SEX-LINKED, RECESSIVE, COLD-SENSITIVE MUTANTS OF *DROSOPHILA MELANOGASTER*: GENETIC AND BIOCHEMICAL STUDIES.

by

HELEN MARGARET MAYOH

B.A., University of British Columbia, 1953
M.A., University of Toronto, 1960
Candidate in Philosophy, University of California, Berkeley, 1969

B.L.S., University of Alberta, 1973

A THESIS SUBMITTED IN PARTIAL FULFILMENT

OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

in the Genetics Division

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September - 1973

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Zoology (Genetics Group)

The University of British Columbia
Vancouver 8, Canada

Date Nov 14, 1973

ABSTRACT

Cold-sensitive mutants of E. coli have been valuable in the study of the structure and assembly of bacterial ribosomes. Some cold-sensitive mutations of E. coli have been mapped to loci coding for ribosomal proteins. Other cold-sensitive mutations have been shown to alter the regulatory properties of bacterial enzymes.

In contrast, little was known about the genetics and biochemistry of cold-sensitive mutants of Drosophila and other eukaryotes. Also, the genetic loci of ribosomal proteins of D. melanogaster were unknown although the ribosomal RNA genes have been located on the X and 2nd chromosomes. Therefore the following questions were asked: Can cold-sensitive lethals of the X chromosomes of Drosophila melanogaster be isolated? If so, what are their genetic properties? Do some have altered ribosomal proteins as in cold-sensitive mutants of bacteria? The study was part of a general search for and characterization of cold-sensitive lethals of all the chromosomes of D. melanogaster.

Among 3,919 EMS-treated X chromosomes, 25 were retained as cold-sensitive lethals or semi-lethals. That is, more than 20% of the flies carrying a cold-sensitive lethal survived at 22° C and none at 17° C, and for cold-sensitive semi-lethals, $>30\%$ survived at 22° C and $<13\%$ at 17° C. The cold-sensitive mutations were not randomly distributed,

7 being located at the X tip and 3 being alleles to the right of car. Over half exhibited female sterility at the permissive temperature and 7 exhibited visible phenotypes characteristic of bobbed and Minute mutations.

As the presence of altered ribosomal proteins has been demonstrated in cold-sensitive mutants of bacteria, the ribosomal proteins from Drosophila mutants and controls were studied by 2-dimensional gel electrophoresis. By this method, it was estimated that 69 - 72 proteins are present in the ribosomes of Drosophila. No qualitative differences were observed in the gel patterns of ribosomal proteins from 5 cold-sensitive, sex-linked mutants and the controls.

A summary of the biochemical testing of the ribosomes from other cold-sensitive mutants of Drosophila is also given. No biochemical evidence to support the hypothesis that cold-sensitive mutants of Drosophila have altered ribosomes was obtained. The limitations of the study and recommendations for future research are discussed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES cont.

1. INTRODUCTION: Cold-Sensitive Mutants

Aim of research: In the present study, sex-linked cold-sensitive mutants of Drosophila were isolated, characterized genetically and examined biochemically for ribosomal alterations. The research was undertaken because it was thought that the selection of cold-sensitive mutants of Drosophila might enhance the recovery of mutations affecting ribosomal proteins as is the case in E. coli (Nomura, 1970). These mutants would be invaluable in answering questions such as: Are the genes for ribosomal proteins clustered near the genes for ribosomal RNA on the X chromosome or scattered throughout the genome of D. melanogaster? Are ribosomes chemically constant throughout the life cycle of Drosophila or are different ribosomal genes transcribed during larval and adult periods? Is the temperature-sensitive period of Drosophila temperature-sensitive mutants related to the initial synthesis of the defective gene product or to a critical developmental event in which the product is required (Tarasoff and Suzuki, 1970)? Are the assemblies of the small and large ribosomal subunits interdependent in Drosophila? Thus problems examined in bacterial studies and also new ones related to the complexities of a higher organism could be investigated with ribosomal mutants.

Cold-sensitive mutants of microorganisms: Cold-sensitive mutants are defined by their viability at "normal" temperatures and inviability at low temperatures. Two types of molecular alterations have been established so far for cold-sensitive mutants of bacteria: loss of allosteric control of enzymes and defective assembly of ribosomes. Several cold-sensitive auxotrophs of E. coli were found to have changes in feedback control of metabolic pathways rather than losses in enzyme activities (O'Donovan and Ingraham, 1965). Other cold-sensitive mutants of bacteria fail to assemble 30S and 50S ribosomal subunits at low temperature (Davies and Nomura, 1972). These are called Sad or subunit assembly defective mutants and some have been studied intensively to elucidate the in vivo assembly of bacterial ribosomes. The molecular defects of other cold-sensitive mutants have not been so thoroughly investigated. Cold-sensitive mutations of viruses, for example, were shown to map in a limited number of genes and to affect viral development but few biochemical tests of the mutants have been done (Cox and Strack, 1971; Scotti, 1968; Dowell, 1967).

Defective feedback control of enzymes of cold-sensitive bacterial mutants: A cold-sensitive mutant of E. coli was shown to have a histidine enzyme with altered regulatory properties (O'Donovan and Ingraham, 1965). The mutant grew well in glucose-salts medium at 37° C but not at 20° C. It required histidine only at the low

temperature. When extracts of the mutant and the parental strain were tested for phosphoribosyl - ATP pyrophosphorylase activity, it was found that mutant grown at 37° C gave values similar to those for wild-type but mutant grown at low temperature had no detectable enzyme activity. This enzyme occurs early in the histidine pathway and normally excess production of histidine is prevented by inhibition of the pyrophosphorylase by the end-product of the pathway. Enzyme from the mutant, however, was found to be 1000 - fold more sensitive to inhibition by histidine than the wild-type enzyme, and bound the inhibitor irreversibly. Both the mutant and the wild-type enzyme were more sensitive to feedback inhibition at 20° C than at 37° C. Apparently the increased sensitivity of the mutant enzyme at low temperature was sufficient to result in a histidine requirement at low temperature.

Mutants resistant to thiazolealanine were derived from the cold-sensitive stock. This analogue of histidine is known to cause feedback inhibition of phosphoribosyl- ATP pyrophosphorylase but cannot substitute for histidine in protein synthesis (Martin, 1963; Sheppard, 1964). Resistance to the analogue alters the regulatory site of the enzyme and not the catalytic site. As predicted if the cold-sensitive mutant had an altered regulatory site, resistance to thiazolealanine abolished the requirement for histidine at the low temperature and the extreme sensitivity

to histidine inhibition (O'Donovan and Ingraham, 1965). It was also shown that derivatives of the cold-sensitive stock selected for prototrophy at low temperature lost the extreme sensitivity to feedback inhibiton.

Increased feedback inhibition seems to occur frequently in cold-sensitive auxotrophs of bacteria. Out of 6 independently isolated cold-sensitive mutants which required histidine at low temperature, all produced phosphoribosyl - ATP pyrophosphorylase that was more sensitive to inhibition by histidine than the parental enzyme (O'Donovan and Ingraham, 1965). Furthermore, 15 cold-sensitive, arginine - requiring mutants were blocked in the first enzyme of the arginine pathway.

It is thought that altered feedback inhibition in the cold-sensitive mutants is due to conformational changes in allosteric enzymes (O'Donovan, et al., 1965). Since repressors also appear to be multimeric, allosteric proteins with regulatory sites, it is predicted that repression and induction will be altered in some cold-sensitive mutants of bacteria.

The structure and in vitro assembly of E. coli ribosomes: Before discussing the Sad mutants in detail, it may be useful to outline the biochemistry of bacterial ribosomes (Kurland, 1972; Davies and Nomura, 1972). They are complex 70S particles which consist of two subunits, the 30S and the 50S subunits. The small subunit of E. coli

contains one 16S RNA molecule and 20-21 unique proteins whereas the large subunit contains one 5S RNA molecule, one 23S RNA molecule, and 27-34 unique proteins. The 2 subunits of E. coli have no proteins in common. The ribosomal RNA species found in the ribosomal subunits are derived from larger precursor forms. The extra oligonucleotides are cleaved during ribosome assembly.

The in vitro reconstitution of 30S ribosomal subunits occurs spontaneously when 16S RNA and ribosomal proteins from 30S subunits of E. coli are mixed under specific ionic conditions at temperatures between 20 and 40° C (Traub and Nomura, 1969). Such reassembled subunits are biologically active in an in vitro protein synthesizing system. At 10° C or less, intermediate 21S particles of wild type subunits accumulate. Heat is required for a structural rearrangement of the intermediate before the remaining ribosomal proteins attach. Thus, reconstitution of 30S ribosomal subunits occurs more readily at high temperatures than at low.

The in vitro reconstitution of 50S subunits from 23S RNA, 5S RNA and 50S ribosomal proteins also involves intermediate ribonucleoprotein particles and does not proceed to completion at low temperatures (Nomura and Erdmann, 1970; Nomura, 1972). Reconstitution studies of enzymes and other multimeric proteins from wild type organisms have demonstrated that the assembly process is cold-sensitive (Van Holde, 1966; Valentine et al, 1966).

Cold-sensitive mutations may, therefore, be regarded as defects which exaggerate a normal reaction toward cold. Cold-sensitive mutations may be specific in the sense that they affect conformational changes of proteins and protein interactions within specific aggregates.

Further studies on the reconstitution of 30S subunits have shown that the process is sequential and cooperative in character (Mizushima and Nomura, 1970). Only certain of the proteins combine directly with 16S RNA. Some proteins only bind if other proteins are already attached and so on. Sometimes binding is improved if two proteins are present simultaneously. Such in vitro studies imply that the information for correct assembly is contained in the structure of the components themselves; i.e. no templates or auxillary enzymes are needed.

Cold-sensitive mutants with ribosomal defects: At low temperatures, the Sad mutants of bacteria grew poorly and accumulated various ribonucleoprotein particles (Guthrie, et al., 1969a; Tai, et al., 1969). At high temperatures they produced 30S and 50S subunits and 70S ribosomes like wild-type strains. Assembled 70S ribosomes from E. coli Sad mutants were not functionally cold-sensitive in an in vitro protein synthesizing system (Guthrie et al., 1969b).

Ribosomal mutants may constitute a large portion of the cold-sensitive mutants of E. coli. Out of 100

cold-sensitive mutants of E. coli, one third to one half gave irregular sedimentation patterns when crude extracts were examined by analytical centrifugation (Guthrie et al., 1969a). Nomura estimated that 20% of the cs mutants were linked to the region of the E. coli chromosome where spectinomycin resistance and streptomycin resistance mutations map. The 2 antibiotic resistance loci are closely linked and code for ribosomal proteins. The genes in the spc-str region may constitute an operon of ribosomal proteins and supernatant proteins which combine with ribosomes (Nomura and Engback, 1972). But ribosomal proteins have been mapped to other areas of the E. coli chromosome (Bollen, et al., 1973). Only a few of the putative Sad mutants of E. coli mapping in the spc-str region have been examined in detail.

