(^{cs}\)* appear to be stable.

**Cosedimentation of wild-type ribosomes and subunits with *l(1)*HM16\(^{cs}\)* and *l(1)*HM20\(^{cs}\)* ribosomes and subunits**: Subtle differences between the sedimentation value of mutant and wild-type ribosomes and subunits would not have shown up in the experiments previously performed. Therefore wild-type females injected with \(^{34}\)C-uridine and labelled for 2 or 20 hours were combined with either

### Table 3

**The 40S:60S ratios of wild type and cs strains labelled at 17\(^{\circ}\) and 25\(^{\circ}\)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>17(^{\circ})</th>
<th>25(^{\circ})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-hour label</td>
<td>20-hour label</td>
</tr>
<tr>
<td>Wild type</td>
<td>.45</td>
<td>.42</td>
</tr>
<tr>
<td>*l(1)<em>HM20(^{cs})</em></td>
<td>.41</td>
<td>.41</td>
</tr>
<tr>
<td>*l(1)<em>TW-2(^{cs})</em></td>
<td>.22</td>
<td>.28</td>
</tr>
<tr>
<td>*l(1)<em>HM16(^{cs})</em></td>
<td>.22</td>
<td>.61</td>
</tr>
<tr>
<td></td>
<td>.17</td>
<td>.64</td>
</tr>
<tr>
<td></td>
<td>.23*</td>
<td>.66*</td>
</tr>
<tr>
<td></td>
<td>.26*</td>
<td>.63*</td>
</tr>
<tr>
<td>*l(1)<em>HM23(^{cs})</em></td>
<td>.25*</td>
<td>.65*</td>
</tr>
<tr>
<td>*l(1)<em>HM5(^{cs})</em></td>
<td>.43</td>
<td>.35</td>
</tr>
<tr>
<td><em>HM16(^{cs})</em> <em>HM20(^{cs})</em></td>
<td>.20</td>
<td>.39</td>
</tr>
</tbody>
</table>

The cpm's in the 40S subunit peak were totaled and divided by the cpm in the 60S subunit peak to give the 40S:60S ratios. All ratios were computed from Mg\(^{++}\)-free sucrose gradients fractionated into TCA except those marked with an asterisk that were from Mg\(^{++}\)-free sucrose gradients fractionated into Triton X-100.
wild type, *HM16<sup>cs</sup>*, or *HM20<sup>cs</sup>* females injected with *H*-uridine and labelled for 2 or 20 hours before preparing a mitochondrial-free extract. Extracts were examined on both Mg<sup>++</sup> and Mg<sup>++</sup>-free sucrose gradients fractionated into TCA. No difference in sedimentation value was found between wild-type and mutant ribosome and subunit sedimentation rates on Mg<sup>++</sup> gradients (Figure 7) or on Mg<sup>++</sup>-free gradients (Figure 8). The differences between wild-type and mutant 40S:60S ratios (Table 3) are reflected in Figure 8 where the ratio of mutant is less than wild type in Figures 8b, 8c, and 8f, and in 8e the long-term label of *HM16<sup>cs</sup>* has a higher ratio. These experiments were also done with the *HM16<sup>cs</sup>* and *HM20<sup>cs</sup>* strains in which most of the original EMS-treated X chromosome had been replaced, and gave results identical to those shown in Figures 7 and 8.

An unexpected result was the interaction between *HM20<sup>cs</sup>* and wild type so that the wild-type flies labelled for 2 hours now appeared to express the mutant phenotype (Figure 7c). The depression of radioactivity in the 40S and 60S particles of wild type relative to the radioactivity in the 60S subunit is not as great as that in *HM20<sup>cs</sup>*; although the effect is quite marked and was seen in each

---

**Figure 7.** Cosedimentation of mitochondria-free extracts on Mg<sup>++</sup>-sucrose gradients fractionated into TCA. Wild type was labelled with *14*C-uridine and combined with either *3*H-uridine labelled wild type (7a, 7d), *3*H-uridine labelled (*1*)HM16<sup>cs</sup> (7b, 7e), or *3*H-uridine labelled (*1*)HM20<sup>cs</sup> (7c, 7f). The 40S subunit peak is in fraction number 15, the 60S subunit peak is in fraction number 22, and the 80S ribosome peak is in fraction number 28. cpm *14*C (— — —); cpm *3*H (— — —).
of 4 gradients. The sedimentation profile of 2-hour labelled wild type in Figure 7b is wild type when cosedimented with HM16cs. However, in two of four gradients the wild-type profile exhibited depressed amounts of radioactivity in the 40S and 80S particles relative to that found in the 60S subunit.