When 45 cold-sensitive mutants of Salmonella typhimurium were screened by transduction for their linkage to the streptomycin resistance locus, 7 showed such linkage (Tai, et al., 1969). Only 2 of the latter were tested for ribosomal defects. Evidence that incomplete ribosomal subunits accumulated in the 2 Salmonella mutants at low temperature was derived from double label experiments. Mixtures of wild type ribosomes labeled with C^{14} uracil and mutant particles labeled with H^3 uracil were analyzed by sucrose gradient sedimentation. When grown at high temperatures with radioactive precursors, wild type and mutants had similar sedimentation profiles of 70S, 50S

and 30S peaks. When labeled with radioactive uracil at 20° C, however, the mutants, but not the parent strain, had altered sedimentation profiles. One Salmonella mutant had a 23S peak and the other mutant had a 30S peak which was increased relative to the other peaks. This increase may have been due to an accumulation of subparticles at the 26S and the 32S regions (Nomua, 1970).

Three putative ribosomal mutants of E. coli, representatives of the 3 patterns of irregular sedimentation, were analyzed by temperature shift experiments (Guthrie, et al., 1969a and 1969b). Cultures grown at 42° C, were transferred to 20° C and labeled with H^3 uracil. The ribosomal supernatant from half of the culture was analyzed by sucrose gradient sedimentation. Thus ribonucleoprotein subparticles synthesized in the cold were labeled and identified by their unusual sedimentation properties. The other half of the culture was washed and incubated at 42° C with cold uracil. Subparticles converted to normal subunits at high temperature resulted in labeled 30S and/or 50S peaks. For example, at 20° C, mutant 68 had considerable label in the 30S peak, none in the 50S peak and considerable in a 43S peak (Guthrie et al., 1969a). When transferred to 42° C with cold uracil, the mutant had considerable label in the 30S and 50S peaks but the 43S peak was greatly reduced.

From such studies, Nomura (1970) concluded that the Sad mutants of E. coli were of 3 types:

(1) mutants that failed to synthesize 50S subparticles and accumulated 32S particles (precursors of 50S subunits),

(2) mutants that failed to synthesize 50S subunits and accumulated 43S particles,

(3) mutants that failed to synthesize both 50S and 30S subunits and accumulated both 32S and 21S particles at 20° C.

The 3 kinds of mutants suggested that the in vivo assembly of 30S subunits proceeded independently of 50S subunit assembly but 50S subunit assembly was dependent on 30S assembly. In contrast, the in vitro studies had shown that both 30S and 50S subunits assembled independently (Traub and Nomura, 1968; Nomura and Erdmann, 1970). The type 3 Sad mutants seemed to indicate the presence of an in vivo control mechanism not apparent in the in vitro studies (Nomura, 1970). Recently a fourth type of Sad mutant was isolated. A heat-sensitive mutant of E. coli accumulated intermediates of 30S subunits but normal 50S subunits were formed (Rosset, et al., 1971). Here 50S subunit assembly appeared to be independent of 30S subunit assembly. No satisfactory theory which explains all the mutants has been published (Davies and Nomura, 1972).

Analyses of the subparticles, however, supports the idea that incomplete ribosomal assembly occurs in the Sad mutants. The 32S and 43S subparticles contained 23S RNA whereas the 21S particles contained 16S RNA (Guthrie, et al.,

1969a). These are the species of RNA expected if the 32S and 43S subparticles are precursors of 50S subunits, and 21S subparticles are precursors of 30S subunits.

The proteins in the 21S particles were identified by carboxymethyl cellulose chromatography or 2-dimensional gel electrophoresis (Nashimoto, et al., 1971). Two type 3 mutants were examined, Sad-410 and Spc-49-1. The latter was a spectinomycin resistance mutant which was also cold-sensitive and accumulated intermediates of 30S and 50S subunits. Nine to 11 unique 30S ribosomal proteins were present in the 21S intermediates depending on the preparation. The analyses for the 2 mutants were similar. The proteins of the subparticles were all proteins involved in the early steps of the in vitro reconstitution of 30S subunits (Mizushima and Nomura, 1970). Again the results support the idea of incomplete ribosomal assembly in Sad mutants.

Mapping of the Sad mutants was accomplished mainly by phage P_1 transduction of cold-sensitivity from the mutant strain, $aroE^+$ spc^s str^r, to a cold-insensitive recipient, $aroE^-$ spc^r str^s (Guthrie, et al., 1969a and 1969b; Nashimoto, et al., 1971). Transductants for $aroE^+$ were selected and tested for growth at 20° C as well as sensitivity to streptomycin and spectinomycin. To test whether cold-sensitivity and the Sad phenotype were related, the sedimentation patterns of ribosomal supernatants from several cold-sensitive transductants were examined.

Four Sad mutants were mapped to the spc-str area of the E. coli chromosome:

(Sad-410, Sad-38) aroE Sad-19 spcA strA k-protein

↓

Spc-49-1

The region between aroE and spcA codes for several 30S and 50S ribosomal proteins (Dekio, 1971). Spectinomycin resistant and streptomycin resistant mutants have altered 30S ribosomal proteins, S5 and S12, respectively (Dekio and Takato, 1969; Bollen and Herzog, 1970; Ozaki, et al., 1969). (The ribosomal proteins of E. coli are identified by Wittmann's nomenclature which has been generally accepted by ribosome researchers (Wittmann, et al., 1971). The numbering system is based on the mobilities of the proteins from the small, S, and the large, L, subunits in 2-dimensional gels.) The known genes for ribosomal RNA are located in sites distant from the spc-str region (Davies and Nomura, 1972). Sad-19, a type 1 mutant which accumulated 32S intermediates of 50S subunits, mapped between aroE and spcA and was closely linked to spcA (Guthrie, et al., 1969b). Type 3 mutants, Sad-410 and Sad-38, mapped to the left of aroE but another type 3 mutant spc-49-1, was located in the spc gene (Nashimoto, et al., 1971). All the phenotypes of the Spc-49-1 mutant, i.e. spectinomycin resistance, cold-sensitivity and accumulation of intermediates of 50S and 30S subunits mapped to the spc locus.

Although the protein components of 21S subparticles from mutants Sad-410 and Spc-49-1 were similar, the two mutations mapped in different regions of the chromosome (Nashimoto, et al., 1971). The mapping results, especially for Spc-49-1, suggest that ribosomal proteins are altered in the Sad mutants. The results also show that a single cold-sensitive mutation in a type 3 mutant causes non-assembly of both subunits.

Generally, cold-sensitivity and the Sad phenotype were associated in the transductants. Sad 68, a type 2 mutant which also mapped to the spc-str region, was an exception; the cold-sensitive transductants did not accumulate 43S particles but synthesized normal 50S subunits (Guthrie et al., 1969a). No explanation was offered for this observation. One may speculate that modifiers in the recipient strain sufficiently complemented the molecular defect to suppress the Sad phenotype in the sedimentation test. The latter may be a less sensitive test than the 20° C growth test.

One other genetic fact which may be important was the dominance of the cold-sensitive phenotype (Guthrie, et al., 1969b). A partial diploid of Sad-19 was formed using a recombination-negative F' episome which covers the mutant region. The mutant phenotype was dominant to the standard phenotype.

The mechanism by which altered protein S5 of mutant Spc-49-1 affects ribosomal assembly is not clear

(Nashimoto, et al., 1971). S5 was not present in the 21S intermediates accumulated by the mutant. Omission of S5 from reconstitution mixtures has a relatively minor effect leading to the formation of 28S particles. Finally the presence of altered S5 from Spc-49-1 in the reconstitution mixture had no adverse effect on the in vitro assembly of 30S subunits. Whether the use of mature 16S RNA in the in vitro studies, rather than precursor 16S RNA, is responsible for the discrepancy between in vivo and in vitro observations is uncertain. The mechanism by which one altered ribosomal protein results in non-assembly of both 30S and 50S subunits is also not understood (Davies and Nomura, 1972).

Other antibiotic mutants have also been examined for cold-sensitivity and the Sad phenotype (Lewandowski and Brownstein, 1969; Nashimoto and Nomura, 1970). A significant fraction of phenotypic revertants from streptomycin dependence to streptomycin independence were cold-sensitive and accumulated 26S and 43S subparticles at low temperatures (Nashimoto and Nomura, 1970). Other studies have shown that streptomycin dependence and streptomycin resistance mutations map in the strA locus and affect the 30S ribosomal protein, S12 (Ozaki, et al., 1969; Birge and Kurland, 1969; Hashimoto, 1960). Phenotypic revertants from streptomycin dependence to independence remain unchanged in protein S12 but show alterations in either one of two 30S ribosomal proteins, S4 or S5

(Birge and Kurland, 1970; Deusser, et al., 1970; Kreider and Brownstein, 1972; Stoffler, et al., 1971). Presumably streptomycin independent mutants which are cold-sensitive, have either altered protein S5 or altered S4 and one of the latter is responsible for the Sad phenotype and cold-sensitivity.

Though much work still remains to be done on the Sad mutants, the following conclusions seem warranted: (1) Some cold-sensitive mutants of bacteria accumulate intermediates of ribosomal subunits. (2) Thus far ribosomal proteins are the only mutational alterations implicated. Sad mutations occur in several genetic loci some of which are known ribosomal protein genes. (3) In vivo assembly of ribosomal subunits is more complex than the in vitro reconstitution studies indicated. Some control mechanism which coordinates 30S and 50S subunit assembly may be involved.

Genetic loci of ribosomal RNA of Drosophila melanogaster.

The ribosomal RNA genes of Drosophila melanogaster have been identified by RNA-DNA hybridization. Drosophila with the bobbed (bb) phenotype were shown to contain reduced amounts of DNA complementary to RNA from Drosophila ribosomes (Ritossa, et al., 1966). Previous cytological studies indicated that the bb locus of the X chromosome was close to the nucleous organizer (NO) in the heterochromatic region (Cooper, 1959). Ritossa and

colleagues regard the bb locus as the mutant counterpart of the NO (Ritossa, et al., 1966; Atwood, 1969). The bb mutants are thought to result from partial deletions in the NO region which contains a large series of duplications of ribosomal DNA. A second bb locus and NO are located on the Y chromosome of Drosophila melanogaster (Cooper, 1959). Hybridization of 5S ribosomal RNA to salivary gland polytene chromosomes of Drosophila melanogaster indicated that the genes for this ribosomal RNA are clustered on the 2nd chromosome (Wimber and Steffensen, 1970). As the total extract of ribosomal RNA used in the bobbed hybridization tests would probably be mainly 18S and 28S RNA, the nucleolus organizer probably codes for 28S and 18S RNA (Tartof and Perry, 1970).

Various genotypes, XO, XX, XY and XXY, contained approximately equivalent amounts of ribosomal RNA (Kiefer, 1968). The results indicate that dosage regulation allows equivalent production of ribosomal RNA from 1 to 3 NO regions. The rate of ribosomal RNA synthesis, however, was reduced in bobbed mutants with less than 1 complete NO (Mohan and Ritossa, 1970). Combinations of extreme bb alleles in females also resulted in reduced egg production. bb stocks have finer, shorter bristles than wild type and etched tergites, i.e. reduced abdominal banding (Lindsley and Grell, 1968). Alleles vary considerably; some exert only a slight effect on the phenotype whereas others are recessive lethals. Variation in the intensity of the

mutant phenotype of bb alleles is attributed to various sizes of deletions but some mutants do not fit this interpretation (Atwood, 1969; Kiefer, 1968). It is assumed that a slower rate of ribosome production and concomitant protein synthesis brings about the numerous abnormalities of the bb mutants.

Previous attempts to identify the ribosomal protein genes of Drosophila. Except for the bb loci and 5S RNA site, no other ribosomal genes of Drosophila have been identified. Several of the approaches used to locate ribosomal protein genes in bacteria have been applied to Drosophila. The Drosophila reports, however, have been brief and preliminary in character.

1. Strain- and species-differences in ribosomal proteins. Mapping of ribosomal proteins by strain- or species-differences was first performed with E. coli B and E. coli K-12 (Leboy, et al., 1964). The two strains showed one major difference in the banding patterns of their ribosomal proteins after one-dimensional gel electrophoresis. Gels of various recombinants of the two strains indicated that the "K protein" locus mapped near the strA locus (Leboy, et al., 1964; Mayuga, et al., 1968). This approach has been successfully applied to the mapping of species differences using E. coli-Salmonella typhimurium and E. coli-Shigella dysenteriae hybrids (Osawa, et al., 1971; Dekio, 1971; Dekio, et al., 1970). The latter studies indicated that

the genes for eight 30S ribosomal proteins and nine 50S ribosomal proteins are clustered in the spc-str region between aroE and spcA.

Ribosomal proteins from various species, strains and genotypes of Drosophila were compared by 1-dimensional gel electrophoresis (Kiefer and Gross, 1969). No qualitative differences were observed among the various D. melanogaster, Samarkand genotypes, XY, XX, XXY, XO and two bobbed strains but some quantitative differences were found. Clear-cut qualitative differences, however, existed between the two species, D. pseudoobscura and D. melanogaster and also between two D. melanogaster strains, Samarkand and Amherst. Mapping of these differences was not reported.

2. Streptomycin mutants. Streptomycin resistant (Str-R) mutants of E. coli were the first bacterial mutants demonstrated to have an altered ribosomal protein definitely associated with a change in ribosomal properties. Ribosomes from Str-R mutants continue to synthesize protein in the presence of streptomycin whereas ribosomes from the streptomycin sensitive (Str-S) parental strains are inhibited (Davies, 1964). Str-R ribosomes bind less streptomycin than Str-S ribosomes (Kaji and Tanaka, 1968). The altered ribosomal protein of strA mutants was identified as S12 by reconstitution studies (Ozaki, et al., 1969; Traub and Nomura, 1968). It should be noted that nonribosomal, streptomycin resistant mutants, not associated with the strA locus, have also been obtained (Roberts and Reeve,

1970; Yamanda and Davies, 1971).

Drosophila are generally not affected by high concentrations of streptomycin in their food. A scarlet eye color (st) mutant stock, however, was found to be sensitive to 1% streptomycin (Duke and Glassman, 1968). The drug arrested development in the early larval stages but did not affect adults of the eye color stock. Unlike the bacterial streptomycin resistance mutations (which are usually recessive), the Drosophila streptomycin resistance mutation was dominant to sensitivity (Breckenridge and Gorini, 1969; Duke and Glassman, 1968). The trait mapped to the 3rd chromosome. No biochemical studies of the ribosomes of the stocks were reported.

In a recent abstract, Lambertsson and Rasmuson (1971) reported that ribosomes from Str-S larvae of D. melanogaster bound 10 times more labeled streptomycin than those from Str-R larvae. Preliminary results indicated that streptomycin inhibited in vivo protein synthesis in the Str-S larvae. In two-dimensional gels of ribosomal proteins, Str-S larvae have at least one ribosomal protein more than Str-R larvae. Resistance was found to be dominant and mapped to the X chromosome. A full account of this work would be of interest because it indicates that drug resistance may be a successful means of obtaining ribosomal protein mutants of Drosophila.

3. Suppressors. It is well established that mutations can be suppressed at the translation level in

bacteria by altered transfer RNAs (Smith, 1972). Since the early studies of the effect of streptomycin on bacteria, it has been suspected that altered ribosomes may also suppress mutations at the translation level (Gorini, et al.,1966). Streptomycin caused misreadings of synthetic messenger RNAs in vitro (Davies, 1966). This observation seemed related to the finding that streptomycin suppressed a number of mutations in vivo (Gorini, et al., 1966). Mutations to streptomycin resistance resulted in less misreading in vitro in the presence of the drug and restriction of the streptomycin effect on suppression in vivo. Streptomycin resistance mutations can also affect the efficiency of suppression by transfer RNAs (Apirion and Schlessinger, 1967); Strigini and Gorini, 1970). These observations led to the idea that ribosomal structure influences the fidelity of translation.

Recently mutants of E. coli were isolated which suppressed other mutations in vivo and contained altered ribosomes with higher translation errors in vitro (Rosset and Gorini, 1969; Gorini, 1971). These were called ram or ribosomal ambiguity mutants and mapped to a locus in the spc-str region of the chromosome. The ram mutants were isolated by looking for mutations that counteract the restriction of suppression by streptomycin resistance mutations on translation suppression. The ram locus of E. coli may code for the 30S ribosomal

protein, S4 (Zimmermann, *et al.*, 1971). It has now been concluded that suppression in bacteria may result from a change in either transfer RNAs or ribosomes.

Some mutations of *Drosophila* have little effect on the phenotype by themselves but cause other mutants to appear closer to wild type (Lindsley and Grell, 1967). They have been designated as suppressors but it is unknown whether their mechanism of action is similar to that of bacterial suppressors. A *Drosophila* suppressor may correct some alleles but not others and may counteract mutations at several loci. At least superficially, Drosophila suppressors resemble the bacterial mutants.

It has been suggested that *Drosophila* suppressors, such as *su(f)*, may code for altered transfer RNAs or altered ribosomes but few suppressors have been investigated biochemically (Schalet, 1973; Dudick and Wright, 1973). The mutation, $su(s)^2$, which suppresses vermillion mutants of *Drosophila* has been correlated with an alteration in the chromatographic properties of a tyrosine transfer RNA (Twardzik *et al.*, 1971). The effect of the suppressor, however, was not at the translation level and $su(s)^2$ is not considered to be the locus of the altered transfer RNA (Jacobson, 1971; White, *et al*; 1973a). As yet no biochemical studies of the ribosomes of *Drosophila* suppressors have been reported although they are being considered (Wright, 1973).

It is interesting to note that ribosomal mutants of yeast have been obtained by selecting for streptomycin suppression and that one of these mutants was cold-sensitive (Bayliss and Vinopal, 1971). Like other eukaryotes, the yeast, Saccharomyces cerevisiae, is insensitive to streptomycin. It was assumed that the insensitivity was equivalent to streptomycin resistance in bacteria, i.e. due to a ribosomal protein. Mutants of a histidine-requiring strain were selected which would grow in low concentrations of streptomycin (50 μg/ml or less) without histidine, i.e. streptomycin sensitive mutations were selected which permitted the suppression of the histidine requirement by streptomycin. Unlike the parental strain, the mutants were sensitive to moderate concentrations of streptomycin (about 500 μg/ml). One of the streptomycin sensitive mutants was also cold-sensitive and had an altered ribosomal profile in sucrose gradients when grown at low temperature. As cold-sensitivity and streptomycin sensitivity were not separated in mapping procedures, the two traits are apparently due to one mutation. Unlike wild type ribosomes, the mutant ribosomes were sensitive to streptomycin in an in vitro protein synthesizing system. It appears that this cold-sensitive mutant of yeast has an altered ribosomal protein.

4. Cold-sensitive mutants. As discussed previously, selection for cold-sensitivity is another means of obtaining

ribosomal mutants of bacteria. Because the bacterial mutants mapped in several ribosomal protein genes it was thought that a number of different ribosomal mutants of Drosophila might be obtained by this approach. Also, when the present study was initiated, little was known about cold-sensitive mutants of eukaryotes. Therefore, the following questions were asked: Can cold-sensitive lethals of the X chromosome of Drosophila melanogaster be isolated? If so, what are their genetic properties? Do some have altered ribosomal proteins? The study was part of a general search for and characterization of cold-sensitive lethals of all the chromosomes of D. melanogaster (Rosenbluth, et al., 1972; Mayoh and Suzuki, 1973; Tasaka and Suzuki, 1973).

II. Genetic Studies of Sex-linked Recessive Cold-sensitive Mutants of *Drosophila melanogaster*.

The utility of conditionally lethal mutations in analyzing a variety of molecular, genetic and developmental phenomena has been extensively documented (Epstein *et al.*, 1963; Hartwell, 1967; Suzuki, 1970). Temperature-sensitive (ts) lethals are probably the simplest class of conditionally expressed mutations to recover and study in diploid eukaryotes. Remarkably, while heat-sensitive lethals map extensively around the phage chromosome (Edgar and Lielausis, 1964), cold-sensitive lethals are highly clustered within a limited number of cistrons (Scotti, 1969). The functional significance of the difference between the two classes of mutations is suggested by the finding that a large proportion of cold-sensitive lethal mutations in *E. coli* affect the self-assembly of ribosomal subunits (Guthrie, *et al.*, 1969 a,b; Tai, *et al.*, 1969). Moreover, cold-sensitive mutations can alter the regulatory properties of proteins (O'Donovan and Ingraham, 1965b).

Clearly, cold-sensitivity as the selective property of mutations restricts the sites and functions of loci detectable in micro-organisms. The possibility of enrichment for regulatory and ribosomal defects by simply selecting for cold-sensitivity has great potential for the study of developmental mechanisms in eukaryotes. Consequently, we decided to determine whether cold-sensitive lethal

mutations are readily recoverable in Drosophila and whether their genetic properties distinguish them from heat-sensitive lethals. The answers to these questions will determine whether it is possible to enrich for ribosomal mutants and other defects such as altered feedback control of enzymes.

MATERIALS AND METHODS

Mutant Screen and Viability Tests. The screening procedure for the detection of recessive cold-sensitive lethal mutations on the X chromosome is shown in Figure 1. Males from an inbred Oregon-R stock were fed for 24 hours with 0.005M or 0.0125M ethyl methanesulfonate (EMS) dissolved in 1% sucrose (Lewis and Bacher, 1968). Approximately 20 EMS-treated males were crossed to 20 attached-X-bearing females (C(1)RM/Y) in each quarter-pint bottle at 22° C (chromosomal rearrangements and markers which are not defined here can be found in Lindsley and Grell, 1968). The use of attached-X females permitted the recovery of mutagenized paternal X chromosomes in F_1 male offspring. As this procedure eliminated all lethals except conditionals in the first step of the screen, it was more efficient than screens in which the mutagenized chromosome is recovered in a heterozygous recipient.