Only some of the wild-type 80S monosomes are lost when wild type is cosedimented with HM16cs and HM20cs. The rest are apparently unaffected by the mutant supernatant, implying there may be two classes of ribosomes, and only one class is affected. HENSHAW, GUINEY and HIRSCH (1973) have shown that free monosomes are relatively inactive in protein synthesis as opposed to the ribosomes associated with polysomes. Perhaps it is one of these two classes which is affected by the mutant supernatant.

Co-sedimentation of wild type and 1(1)HM16cs and 1(1)HM20cs RNA: In order to determine whether or not the rRNA’s of mutant and wild-type ribosomal particles are the same, wild-type females were injected with 14C-uridine and wild type, HM16cs, and HM20cs females were injected with 3H-uridine. After a 2-hour labelling period at 17° the 3H-injected and the 14C-injected flies were combined and a mitochondria-free extract prepared. RNA was extracted from
FIGURE 9.—Cosedimentation of \(^{14}\text{C}-\text{uridine} \) 2-hour-labelled wild-type RNA combined with \(^{3}\text{H}-\text{uridine} \) 2-hour-labelled RNA isolated from either wild type, \(l(1)\text{HM16}^{cs} \), or \(l(1)\text{HM20}^{cs} \) mitochondria-free extracts (9a–9c) and 40S subunits (9d–9f). Gradients were fractionated into TCA. The 18S rRNA peak is in fraction number 21 and the 28S rRNA peak is in fraction number 33. cpm \(^{14}\text{C} \) (—); cpm \(^{3}\text{H} \) (--.--.--).}

an aliquot of the mitochondria-free extract. The rest of the supernatant was centrifuged on a Mg\(^{+} \)-sucrose gradient, the 40S, 60S, and 80S fractions were collected, RNA was extracted from these fractions and then analyzed by sucrose gradient centrifugation. The gradients were fractionated onto glass fiber filters and the RNA precipitated with TCA.

In no case (Figures 9 and 10) was any qualitative difference found between the sedimentation of wild-type and mutant rRNA. Quantitatively the gradients were almost superimposable, with the exception of a relative excess of some low molecular weight RNA from the \(\text{HM16}^{cs} \) mitochondria-free extract (Figure 9b) and the 80S monosome (Figure 10e). The significance of this excess RNA is unknown.

*Wild-type l(1)TW-2\(^{cs} \), l(1)HM16\(^{cs} \), and l(1)HM20\(^{cs} \) ribosome synthesis at 25\(^{\circ} \):* Since TW-2\(^{cs} \), HM16\(^{cs} \), and MH20\(^{cs} \) are viable and fertile at the permissive temperature of 25\(^{\circ} \), the synthesis of their ribosomes at the permissive temperature is of interest. Females were injected at 25\(^{\circ} \) with \(^{3}\text{H}-\text{uridine} \) for either \(3/4 \) or 7\(1/2\)
hours and mitochondria-free extracts were prepared and analyzed on both Mg++-sucrose gradients and Mg++-free sucrose gradients.

A comparison of \(3 / 4\)-hour labelled wild type (Figure 11a), TW-2cs (Figure 11d), and HM16cs (Figure 11c) radioactivity sedimentation profiles on Mg++ gradients show that there is a slight depression of radioactivity in the 80S monosome and 40S subunit relative to that in the 60S subunit of the cs mutants. Although significant, this depression is not as great as that found with a 2-hour label at 17° (see Figure 5b). Also on Mg++-free gradients at 25° the 40S:60S ratio for TW-2cs is lower than wild type with a \(3 / 4\)-hour label and higher with a 7½-hour label (Table 3). On the other hand, at 25° HM16cs does not differ from wild type on Mg++-free gradients with both \(3 / 4\)-hour and 7½-hour labelling times (Table 3). HM20cs sedimentation profiles differ from wild type, being similar to those obtained at 17° (Figures 5c, 5f, and 11b).