Single F_1 males were mated at 22° C to 3 or 4 C(1)RM/Y females in vials (Step 2, Figure 1). After 4 days, they were transferred to fresh replicate vials incubated at 17° C. Offspring emerged after 2 weeks at 22° C and after one month at 17° C. The 17° C vials were scanned briefly without etherizing the flies (Step 3, Figure 1). When few or no males were observed in a 17° C vial, the replicate vial at 22° C was inspected. If a greater proportion of males occurred at 22° C than at 17° C, the strain was retained as a putative cold-sensitive lethal. In some experiments, the flies in the 17° C cultures were

etherized and examined for conditionally expressed phenotypes, i.e., an abnormal phenotype at 17° C and normal appearance or reduced penetrance of the abnormality at 22° C. The mutations are designated, in the order of detection as $\ell(1)1^{cs}$, $\ell(1)2^{cs}$, etc. They have also been referred to by an abbreviation, the number of the mutation and the cs superscript ($\ell(1)2^{cs}$ will be 2^{cs}).

Males of the putative cold-sensitive strains were retested by repeating steps 2 and 3 (Figure 1). Six to 12 males were individually tested in each trial. The relative viabilities of the mutant chromosomes were assessed by determing the sex ratios at 22° C and 17° C. All stocks were tested at least twice and some were checked several times within a period of three years. The confirmed cs mutations were maintained on the X chromosome of males mated to C(1)RM/y and C(1)DX/Y females at 22° C in bottles. When heterogeneity was observed in a viability test, those vials yielding the lowest viabilities at 17° C but high values at 22° C were used to perpetuate the stock.

Testing Female Fertility. Females homozygous for the mutations were generated and tested for fertility at 22° C by mating them with FM6/Y and mutant males at 22° C. In some experiments, one to three females 1 to 6 days old were present in each vial along with 3 males of each type. In other tests, females were mated separately with the

two types of males. An average of 25 females was tested for each mutant. Adults were discarded after 1 week and progeny scored for at least 9 days after eclosion began. When few offspring occurred, the vials were briefly scanned for signs of lethality during development, i.e., unhatched eggs, dead larvae or pupae, and tunnelling of the medium without the occurrence of pupae or adults.

Genetic Mapping. The cs mutants were mapped using the following markers listed with their symbols and genetic positions: yellow - y, 0.0; crossveinless - cv, 13.7; vermilion - v, 33.0; forked - f, 56.7; and carnation - car 62.5. These markers span most of the euchromatin of the X chromosome from the distal tip to the proximal area but do not include the heterochromatic region around the centromere. $\ell(1)^{cs}/Y$ males were crossed at 22° C to homozygous y cv v f car females, and the F_1 females were testcrossed in quarter-pint bottles at 17° C. Male offspring were scored for 20 days. Males from the Oregon-R wild-type stock were mated in the above scheme as controls. Those mutants exhibiting abnormal phenotypes at 22° and lethality at 17° C were mapped at both temperatures.

Mutations which mapped to the right of carnation were tested further by combining them with a duplication or various deletions that encompass most of the proximal heterochromatin. The rearrangements used were the X-Y

insertion, $\underline{y^+}$ Y $\underline{mal^{106}}$, and the deleted X chromosomes, $\underline{Df(1)mal^{12}}$, $\underline{Df(1)mal^{6}}$ and $\underline{In(1)sc^{4L}sc^{8R}}$ (Figures 2 and 3). Extensive descriptions of the chromosomes can be found in Lindsley and Grell (1968) and Schalet and Finnerty (1968a,b). All crosses were carried out at 17° and 22° C with a view to demonstrating pseudodominant expression of the mutant phenotype at 17° C (either lethality or a visible phenotype) with the proximal deletion or a wild-type phenotype with the duplication. None of the mutations tested fell in the regions covered by the deletions or duplication.

RESULTS

Screen and Viability Tests. From the progeny of 3,919 individually-tested F_1 males, 151 stocks were retained as possible cold-sensitive mutants. Of these, only 32 had a male to female ratio greater than 0.29 at 22° and less than 0.16 at 17° C in the first viability test. Only 25 stocks fitted these requirements in at least two viability tests. In addition, two mutants with a cold sensitive phenotypic effect were recovered and verified.

The Oregon-R♂ x C(1)RM/Y♀ control crosses showed little change in sex ratio at the two temperatures. The male to female ratio of the control was 1.37 (1,252 flies scored) at 22° C and 1.32 (1,095 flies scored) at 17° C when the flies were raised in vials (Table 1). These ratios were considered to represent the standard viability against which the ratios of the mutants were compared. This was done by the equation (r_1/r_o) 100 where r_1 = male/female ratio in the mutant cross and r_o = male/female ratio in controls. The limits for retention of the mutants were arbitrarily set at greater than 21% relative viability at 22° and less than 13% at 17° C.

Twelve of the 25 mutants retained were lethal at 17° C and had viabilities greater than 20% at 22° C in the first viability test (Tables 1 and 11). The remaining 13 were semi-lethal with greater than 0% but less than 13%

viability at 17° C and greater than 30% viability at 22° C. The semilethals showed at least 4.6 times greater viability at the permissive temperature than at the restrictive temperature. In subsequent tests, the mutants exhibited increasing viabilities at 17° C despite selection for cold-sensitivity. The lethals, for example, became semi-lethals on or before the third trial. Viabilities at 22° C also changed, either increasing or decreasing or both in subsequent tests. The viabilities of the two types of stocks, C(1)RM/Y and C(1)DX/Y, of the same mutant, generally differed although the viability tests were carried out with C(1)RM/Y females

The reasons for the instability of the stocks can only be surmised. The initial drastic decline from 151 putative mutants to 34 cold-sensitive lethal stocks after the first viability test was due in large part to the technical difficulty of accurately assessing the month-old vials kept at 22° C. Consequently, stocks that were sub-vital at both temperatures were probably retained. In screens for heat-sensitive mutants induced by EMS, it has also been noted that many stocks are no longer heat-sensitive lethals in the first retest. For example, out of 186 putative heat-sensitive lethals of the X chromosome, only 90 were confirmed on the first retest (Suzuki, et al., 1967). This initial unreproducibility seems to be characteristic of mutations induced by EMS and has been attributed to mosaicism (Jenkins, 1967; Epler, 1966).

The instability of ts strains has also been reported for Habobracon (Smith, 1968) and Paramecium (Igarashi, 1966). Other causes of variability of the cold-sensitive stocks may be changes in the genetic background and inherent instability of cold-sensitive mutations. In fact, T.R.F. Wright (1973) has made similar observations and has found that changes in viability of cs lethals are most often the result of accumulation of genetic modifiers elsewhere in the genome. Leakiness and reversion of cs mutants of microorganisms has also been reported (Cox and Strack, 1971).

Phenotypes. Some of the mutants at 22° C exhibited abnormal phenotypes which resembled the phenotype of bobbed, bb, and abnormal abdomen, a. The males and females homozygous for 5^{cs}, 6^{cs} and 15^{cs} had missing or reduced sternites, etched, missing or angled tergites, and black specks on the ventral surface of the abdomen. The mutants sometimes exhibited pinching of the last segment near the genital plate. The occurrence of these traits was more frequent and extreme among survivors at the restrictive temperatures than among the offspring at the permissive temperature (Table II). Survivors at 17° C also had extra wing veins. Mutants 26^{cs} and 27^{cs} also exhibited abdominal abnormalties similar to 5^{cs}, 6^{cs} and 15^{cs} and the traits were more frequent and severe at 17° C than at 22° C (Table II). In addition, flies carrying mutant 26^{cs} sometimes exhibited a rotated genitial plate. Although mutants 26^{cs} and 27^{cs}

were slightly less viable at 17° than at 22° C, they were not lethal and therefore are referred to as cold-sensitive phenotypic mutants. The frequencies of the abnormal abdomen trait in these 5 mutants was extremely variable (Table II) as was noted previously for their viabilities.

The visible phenotypic abnormality of mutations $\underline{26^{cs}}$ and $\underline{27^{cs}}$ were mapped at 17° C, the temperature at which the abnormal abdomen trait was most penetrant (Table III). Mapping demonstrated that these two mutations were located in different areas from one another as well as from the other mutants exhibiting abnormal abdomens ($\underline{5^{cs}}$, $\underline{6^{cs}}$ and $\underline{15^{cs}}$). The abnormal abdomen trait of mutant $\underline{26^{cs}}$ mapped to the region between y and cv, and that of mutant $\underline{27^{cs}}$ in the tip of the X near y. Note that in the cross involving $\underline{26^{cs}}$, all classes carrying cv were reduced in number when compared with their respective reciprocal crossover classes. The basis for this observation is uncertain but it may indicate the presence of a segregating autosomal mutation which produced a detrimental effect in combination with cv. In addition, 8% of the female offspring exhibited the abnormal abdomen trait as compared to 21% of the male siblings. The appearance of the trait in the females, however, was fairly evenly distributed among the various crossover classes and therefore, could not be attributed to a specific region of the X chromosome. It was also noted that in the viability tests of mutant $\underline{26^{cs}}$ a small percentage of attached-X female offspring showed

the abnormal abdomen trait. Again, the presence of an autosomal factor in mutant $\underline{26^{cs}}$ is indicated. Clearly mutant $\underline{26^{cs}}$ is compled and its properties cannot be attributed to a single mutation.

The similarity in the phenotypes of mutants $\underline{5^{cs}}$, $\underline{6^{cs}}$ and $\underline{15^{cs}}$ and the increased penetrance of these traits at 17^{o} C, suggested that the three mutants might be alleles with the abnormal phenotype related to the cold-sensitive lethality. Indeed, the mapping data supported these ideas. Lethality at 17^{o} C mapped to the right of car in each case (Table lll). Moreover, the few abnormal abdomen flies occurring at 17^{o} C were in crossover classes consistent with this location. Most of the 22^{o} C mapping data also supported the conclusion that the abnormal abdomen trait was located to the right of car. The small number of exceptions for mutants $\underline{5^{cs}}$ (6/856) and $\underline{6^{cs}}$ (2/1,199) were distributed among all the crossover classes and may have been due to a second crossover to the right of the cs mutation, or the exceptions may have been due to selection of additional factors during the maintenance of the stocks.

The locations of mutations $\underline{5^{cs}}$, $\underline{6^{cs}}$, and $\underline{15^{cs}}$ were more specifically defined by duplication-deficiency mapping (Table IV). None of them fell within the region spanned by the rearrangements discussed earlier. Only two small sections of the X chromosome were not covered by the duplication-deficiency mapping; the heterochromatic region

immediately adjacent to the centromere (Figure 2) and the area between car and ℓ34 (Figure 3). As the mutations 5^{cs}, 6^{cs} and 15^{cs} behaved similarly in all the mapping procedures, it was concluded that they were located to the right of car in one of the above two regions.

Mutations 5^{cs}, 6^{cs} and 15^{cs} behaved like alleles in complementation tests (Table V). Different cold-sensitive mutations were combined in trans-heterozygotes by crossing FM6/$\ell(1)^{cs}$ females of one strain with $\ell(1)^{cs}$/Y males of a different strain at both 22^{o} and 17^{o} C. The ratio of trans-heterozygous females for the two lethals to FM6-bearing females was used as a measure of the viability of the trans-heterozygote. The figures in brackets in Table V indicate the number of flies involved in the calculation of each viability ratio. If a trans-heterozygote gave a low viability ratio at 17^{o} C and a high frequency of abnormal abdomen trait at 22^{o} C, to an extent comparable to both of the two homozygous stocks, then the two mutants were considered to be allelic. For example, the trans-heterozygote of mutants 5^{cs} and 15^{cs} had a viability ratio of 0.0364 at 17^{o} C and 45% abnormal abdomen trait at 22^{o} C (the same trans-heterozygote from the reciprocal cross, had a ratio of 0.141 and 52% abnormal abdomen). The homozygotes of mutants 5^{cs} and 15^{cs} had properties similar to the trans-heterozygote; viability ratios of 0.0441 and 0.0460 and 52% and 62% expression of abnormal abdomen, respectively. On the other hand, the trans-heterozygotes

formed from either mutant with Oregon-R or mutants $\underline{26}^{cs}$, $\underline{16}^{cs}$, $\underline{27}^{cs}$ and $\underline{21}^{cs}$ had high viability ratios, i.e., $>$ 0.75 and $<$1% abnormal abdomen. Of the seven mutants tested, only mutants $\underline{5}^{cs}$, $\underline{6}^{cs}$ and $\underline{15}^{cs}$ did not complement one another.

All flies in two other stocks, $\underline{16}^{cs}$ and $\underline{20}^{cs}$, exhibited fine short bristles and some etching of tergites at 22^{o} C. In addition, flies carrying $\underline{16}^{cs}$ occasionally had indented and reduced eyes and indented wings. Both $\underline{16}^{cs}$ and $\underline{20}^{cs}$ were lethal at 17^{o} C. The visible phenotypes of the five mutants so far considered are similar to the effects of various alleles of bb (see Lindsley and Grell, 1968). The known role of bb in the specification of ribosomal RNA (Ritossa, et al., 1965) suggests that a bobbed phenotype might reflect defective ribosome production.

In addition, $\underline{10}^{cs}$ flies when exposed to cold shocks during development, exhibited abdominal tumors, a small incidence of plexus and blistered wings and occasional loss of external adult genitalia (W. Willis, personal communication). $\underline{8}^{cs}$ males and females had upheld wings at both 22^{o} and 17^{o} C (escapers). The alulae of the wings appear normal and are not involved in the mutant phenotype.

Any phenotypic abnormality observed within a cs mutant stock could be a pleiotropic effect of the mutation being studied or the expression of a second mutation

induced elsewhere on the chromosome. Consequently, it is necessary to map both the cs lethal defect at 17° C and the visible phenotype at 22° C (Table III). It can be seen that in every case, the position of the cs lethal corresponds very closely to the region in which the mutation exhibiting the visible defect also maps (Table III).

Viability of Mutant Females. Theoretically, the screening protocol (Figure 1) should allow the recovery of dominant as well as recessive cold-sensitive lethal mutations. In fact, all of the mutants isolated proved to be recessive as shown by the viability of all $\ell(1)^{cs}$/FM6 and $\ell(1)^{cs}$/+ females at 17° C. This is not surprising since all mutations but one selected by their dominant heat- or cold-sensitive lethality have been found to be recessive lethals at the permissive temperature (Suzuki and Procunier, 1969; Rosenbluth, et al., 1972; Holden and Suzuki, 1973). The absence of dominant cs mutants in the X chromosome screen may, therefore, have been due to lethality of dominant cs mutations in the hemizygous state at the permissive temperature. Moreover, after selection of 400 recessive ts lethals in yeast, Hartwell (1967) found that only 4 behaved as dominant ts lethals in diploids.

A comparison of survival of $\ell(1)^{cs}$ heterozygotes and homozygotes was made by crossing $\ell(1)^{cs}$/FM6 females to $\ell(1)^{cs}$/Y males for 4 days at 22° C then at 17° C for a week. FM6/$\ell(1)^{cs}$ offspring occurred in large numbers at both

temperatures whereas males and homozygous females generally were greatly reduced in numbers at 17° C (Table VI).

The viabilities of the offspring from the above cross were assessed by the ratio of $\underline{\ell(1)^{cs}}$/Y males or $\underline{\ell(1)^{cs}}$ females to $\underline{\ell(1)^{cs}}$/FM6 females. FM6/Y males were not used as a standard because they were subvital at both temperatures. The viability ratios of the Oregon-R control differed little at the two temperatures; for the males, the ratios were 0.92 at 22° C and 0.91 at 17° C, and for the homozygous females, 1.02 at 22° C and 1.03 at 17° C. The four values were derived from 867, 889, 842 and 947 flies, respectively. The ratios for the Oregon-R control were considered to represent 100% viability. Relative viabilities of the mutants have been calculated as follows: for males, ($\underline{\ell(1)^{cs}}$/Y♂)/(FM6/$\underline{\ell(1)^{cs}}$♀) x 100 (FM6/X♀)/Oregon-R♂) and for females, ($\underline{\ell(1)^{cs}}$/$\underline{\ell(1)^{cs}}$♀)/(FM6/$\underline{\ell(1)^{cs}}$♀) x 100 (FM6/X♀)/(Oregon-R♀). The figures in brackets below the relative viabilities of Table VI indicate the number of flies involved in the calculation of each viability ratio.

Generally, male and female viabilities in each $\underline{\ell(1)^{cs}}$ stock were similar both at 22° and 17° C (Table VI). The most striking exception to this generalization was mutant $\underline{2^{cs}}$. The relative viability of the homozygous $\underline{2^{cs}}$ females was twice as great as that of males at 22° C and although female viability declined moderately at 17° C, the value was still 13 times greater than that for the

males at 17° C. This mutant was also unusual in that the homozygous females were the most frequent class of offspring at both 22° C and 17° C and exceeded the heterozygous females in viability. Sexual dimorphism has previously been reported for heat-sensitive mutants (Tarasoff and Suzuki, 1970) and non-conditional lethals (Thompson 1921; Redfield, 1926) of Drosophila. Whether such a mechanism is involved with mutant 2^{cs} remains to be determined by further tests.

Spofford (1961) has shown that genetic expression can be affected by the parental source of a chromosome. Indeed, functioning of ribosomal genes of maternal chromosomes during oogenesis has been extensively documented (Brown and Dawid, 1968). If cs lethals do, in fact, enrich for selection for ribosomal defects, one might expect an effect of parental source of the mutation on its viability. It can be seen that each $\ell(1)^{cs}$ is paternally (Tables 1 and 11) and maternally (Table VI) inherited so that a comparison can be made. While viability varies from test to test (compare Tables 1 and VI), there is no striking parental effect on survival of $\ell(1)^{cs}$/Y males.

Female Fertility. During oogenesis of some insects and amphibians, extrachromosomal ribosomal RNA is extensively amplified in the oocyte (Brown and Dawid, 1968; Gall, 1969). These molecules are then transcribed to provide a ribosome-rich cytoplasm for the egg. In other organisms, where

the synthesis of RNA for the oocyte is taken over by nurse cells, selective replication of oocyte ribosomal DNA is less common. In D. melanogaster, for example, 15 highly polyploid nurse cells adjacent to the oocyte, serve to multiply the rDNA for transcription by thousands of times (King, 1970). Autoradiographic studies show that RNA is transported over cytoplasmic bridges between the nurse cells and the oocyte. Hence genetic defects in ribosome production may be detectable by decreased production or survival of eggs from mutant females. It has been shown, for example, that Drosophila females with extensive deletions in N.O. regions produce fewer eggs than wild-type females (Mohan and Ritossa, 1970). Sterility or low fertility, therefore, can be another crude criterion for selection of a ribosomal defect.

The results of crosses of individual females with FM6/Y or $l(1)^{cs}$/Y males separately or both together are summarized in Table VII. The average number of progeny per female in a seven day egg laying period is shown with the number of females tested listed in brackets. Of the 27 stocks, 8 produced an average of 51 progeny or more per female whereas 7 were completely sterile. The rest varied from almost complete sterility (see mutant 11^{cs}) to about a quarter the fertility of the wild-type control. Thus, 70% (19/27) of the mutations selected on the basis of cold-sensitivity had reduced or no fertility. This

is in contrast to the observation that 57% (50/88) of all EMS-induced heat-sensitive lethals produce viable and fertile homozygous females (Suzuki and Piternick, 1972).

No eggs or other indications of offspring were observed in the vials containing females mutant for $\underline{12^{cs}}$ and $\underline{17^{cs}}$. Also, at least half of these females were dead when the fertility test vials were cleared (9 days after the females had emerged). The infertility of these females is apparently just one aspect of their abnormal physiology at the permissive temperature. Sterile mutants $\underline{5^{cs}}$, $\underline{13^{cs}}$ and $\underline{15^{cs}}$ produced eggs which failed to hatch. Even at the permissive temperature, the eggs of these females failed to support normal embryogenesis regardless of the genotype of the fertilizing sperm. Also, mutant females $\underline{6^{cs}}$, $\underline{16^{cs}}$, $\underline{20^{cs}}$ and $\underline{24^{cs}}$ which were poorly fertile frequently laid eggs which failed to hatch. Sterile mutants $\underline{3^{cs}}$, $\underline{10^{cs}}$, $\underline{11^{cs}}$ and $\underline{26^{cs}}$ exhibited evidence that development sometimes progressed as far as the larval and pupal stages. As more than one mutation may be present in each stock, the observed infertility may not be due to the mutation causing cold-sensitive lethality.

Of those homozygous females which were fertile, all except mutant $\underline{23^{cs}}$ yielded all expected types of offspring. Mutant $\underline{23^{cs}}$ females, when crossed to mutant males and FM6 males, only yielded $\underline{FM6/\ell(1)23^{cs}}$ offspring. $\underline{\ell(1)23^{cs}}$

males and probably females failed to develop normally. This observation suggests that the addition of the wild-type allele to the zygote at fertilization was necessary, even at the permissive temperature, to overcome some deficiency in the eggs laid by homozygous *l(1)23^{cs}* females. A similar pattern of limited fertility has been reported for the sex-linked, recessive visible mutants *dor*, *fu* and *r* (Counce, 1956a,b,c) and a ts lethal (Tarasoff and Suzuki, 1970).