**Ribosome synthesis at 17° in the double mutant strain 1(1)HM16cs 1(1)HM20cs:** In order to demonstrate possible interactions between mutants HM16cs and HM20cs, the double mutant strain HM16cs HM20cs was labeled for 2 and 20 hours at 17° with \(^3\text{H}\)-uridine, and a mitochondria-free extract was
FIGURE 11.—Incorporation of $^3$H-uridine into wild type, l(1) TW-2cs, l(1) HM16cs, and l(1) HM20cs ribosomes in mitochondria-free extracts of flies labelled for 3/4 hour at 25°C. Mg$^{++}$-sucrose gradients fractionated into TCA. Optical density at 260 nm (---); cpm $^3$H (----).

prepared and was then centrifuged on both Mg$^{++}$- and Mg$^{++}$-free sucrose gradients.

The optical density sedimentation profile of the HM16cs HM20cs strain was found to be similar to that of mutant HM16cs in that there was no excess of free 60S ribosomal subunits as seen in HM20cs (Figure 6), whereas the radioactivity sedimentation profile of extracts of 2-hour labelled flies exhibited a decreased level of radioactivity in the 80S monosome relative to the 60S subunit. The decrease does not appear to be as severe as that seen in either HM16cs or HM20cs (Figures 7b and 7c), suggesting that there may be some interaction between the two cs gene products. The 20-hour radioactivity sedimentation profile was the same as that of HM16cs (see Figure 7e).

A two-hour label of HM16cs HM20cs resulted in a 40S:60S ratio similar to both parental strains (Table 3). A 20-hour label, which would have given a clear indication of which strain is epistatic, gives a 40S:60S ratio intermediate between both parental strains. Thus there is no clear-cut epistasis and both genes contribute to the final phenotype.

Ribosome synthesis in l(1)su(f)$^{ts67g}$: There is some evidence that su(f) may code for a ribosomal protein (FINNERTY et al. 1973). A temperature-sensitive allele, l(1)su(f)$^{ts67g}$, which is female-sterile at 29°C (DUDICK et al. 1974), made it possible to carry out an analogous time study of ribosome synthesis at 29°C for this mutant. Wild-type females and females from two mutant strains, car l(1)su(f)$^{ts67g}$ and f$^*$ l(1)su(f)$^{ts67g}$, were injected with $^3$H-uridine and placed at 29°C for either 3/4 hour or 7 1/2 hours. Mitochondria-free extracts of wild type and the two ts strains were made and centrifuged on both Mg$^{++}$-sucrose gradients
and Mg\(^{++}\)-free sucrose gradients. In all cases the mutant radioactivity sedimentation profiles did not differ from wild type and ribosome synthesis was normal within the limitations of the above analysis.

*Ribosome synthesis in a bobbed mutant:* Since the bobbed gene is deficient in ribosomal DNA (Rtrossa, Atwood and Spiegelman 1966), the synthesis of ribosomes in a bobbed mutant at 17\(^\circ\)C was of interest. A \(y^{2} b b^{s}\) strain was labelled for 2 and 20 hours at 17\(^\circ\)C in the same manner as the cs strains tested in the screen. All gradients exhibited wild-type radioactivity and optical density sedimentation profiles.

**DISCUSSION**

*Sucrose gradient analysis of cold-sensitive lethal, female-sterile mutants*

40:60 ratios: The screen of the cs mutants revealed four categories of 40S:60S-ratios (Table 3): (1) a 40S:60S ratio lower than wild type with both a 2-hour and a 20-hour label at the restrictive temperature (HM20\(^{cs}\)). (2) A 40S:60S ratio lower than wild type with a 2-hour label and higher than wild type with a 20-hour label at the restrictive temperature (the alleles TW-2\(^{cs}\), HM16\(^{cs}\), and HM23\(^{cs}\)). (3) A wild-type 40S:60S ratio with a 2-hour label and a ratio lower than wild type with a 20-hour label at the restrictive temperature (HM5\(^{cs}\)). (4) A 40S:60S ratio that was either wild type or not sufficiently different from wild type to be detected by the methods used in the screen (TW-1\(^{cs}\), TW-5\(^{cs}\), HM1\(^{cs}\), HM6\(^{cs}\), HM10\(^{cs}\), HM13\(^{cs}\), HM15\(^{cs}\), HM21\(^{cs}\) and HM24\(^{cs}\)).