All the visible phenotypes were recessive except for the abnormal abdomen trait of mutant *26^{cs}*. The latter can be considered semi-dominant because at 22° C, 5% of 91 *l(1)26^{cs}/FM6* females exhibited the trait, and at 17° C, 48% of 62 females were affected. In the same cross, 39% of 106 homozygous females and 9% of 91 hemizygous males exhibited the trait at 22° C, whereas at 17° C, 69% of the homozygous females (48) and 44% of the males (41) were affected.

Mapping. Genetic localization of the 27 mutants was carried out at 17° C relative to the markers: *y*, *cv*, *v*, *f* and *car* (Tables III and VIII). The region between *y* and *cv* was designated as 1, between *cv* and *v* as 2, and so on. Twenty-four of the cold-sensitive mutations were readily localized to a region of the X chromosome. Two mutants were too leaky (i.e., yielded surviving mutants at 17° C) to map precisely, and two other mutants no

longer behaved as lethals upon outcrossing to the marker stock. The latter observation has been reported for ts mutations (Suzuki, et al., 1967) and is presumed to result from the introduction of genetic modifiers from the marker stock. Although mutant 19^{cs} belonged to the latter group, it still could be mapped because surviving males carrying the mutation emerged 9 days later than those with the wild-type allele (Table VIII).

The crossover distances at 17^{o} C in each region of the Oregon-R control were similar to the standard values (Lindsley and Grell, 1968). The mutants, however, freqeuntly showed discrepancies when compared with the control values. In such cases, crossover values were checked in females and all showed values similar to standard values. None of the cs-bearing chromosomes gave any evidence of chromosome rearrangement.

DISCUSSION

The frequency of sex-linked lethal induction by EMS can be estimated by comparing the sex-ratios of progeny of treated and untreated males crossed to attached-X-bearing females. On this basis, it was found that about 36% of all X chromosomes exposed to 0.005M EMS carry an induced lethal. At this level of mutation-induction, a proportion of the lethal chromosomes will carry more than 1 newly-induced lethal mutation. Mapping studies show that the cs lethals are recovered as single mutation events. Thus, an estimate of the rate of cs production should be based on their proportion among those chromosomes carrying only one induced mutation (Baillie et al, 1968). In previous tests of EMS-induced lethals, it was estimated that of the chromosomes bearing a single mutation, 10-12% were heat-sensitive (Suzuki et al., 1967; Baillie et al.,1968). In the present experiments it was calculated that 1.5-3.0% of all single lethal chromosomes are cold-sensitive. (See appendix for calculation.)

It is obvious that sex-linked cs lethals were much less frequent than heat-sensitive lethals (1.5 - 3% as compared to 10 - 12%). Part of the greater proportion of heat-sensitive lethals, however, may reflect the larger temperature range used to screen (29° to 17° and 22° C for heat-sensitives, 22° to 17° C for cold-sensitives).

The most meaningful comparison of frequencies of ts mutants come from recent experiments where the same set of mutagenized 3 rd chromosomes was screened for both heat- and cold-sensitivity (Tasaka and Suzuki, 1973). Flies carrying each mutagenized chromosome were tested for viability at 17°, 22°, and 29° C. Twenty recessive mutations were lethal at 29° C but viable at 17° C, i.e. were heat sensitive, and an equal number of mutations were lethal at 17° but viable at 29° C, i.e. were cold-sensitive. All these mutants were viable at 22° C. Three other mutations were viable at one of the extreme temperatures but were lethal at the other two temperatures. In total, 21 heat-sensitive mutants, 22 cold-sensitives, and 10 mutants sensitive to both 17° and 29° C but viable at 22° C were found. Hence the frequencies of recessive cold-sensitive and heat-sensitive mutations were very similar on the third chromosome of Drosophila.

Comparisons of the genetic distributions of dominant autosomal heat- (DTS) and cold-sensitive (DCS) lethals can be made. On chromosome 3, DTS lethals which map at a number of sites have been recovered (Holden and Suzuki, 1973) whereas no DCS lethals have been found (Rosenbluth et al., 1972; Tasaka and Suzuki, 1973). On chromosome 2, a large cluster of DTS lethals was found near dp in 2L but other sites were also found (Suzuki and Procunier, 1969).

DCS lethals were found on chromosome 2 only in the cluster (Rosenbluth et al., 1972). Clearly, DCS lethals are more rare and distributed quite non-randomly in contrast to DTS mutations.

On the X chromosome, about 4% of EMS-induced non-conditional and heat-sensitive lethals map to the right of car (Suzuki and Piternick, 1972) whereas 12% (3/24) of the cs mutants mapped fell to the right of car. Of the heat-sensitive lethals, 7.4% (7/95) mapped at the tip of the X chromosome (Suzuki and Piternick, 1972) whereas 29% (7/24) of the cs lethals map in that region (Figure 4). Clearly, selection for cold-sensitivity of lethal mutations yielded a different distribution of sites from lethals selected by heat-sensitivity.

The 3 cs mutations (5^{cs}, 6^{cs} and 15^{cs}) mapping to the right of car were allelic (Table VIII). Mutation 15^{cs} was recovered in a completely different experiment from 5^{cs} and 6^{cs} and the latter are phenotypically distinct (Table IX). It was concluded, therefore, that each allele represented a different induction event. Moreover, a similar cs mutant mapping to the right of car has been isolated in another lab (Wright, 1973). Among the 7cs mutants isolated by Wright, a fine bristle mutant similar to 16^{cs} and 20^{cs}, was also found. Complementation tests of the 2 sets of mutants are now in progress (T. Wright, personal communication). It appears

that selection for cold-sensitivity on the X chromosome enriches for mutations in some sites.

Whether cold-sensitivity in fact enriches for ribosomal or regulatory defects in Drosophila must be determined by biochemical tests. Biochemical and genetic evidence that the bobbed locus on the X chromosome codes for ribosomal RNA is very good (Ritossa et al., 1965; Mohan and Ritossa, 1970). Mutants for extreme alleles of bobbed are inviable and less extreme alleles may produce a phenotype of slender bristles, etching of abdominal tergites and prolonged development. This has led to the suggestion that Minute mutations which exhibit some of these phenotypes may have altered tRNAs and thereby affect the translational apparatus. Thus, it is tempting to suggest that the phenotypes of bb and Minute flies might be characteristic of translation defects. Seven of the 27 cs mutations (5^{cs}, 6^{cs}, 15^{cs}, 16^{cs}, 20^{cs}, 26^{cs}, 27^{cs}) exhibited tergite etching and/or slender bristles.

Maternally-produced ribosomes constitute the bulk of the ribosomes present during early embryogenesis (Brown and Dawid, 1968). Defective ribosome synthesis in a female might be reflected in female sterility due to the production of eggs which cannot develop. In fact, 7 of the 27 cs mutations retained yielded sterile females and 10 more produced females with low fertility (Table V). Moreover, all but one (27^{cs}) of the mutations which had a bristle or abdominal phenotype were also sterile or

poorly fertile. (See Table IX for a summary of the properties of the mutants.) The possibility that the combined properties of specific visible phenotypes, female sterility and cs lethality are sufficient to define many defects in ribosomal proteins is an intriguing one which demands further biochemical studies for verification.

Unlike heat-sensitive lethals, the cs mutations reported are quite variable in phenotype and apparently quite sensitive to genetic and environmental differences. Viability ratios recovered in different experiments performed with different stocks or at different times can vary widely. Outcrossing cs lethal stocks can also greatly affect viability of the mutant chromosome (Wright, 1973). We would suggest that cs lethals must be rigidly selected for their complete lethality at 17° C and stability from test to test. Otherwise, the variability and the very long generation time at 17° C render the cs class of mutations very difficult to work with.

III. Cold-sensitive Sex-linked Mutants of *Drosophila melanogaster*: Two-dimensional Gel Electrophoresis of Ribosomal Proteins.

INTRODUCTION

Cold-sensitive mutants of *Drosophila* may produce altered ribosomal proteins, enzymes, or other defective components. Though mutant enzymes would be of interest, their biochemical detection would probably be difficult. Which of the many known enzymes should one assay? Unless some general pretests could be devised to detect faulty biochemical pathways, choosing a relevant enzyme assay would be a matter of chance. On the other hand, Nomura's studies indicated that ribosomal mutants constitute a large portion of cold-sensitive lethals of bacteria (Guthrie, *et al.*, 1969b). Another advantage of investigating ribosomes is that ribosomal assays such as sedimentation in sucrose gradients or gelelectrophoresis of ribosomal proteins, test many components in one procedure.

Ribosomal proteins from five of the cold-sensitive *Drosophila* mutants and Oregon-R controls have been examined by two-dimensional gel electrophoresis. The selected mutants exhibited female sterility and/or phenotypes similar to *bobbed* mutants. As the latter mutants are deficient in DNA coding for ribosomal RNA, it was thought that cold-sensitive mutants with similar phenotypes but mapping in different areas of the X chromosome, might have

altered ribosomal proteins.

Two-dimensional gel electrophoresis was chosen because it has been a useful tool in demonstrating altered ribosomal proteins in mutants and various strains of bacteria (Kaltschmidt, et al., 1970c; Deusser, et al., 1970; Bollen, et al., 1973). One-dimensional gel electrophoresis was previously used to identify and genetically map the "K ribosomal protein" of E. coli K 12 (Leboy et al., 1964). Two-dimensional gel electrophoresis is a recent adaptation of one-dimensional gel electrophoresis and has about double the resolving capacity of the older method (Kaltschmidt and Wittmann, 1970b). E. coli ribosomal proteins can be separated into about 25 bands in one-dimensional gels. In two-dimensional gels, however, more than 50 protein components are evident.

METHODS AND MATERIALS

Introduction. A previously reported procedure for the isolation of ribosomes from Drosophila larvae was used (Boshes, 1970). The method separated "soluble" or "free ribosomes" from ribosomes attached to the endoplasmic reticulum. Both the "free ribosomal" and "attached ribosomal" preparations were shown to contain polysomes and ribosomes as well as 18S and 28S RNA. The "free ribosomal" preparation, however, was found to accumulate radioactive uridine into 18S and 28S RNA more rapidly than the "attached ribosomal" preparation. The latter fraction exhibited a higher rate of amino acid incorporation. The results suggest that "soluble ribosomal" preparations may represent newly formed ribosomes and polysomes not yet engaged in protein synthesis. Greater protein synthesis by the "attached polysomes" than by the soluble polysomes has been observed in numerous organisms and it is thought that the "attached" fraction synthesizes proteins for transport through the endoplasmic reticulum (Birnie, 1972). In the present study of ribosomal proteins, "soluble ribosomal" preparations were used because they may be relatively free of nascent proteins. The method also separates mitochandria from "soluble ribosomes".

Though Boshes procedure was adopted for our use, nascent proteins are not considered to be a serious source of contamination of ribosomes. The tissues examined

were of diverse types and their ribosomes were, no doubt, engaged in the synthesis of many proteins at numerous stages of partial completion. It is unlikely that the concentration of any one of these proteins or partial proteins would be large enough to show up in the gels of the ribosomal proteins.

The method outlined below, which is an adapation of Boshes method, was previously used in this laboratory to isolate ribosomes from adult Drosophila. Some of Boshes' observations were repeated (M. Gould-Somero, personal communication). Adults were fed radioactive uracil or labeled amino acid overnight. The sedimentation of particles in the post-mitochondrial supernatant were examined in sucrose gradients. Particles with sedimentation properties like ribosomes and polysomes had incorporated the precursors of RNA and protein. It seems reasonable to assume that the method outlined isolates ribosomes from adult Drosophila. The degree of purity of the ribosomal preparation, however, is uncertain.

Isolation of ribosomes. In the preliminary experiments, ribosomes were isolated from mixed samples of Oregon-R, adult males and females. As females homozygous for some of the cold-sensitive mutations produced few or no offspring even at the permissive temperature, mutant males from stocks maintained at 22° C with C(1)RM/Y females, were used. Also, Oregon-R males and their C(1)RM/Y sibs

were separately examined. Etherized or CO_2 anesthetized, adult Drosophila melanogaster were sorted according to sex and collected in vials cooled in Dry Ice and ethanol. The flies were stored at -30° C for a week to 7 months before being used for ribosome isolations. No change in the pattern of ribosomal proteins in the two-dimensional gels was observed with increasing storage times. Ribosomes were prepared in a cold room at 5° C and all equipment and solutions were pre-chilled.

Five to 20 gm of flies were homogenized in freshly prepared TKM-S (0.26 M sucrose, 0.03 M Tris-HCl, pH 7.8, 0.1M KCl, 0.007 M Mg acetate, and 0.007 M mercaptoethanol) for 20 seconds in an Omni-mixer operated at top speed. Twenty ml of TKM-S solution was added for each gram of tissue. This high ratio of solution to tissue seemed to produce a clearer, less colored ribosomal pellet than did concentrated homogenates. The homogenate was filtered through coarse nylon mesh to remove most of the insect exoskelleton. The filtrate was then homogenized by five strokes with a B sized, teflon pestle in a Dounce glass homogenizer to ensure that cells were ruptured. Failure to remove hard tissue by filtering made the latter step difficult.

The homogenate was centrifuged at 18,000 rpm for 30 minutes in a fixed angle, Sorvall rotor (39,100 g). Fatty material on top of the supernatant was removed and the

precipitate was discarded. The post-mitochondrial supernatant was treated with 1 ml of 10% Triton-X-100 detergent per 10 ml supernatant to solubilize any microsomal membrane present. The mixture was left for 1/2 to 1 hour at 5^{o} C and again centrifuged at 18,000 rpm. Any fat-like topping or precipitate was discarded.

The clear orange supernatant was centrifuged at 42,000 rpm for 90 minutes in the Bechman 50.1 angle rotor (167,000 g). A slightly orange, translucent pellet was obtained. The centrifuge tubes were inverted and thoroughly drained. The inside walls of the tubes were dried with Kimwipes to remove as much supernatant as possible from the ribosomal pellet. The ribosomal pellet was usually frozen overnight with a small amount of TMA solution (0.01 M Tris- HCl, pH 7.6, 0.001 M $MgCl_2$, 5 X 10^{-2}M NH_4Cl, and 0.007 M mercaptoethanol).

Solubilization of ribosomal proteins.

(1) HCl-acetone. Several methods of preparing ribosomal proteins for 2-dimensional gel electrophoresis were tested in initial experiments. The HCl-acetone method, however, was used for the mutants, Oregon-R males, and C(1) RM females (Lambertsson, et al., 1970). Ribosomes from 10 gm tissue were suspended in a total of 1.4 ml cold TMA II . Cold 1 M HCl (0.47 ml) was added to the chilled suspension to give a final concentration of 0.25 M HCl. The mixture was stirred at 5^{o} C for 1 to 2 hour. The white, flocculent precipitate of RNA which formed was removed

by centrifuging at 15,000 rpm for 20 minutes in the Sorvall. The supernatant was saved and the precipitate was re-extracted with 0.6 ml of 0.25 M HCl in TMA II. When smaller amounts of tissue were used, the volumes of reagents were reduced proportionally.

To precipitate ribosomal proteins, 5 volumes of cold acetone (11.5 ml) was added to the supernatant and washings (2.3 ml). The mixture was left in the cold for an hour or longer. The white protein precipitate was separated from the colored supernatant by low speed centrifugation. The protein pellet was then washed with cold acetone, twice with cold 95% ethanol, twice with ether, and air-dried at room temperature.

(2) Mg-acetic acid. The ribosomal pellet from 20 gm tissue was frozen overnight in TSM (0.01 M tris, pH 7.8, 0.003 M succinic acid, 0.01 M $MgCl_2$, and 15 mM mercaptoethanol). To 4.5 ml ribosomes suspended in TSM, was first added 0.45 ml 1 M $MgCl_2$, then 9.0 ml cold glacial acetic acid (Hardy, et al., 1969). The mixture was stirred for an hour in an ice bath. The white precipitate of RNA was removed by low speed centrifugation and the supernatant saved.

In initial trials, the supernatant was dialyzed against sample gel as suggested by Kaltschmidt and Wittmann (1970b). To keep the final volume of sample gel and ribosomal protein small, dialysis was performed with a greater pressure inside the dialysis sac than outside.

The procedure proved technically difficult in my hands and was replaced by standard dialysis. In later trials, the supernatant was first dialyzed against 66% (v/v) acetic acid and 1 mM mercaptoethanol for 24 hours, then 5% (v/v) acetic acid and 1 mM mercaptoethanol for a further 24 hours, and finally against 1 mM mercaptoethanol for 16 hours (Welfle, et al., 1971). The final dialyzate was freeze-dried. This procedure was reported to release 80% of the protein from rat liver ribosomes.

(3) LiCl. The ribosomal pellet from 10 gm tissue was resuspended in 2.3 ml TKM (0.05 M KCl, 0.0015 M $MgCl_2$, 0.001 M Tris, pH 7.8). To 2.3 ml cold ribosome suspension was added 2.3 ml cold 4 M LiCl. The mixture was allowed to stand for 40 hours in the cold. The large orange gelatinous precipitate of RNA was removed by low speed centrifugation. The supernatant was dialyzed against water for 28 hours. The dialyzate was freeze-dried. This procedure was reported to give complete recovery of proteins from rabbit reticulocyte ribosomes (Mathias and Williamson, 1964).

(4) Cleland's reagent. To a small ribosomal pellet from 1-2 gm tissue was added 50 lambda of Cleland's reagent (4 M LiCl, 8 M urea and 0.005 M dithiothreital). The reagent was prepared immediately before using. The pellet was left in contact with the reagent overnight without mixing. The ribosomal RNA precipitate was removed by centrifugation and the supernatant, containing ribosomal proteins, was saved. The supernatant was mixed

with 0.2 ml of sample gel and 0.1 to 0.15 ml of the mixture was applied to the 1-dimension gel column.

Two-dimensional Gel Electrophoresis. Two-dimensional gel electrophoresis of the extracted ribosomal proteins was performed as described by Kaltschmidt and Wittmann (1970a) except for some minor changes. The main steps in the procedure were as follows. The extracted proteins were placed in the middle of a gel (8% acrylamide, pH 8.6) polymerized in a glass tube (14 x 0.5 cm). Unlike conventional methods, this arrangement allowed the proteins to migrate to either pole and still remain on the gel. The gel column was removed from the tube and equilibrated in buffer for the second electrophoresis. The gel column was attached along the top edge of a gel slab (14 cm x 10 cm x 0.5 cm) composed of 18% acrylamide, pH 4.6. After electrophoresis in the second dimension, the proteins were stained with Amido Black or Coumassie Brilliant Blue. The concentrations of gels and the pH values of the buffers were selected to emphasize separation by change in the first dimension, and by size in the second dimension.

First dimension electrophoresis. The following reagents were used (Addendum, Kaltschmidt and Wittmann, 1970a).

Separation gel, pH 8.6

54 gm urea

6.0 gm acrylamide (recrystallized in chloroform)

0.2 gm N, N'-methylenebisacrylamide (bisacrylamide).

1.2 gm disodium salt of ethylenediaminetetraacetic acid ($EDTA\text{-}Na_2$)

4.8 gm boric acid.

7.3 gm tris(hydroxymethyl)aminomethane (Tris).

0.45 ml N, N, N', N'-tetramethylethylenediamine (TEMED).

The solution was made up to 148.5 ml with water. To polymerize the above mixture, 1.5 ml freshly made 7% ammonium persulfate was required.

Sample gel

48 gm urea
4.0 gm acrylamide
0.2 gm bisacrylamide
0.085 gm $EDTA\text{-}Na_2$
0.32 gm boric acid
0.06 ml TEMED

The solution was made up to 99 ml with water. To polymerize the above solution, a freshly made mixture of 0.5 mg riboflavin and 5 mg ammonium persulfate in 1 ml water was added.

Electrode buffer, pH 8.6

360.0 gm urea
2.4 gm $EDTA\text{-}Na_2$
9.6 gm boric acid
14.55 gm Tris

The solution was made up to 1 liter with water. The reagents were kept at 4° C up to two weeks.

Glass tubes, 18 cm long and 0.5 cm inner diameter,

were thoroughly washed with detergent, rinsed, dipped in dilute Photoflow solution (1 ml/200 ml water) and drained dry. The tubes were closed at the bottom with rubber caps and filled to 9 cm with first-dimension separation gel. The gel solution was overlayered with a few drops of water to prevent the gel from forming a curved surface. After the gel polymerized the water was removed with absorbant paper.

Two to 3 mg of ribosomal proteins was dissolved in 0.1 ml sample gel and applied to the top of the separation gel with a long capillary tube. The transfer was not quantitative but losses were kept to a minimum by using small equipment. Increasing the volume of sample gel or decreasing the amount of protein produced inferior results. The sample gel was overlayered with water and polymerized with U.V. light. After removal of water from the tube, separation gel was added up to 14 cm.

The tubes were electrophoresed in Canalco 1-dimensional gel equipment. The tubes were inserted so that the longer section of separation gel projected into the bottom cathode chamber and the shorter section, into the top anode chamber. When the sample was E. coli ribosomal protein, the lower section of separation gel was 5.5 cm and the upper section, 8.5 cm.

Electrophoresis was carried out in a cold room at 5^{o} C. Four samples were run in each experiment. Three

mamp current was applied per tube for 10 to 23 hours. The current was kept constant at 12 mamp. The voltage was initially 110-150 volt but increased with time. The pH of the electrode buffer was unchanged at the end of a run.

The gel columns were removed from the tubes by placing them under water and moving a long hypodermic needle (22 gauge, 4 inches) gently between the glass and the gel. The gel could then be removed intact by applying a water-filled rubber bulb on the end of the tube. The procedure was routinely successful if the glass tubes had been washed and treated as described.