*Category 1:* The altered 40S:60S ratios observed with HM20\(^{cs}\) (lower than wild type with a 2- and 20-hour label) cannot be explained by different degradation rates for the two subunits. Since there is little incorporation of radioactive uridine into wild type and mutant ribosomes at 20 hours at 17\(^\circ\)C (relative to the amount already present, see Figure 4f), the 20-hour labeling period serves as a rough pulse-chase experiment. In HM20\(^{cs}\) if the half life of the 40S subunit were less than that of the 60S subunit, then the 20-hour 40S:60S ratio should be lower than the 2-hour label. Actually the ratios are quite comparable (Table 3). On the other hand, a possible explanation of these altered 40S:60S ratios is that a 40S subunit protein has been mutated in the HM20\(^{cs}\) strain so that at 17\(^\circ\)C 40S subunits are made at a reduced rate compared to 60S subunits.

*Category 2:* The observation that the 40S:60S ratios of the alleles TW-2\(^{cs}\), HM16\(^{cs}\), and HM23\(^{cs}\) change from less than wild type with a 2-hour label to higher than wild type with a 20-hour label argues against a hypothesis that the mutant ribosomal subunits have different half lives. If the 40S subunit were less stable than the 60S subunit, which would be predicted from the low ratio obtained with a 2-hour label, then one would expect the ratio with a 20-hour label to be still lower than wild type. It is possible that the 40S subunit is made at a lower rate than the 60S subunit due to preferential degradation of nascent 40S subunits, and that either the cs 60S subunit is more labile than the wild-type 60S subunit, or that although it is synthesized more slowly, once it is formed the mutant 40S subunit is more stable than the wild-type 40S subunit.
The relatively low amount of radioactivity incorporated into the 80S monosome with a 2-hour label of TW-2cs, HM16cs, HM23cs, and HM20cs at 17° (Figure 7b and 7c) could be due to reduced amounts of the 40S subunit available. Since the radioactivity in the 2-hour labelled 80S monosome comes from rRNA in both the 40S and 60S subunit (Figure 10d, 10e, and 10f), a decrease in the amount of radioactivity in the 40S subunit will result in a decrease in the amount of radioactivity in the monosome.

Category 3: The modification of 40S:60S ratios, from a wild-type ratio with a 2-hour labelling period to lower than wild type with a 20-hour labelling period in the moderate mutant HM5cs, could be explained most simply if the 40S subunit were less stable than the 60S subunit. The decrease of radioactivity in the 80S monosome of the 2-hour labelled moderate mutants, TW-1cs, HM5cs, HM10cs, and HM21cs (Table 2) could be due to a number of causes. For example the rate of initiation could be lower than wild type due to a defect in one of the ribosomal subunits or initiation factors resulting in an excess of radioactivity in the ribosomal subunits relative to that in the ribosomes. In the case of HM5cs this could also lead to a destabilization of the 40S subunit.

Two other studies of cold-sensitive mutants found altered subunit ratios. WALDRON and ROBERTS (1974) found that of 69 cs mutants in Aspergillus nidulans 4 located at 3 loci had 40S:60S ratios higher than wild type. These mutants most closely resemble HM16cs. SCHLITT and RUSSELL (1974) found that one of 14 cs mutants of Neurospora crassa had an altered 40S:60S ratio of 0.14 as compared with a wild-type ratio of 0.43. The N. crassa mutant was labelled for 48 hours at the restrictive temperature of 10° and would probably correspond to our long-term label of 20 hours. This mutant most closely resembles mutant HM20cs, which has a low 40S:60S ratio with a 20-hour label at 17°.

In a temperature-sensitive line of hamster BHK21 cells TONIOLO, MEISS and BASILICO (1973) found that at the restrictive temperature the mutant had a block in the processing of 32S precursor rRNA into 28S rRNA while the synthesis of the 18S rRNA was almost normal. This would have led to a high 40S:60S ratio.

In a number of other studies a variety of experimental manipulations have resulted in changes in 40S:60S ratios. These include the administration of β-ecdysone to Drosophila imaginal discs in vitro (PETRI et al. 1971), the maturation of ribosomal subunits in HeLa cells starved for various amino acids (MADEN 1969), and the comparison of 40S:60S ratios in growing vs. resting 3T3 and 3T6 cells (ABELSON et al. 1974) and regenerating vs. non-regenerating rat liver (TSURIGI, MORITA and OGATA 1974). In addition, MADEN and VAUGHAN (1968) have evidence that a decrease in the amount of some of the 40S subunit proteins relative to the amount of 60S proteins can result in a decreased 40S:60S ratio. This observation may have some bearing on how the cs genes cause altered 40S:60S ratios.

Accumulation of precursor ribonucleoprotein particles in E. coli (GUTHRIE, NASHIMOTO and NOMURA 1969) and Salmonella typhimurium (TAI, KESSLER and INGRAHAM 1969) most of the cold-sensitive ribosome assembly mutants
accumulated intermediate particles in ribosome assembly. The accumulation of a 28S particle in a cold-sensitive strain of yeast (Bayliss and Vinopal 1971; Bayliss and Ingraham 1974) is one case in which a cold-sensitive mutant in eukaryotes accumulates a ribonucleoprotein particle other than the apparently normal ribosomal subunits. Such particles are not found in Neurospora (Schlitt and Russell 1974) or Aspergillus (Waldron and Roberts 1974). No such particle was observed in any of the Drosophila melanogaster cs mutants screened and the sedimentation values of wild type and HM16cs and HM20cs ribosomal particles were the same (Figures 7 and 8). RNA extracted from the 40S, 60S, and 80S particles of HM16cs or HM20cs was also found to have the same sedimentation value as wild type (Figures 9 and 10). Either the nucleolus was not broken up in the extraction procedure, intermediate particles in ribosome synthesis were rapidly degraded, or intermediate particles do not accumulate in the cs mutants examined.

Screen for ribosome assembly mutants

Number of mutant loci defective in ribosome assembly: Of 13 cold-sensitive lethal, female-sterile mutants screened by means of sucrose gradient centrifugation, 8 mutants located at 6 different loci were found to be defective in ribosome assembly (Table 2). Mutants in two of the loci resulted in a severe effect on ribosome assembly and are located at 1-41.8 with three alleles (TW-2cs, HM16cs, and HM23cs), and at 1-57 with one allele (HM20cs). Mutants at the other 4 loci (TW-1cs, HM5cs, and HM21cs) produced a moderate effect on ribosome assembly (Table 2). Even though mutant HM5cs is allelic to TW-5cs, HM6cs, and HM15cs, the last 3 alleles appeared to be wild type for ribosome assembly in the screen.

From the above data one can ask the questions: (1) Is the X chromosome saturated with cold-sensitive lethal, female-sterile mutant loci? (2) Are the two loci with three and four alleles mutational hot spots? If one assumes that the mutational events are random processes and have equal probabilities of happening, they should follow a Poisson distribution. Unfortunately there are not enough cs strains to do a statistically valid chi-square test on a Poisson distribution or to compute a statistically valid Poisson distribution. One can proceed, however, and generate an expected Poisson distribution in order to obtain a rough idea of what the results should have been (Table 4). From the table it appears that there could be an excess of the observed number of 3 and 4 alleles per locus class, especially when one considers the deficit in the 2-allele-per-locus class. It may be that the loci at 41.8 (3 alleles per locus) and 63.8 (4 alleles per locus) are particularly susceptible to mutation to cold-sensitive lethal, female-sterility alleles, i.e. hot spots. If one disregards the 3 and 4 class because they may be hot spots, one is left with only the 1 class. This, plus the lack of any loci in the 2 class, implies that there may be a relatively large 0 class, indicating that the X chromosome probability has not been saturated by the cold-sensitive lethal, female-sterile loci found to date.

Are defects in ribosome assembly generalized, secondary pleiotropic effects of female-sterile mutants? Since so many cs mutants (6 out of 8 loci) which alter
RIBOSOME ASSEMBLY IN DROSOPHILA

TABLE 4

Number of cold-sensitive lethal, female-sterile alleles per locus

<table>
<thead>
<tr>
<th>Number of alleles per locus</th>
<th>Observed</th>
<th>Expected*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>4.2</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>4.5</td>
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<tr>
<td>2</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>.8</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>.2</td>
</tr>
</tbody>
</table>

* Expected distribution of alleles per locus calculated by means of a truncated mean which permits a calculation of the number expected in an unobserved 0 class (COHEN 1960). The expected frequencies were calculated so that the number of loci in the 1 class through the 4 class would equal 8 and thus be comparable to the observed frequencies.

Ribosome assembly were found, an obvious question arises: Are the defects in ribosome assembly that have been found generalized, secondary pleiotropic effects of female-sterile mutants or are they fairly direct phenotypes produced by the mutant genes that are specifically concerned with ribosome assembly and/or function?

The selection of mutants to be screened was designed to ensure as high a rate of recovery of mutants affecting ribosome assembly as possible. Not only were cold-sensitive mutants picked, which yield a high frequency of ribosome-assembly-defective mutants in bacteria (GUTHRIE, NASHIMOTO and NOMURA 1969), but the further constraint of cold-sensitive female sterility was placed on the selection process. The reasoning was that females defective in ribosome assembly would not be able to lay fertile eggs at the restrictive temperature. Indeed ribosome assembly in the two female-fertile strains examined, HM3cs and HM24cs, appeared to be wild type.

It is unlikely that the results are due to secondary effects of female-sterile mutants since some of the strains, TW-1cs, HM16cs, HM23cs, and HM20cs, are almost completely lethal and die during development at 17° in addition to being female-sterile. Another indication that the mutants are due to some defect in ribosomes is the visible phenotype of some of the strains. Short thin bristles is a phenotype characteristic of bobbed mutants which are deficient in the amount of ribosomal DNA they contain (RITOSSA, ATWOOD and SPIEGELMAN 1966) and has nothing to do with female sterility. Bobbed-like phenotypes have been observed in the following strains: TW-2cs escapers at 17°, HM16cs flies at 25°, HM20cs escapers at 17°, and HM20cs flies at 25°.

In addition there are a number of facts which are consistent with the hypothesis that the mutants are defective in ribosome assembly or efficiency of translocation. CHURCH and ROBERTSON (1966) found that D. melanogaster accumulates vast amounts of RNA during larval growth and that this accumulation ceases during pupation. Most of this accumulation is ribosomal RNA (Hollings 1972). The temperature-sensitive period (TSP) for strains TW-1cs, HM16cs, and HM20cs extends from embryogenesis to mid-pupation (Figures 1, 2, and 3). Since most
of the ribosomes in the organism are synthesized prior to pupation, one would predict that the end of the TSP for a cold-sensitive ribosome-assembly-defective mutant would be during pupation.

Another argument against the results being due to a generalized secondary effect of female-sterile mutants is the observation that $HM16^{cs}$ and $HM20^{cs}$ are very different mutants. While they both have low 40S:60S ratios with a 2-hour label at $17^\circ$, the ratio for $HM20^{cs}$ with a 20-hour label is lower than wild type, in contrast to $HM16^{cs}$ which has a higher ratio than wild type with a 20-hour label.

For reasons discussed above it is unlikely that differential rates of decay of the two subunits are the cause of the differences observed between strains $HM16^{cs}$ or $HM20^{cs}$ and wild type. Another argument against decays being responsible for the differences is the appearance of the 20-hour radioactivity sedimentation profile of flies labelled at $17^\circ$. In all cases except $HM20^{cs}$ the cs profile is the same as wild type, i.e. the vast majority of the label is in the monosomes. In order for ribosomal subunits to form monosomes they must first appear in the polysomes (GIRARD et al. 1965; MADEN 1971). Active polysomes do not seem indicative of a degenerative system. Even in mutant $HM20^{cs}$ the majority of label is in the monosome.

In conclusion, $HM16^{cs}$ ($TW-2^{cs}$ and $HM23^{cs}$) and $HM20^{cs}$ affect ribosome assembly and are probably ribosome-specific mutants. The moderate effect of $TW-1^{cs}$, $HM5^{cs}$, $HM10^{cs}$, and $HM21^{cs}$ on ribosome assembly could be due to generalized, secondary pleiotropic effects or could be due to secondary effects of ribosome-specific mutants which primarily affect ribosome function.

We would like to thank HELEN MAYOH and DAVID T. SUZUKI for making available to us all their X-linked, cold-sensitive lethal mutations.

**LITERATURE CITED**


RIBOSOME ASSEMBLY IN DROSOPHILA


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