Second dimension electrophoresis. The reagents of Kaltschmidt and Wittmann (1970a) were modified as indicated below.

Equilibration buffer

300 gm urea
0.67 gm KOH

Approximately 3 ml glacial acetic acid, i.e. sufficient to make the solution pH 4.6.

The solution was made up to 1 liter with water. The quantity of urea was decreased from 480 gm because annealing of gel columns to gel slabs was improved. The volume of acid was varied because the original amount gave a high and variable pH.

Separation gel, pH 4.6

360 gm urea

180 gm acrylamide (electrophoresis grade, not recrystallized)
5.0 gm bisacrylamide
2.7 gm KOH
5.8 gm TEMED

Approximately 60 ml glacial acetic acid, i.e., sufficient to make the solution pH 4.6.

The solution was made up to 967 ml with water. Thirty-three ml of 10% ammonium persulfate was added to polymerize the above mixture. In initial experiments, acrylamide recrystallized from chloroform was used. As the quality of the reagent did not have an obvious effect on results and recrystallization was expensive and time-consuming, electrophoresis grade acrylamide was used as purchased.

Electrode buffer, pH 4.6

140 gm glycine

Approximately 15 ml glacial acetic acid, i.e. sufficient to make the solution pH 4.6.

The solution was made up to 10 liters with water. The equilibration buffer and separation gel were generally prepared just before use. The electrode buffer was stored for several weeks at 5° C.

Amido Black Stain

5.5 gm Amido Black
50 ml glacial acetic acid

The solution was made up to 1 liter with water, mixed well and filtered. The same solution was used

repeatedly to stain several gels.

Coumassie Brilliant Blue

0.3 gm Coumassie Brilliant Blue
15 ml methanol
980 ml 10% trichloroacetic acid (TCA).

The dye was dissolved in methanol which was then added to the TCA solution. The mixture was filtered. The stain was unstable and had to be made up fresh for each experiment.

In preparation for the second electrophoresis, the 1-dimensional gel columns were equilibrated in pH 4.6 buffer for 1 to 3 hours at room temperature. Variations in the time between the end of the first electrophoresis and start of the second had no apparent effect on results.

The two-dimensional gel electrophoresis apparatus was identical to that of Kaltschmidt and Wittmann (1970a) except that 4 gel chambers, each 14 cm long x 0.5 cm wide and 10 cm high were present instead of 5 chambers, 20 cm x 0.5 cm x 20 cm. Essentially the apparatus consisted of five plastic forms which when bolted together created four rectangular chambers open at top and bottom.

The bottoms of the gel chambers were sealed by placing the assembled apparatus in a tray containing a half inch depth of separation gel and persulfate. (Overlayering with water as prescribed by Kaltschmidt and Wittmann was omitted as the procedure was difficult and unnecessary.) After the bottom layer of gel had polymerized, gel

mixture was poured into the sealed chambers to form the gel slabs.

After heat from the polymerization reaction had subsided, the 1-dimensional gel columns were attached along the top edges of the slabs with more gel mixture. During these manipulations, polymerization of the flask of gel solution and persulfate was delayed by keeping the flack in ice water.

Attempts to attach the gel columns to the gel slabs while they were polymerizing as prescribed by Kaltschmidt and Wittmann (1970a) were unsuccessful. The gel columns tended to sink into the fluid gel. And extensive protein residues were left in the gel columns or other artifacts occurred.

Excess gel used to seal the bottom of the gel chambers was trimmed from the apparatus. It was then placed in the electrode chamber containing chilled pH 4.6 electrode buffer. The anode was at the top of the gel chambers and cathode at the bottom. Electrophoresis was carried out in a 5^{o} C cold room. A constant current of 200 mamps was applied for 12 to 22 hours. The voltage, initially 60 to 100 volts, increased with time. As the pH of the electrode buffer changed during long runs, it was sometimes replaced within the first 10 hours of a run with fresh buffer.

After electrophoresis, the gel slabs were removed from

the apparatus. The bolts holding the parts together were loosened and water was injected between the gel slabs and the plastic walls with a hypodermic syringe.

The slabs were usually stained with Amido Black. They were placed in the stain for 15 minutes and rinsed for an hour in running water. The background was destained by placing the gels in warm, 1% acetic acid for several hours, and then in fresh 1% acetic acid for several days at room temperature. Plastic stacking trays (8.5 inches x 5.5 x 2) obtained from a hardware shop were useful for staining and storing the gels. Gels stained with Amido Black faded only after several months and could be readily restained. Also the gels, if properly destained, photographed well with black and white film.

Staining with Coumassie Brilliant Blue (CBB) was faster but the results were not as satisfactory as with Amido Black. The gel slabs were placed in CBB stain overnight. The dye solution was poured off and replaced by warm 10% TCA. The latter sharpened the stained spots and destained the background. A second change of TCA was necessary for maximum destaining. The proteins appeared as bright blue spots on the gels which had a grey coat on the surface. The spots were clearly distinguishable visually but showed up indistinctly in black and white photographs. Also the gels faded and shrank within a month.

The gels were best viewed on a frosted light table. However, attempts to photograph them with transmitted light were unsuccessful because of the uneveness of the light. The gels were best photographed with special overhead lighting apparatus (Linhof, setting 1/2) that produced an even light intensity. The camera was placed close to the gels, about 8.5 inches between lens and gel, in order to reduce shadow artifacts in the photographs. Fine grain, black and white film (16 DIN, Kodak) was used. Under these conditions, camera settings of 3 f and 1/250 gave the best negatives. High contrast, #4 paper gave the best prints.

RESULTS

Preliminary experiments. The following methods of preparing Drosophila ribosomal proteins for gel electrophoresis were tried: (1) LiCl, (2) HCl-acetone, (3) Mg-acetic acid and (4) Cleland's reagent. The protein residue from the LiCl extraction dissolved well in sample gel solution initially but later precipitated out. In addition, the sample gel solution failed to polymerize. Methods 2 and 3 produced suitable residues which dissolved well in sample gel solution and permitted polymerization. Method 4 was considered unsuitable because diffuse bands of protein were produced in the first dimension.

To determine the suitability of the Kaltschmidt and Wittmann gel system for studying Drosophila ribosomal proteins, samples were placed in the centres of first dimension gel columns and electrophoresed for 16 hours at pH 8.6. When the gel columns were stained, it was found that most of the proteins had migrated to the cathode. On the anionic half of the columns, a few faint bands close to the origin were observed. The experiment was performed with HCl-acetone and Cleland's reagent preparations. Thereafter, the origin for Drosophila samples was shifted to the anionic side of the gel so that an increased area of the gel slab was devoted to proteins migrating to the cathode in the first dimension (Fig. 5).

Time studies were conducted to find optimal electrophoretic conditions for Drosophila proteins on gel slabs

of 10 cm x 14 cm. At least two runs were necessary to characterize the Drosophila ribosomal proteins, a long run to fully develop slow-moving spots and a short run to keep fast-moving proteins on the gels (Fig. 6a to 7C).

Comparison of Drosophila and E. coli ribosomal proteins. That Drosophila and E. coli ribosomal proteins are different is evident in 2-dimensional gels (Fig. 8, 6b, and 6C). The migration pattern shown here for E. coli (Fig. 8) is similar to photographs published by Kaltschmidt and Wittmann (1970b). Although most of the E. coli and Drosophila proteins have isoelectric points higher than pH 8.6 and migrate to the cathode in the first dimension, the basic proteins of the two species produce quite different migration patterns. Several major components of the E. coli samples, however, occur on the anionic side of the gel (Fig. 8). In contrast, ten faintly staining spots are found on this side of the Drosophila gels and form a different pattern than that for the E. coli proteins (Fig. 6c and 7c).

The electrophoretic pattern presently found for Drosophila ribosomal proteins is similar, but not identical, to recently published photographs of Drosophila 2-dimensional gels (Lambertsson, 1972). As in the present study, Lambertsson used HCl-acetone preparations of ribosomal proteins from adult D. melanogaster. In his gel procedure, however, he excluded proteins with isoelectric points

lower than pH 8.6. because they occurred in low concentration. Some differences in Lambertsson's gels and the present ones are also apparent on the cationic side of the gel near the origin. Lambertsson found 52 basic proteins whereas the present study suggests 58. The difference in number seems to be due mainly to minor components. Whether these are contaminants or ribosomal proteins present in low concentration is uncertain. As in the present study, the degree of purity of the ribosomes used by Lambertsson is unknown.

Comparison of HCl-acetone and Mg-acetic acid preparations of ribosomal proteins. HCl-acetone and Mg-acetic acid preparations of Drosophila ribosomal proteins produced similar, but not identical patterns of migration in 2-dimensional gels (Fig. 6a to 7c). Three major differences were noted: spots 24 and 26, 5 and 10, and 6 and 17. The differences in the gel patterns may be attributed to differences in the extractibility of proteins by the two procedures or to alterations of proteins by the two treatments. In protein studies, artifacts can be created through disulfide bond formation; aggregation of monomers into dimers, trimers, etc.; alteration of amino acid residues by isocyanate which is a degradation product of urea; and so on (Nomura, 1970). A definite conclusion about the nature of the differences cannot be reached but an interpretation can be attempted.

The appearance and locations of members of the two pairs, spots 24 and 26 and spots 5 and 10, suggests that each pair may represent one unique protein, i.e. one polypeptide chain, and that 2 spots may be present because of a conversion. Spots 24 and 26 are adjacent to one another (Fig. 7a) and spots 5 and 10 are also very close to one another (Fig. 6a). In 2-dimensional gels of E. coli ribosomal proteins, displacements of about this extent are obtained when mutant proteins are compared with wild type proteins (Deusser, et al., 1970; Bollen, et al., 1973). In such cases, a single amino acid difference (Kahan, et al., 1973) or a shortening of the polypeptide chain (Funatsu, et al., 1972) has later been demonstrated by column chromatography and chemical analysis. In the present study, the closeness of the members of each pair suggested to me that the members of each pair might be chemically related.

The inverse relationship between members of a pair also suggested that the two members were related. In the Mg-acetic acid gel, spot 24 was large and dark and spot 26 was barely visible (Fig. 6a). In the HCl-acetone gel, spot 24 was clearly diminished and spot 26 was clearly increased (Fig. 7a). This relationship suggested to me that spots 24 and 26 may represent alternate forms of one unique protein and that a conversion may occur between the two forms. Spots 5 and 10 also exhibited an inverse relationship but it is less clearly demonstrated in the photographs than the previous example. In the

HCl-acetone gel, spot 5 was relatively large and dark and spot 10 was barely visible (Fig. 7a). In the Mg-acetic acid gel, spots 5 and 10 were small and about equal in size (Fig. 6a). Spots 5 and 10 may also represent a conversion between two forms of one unique protein.

But one can also assume that the two members of a pair represent two unique proteins and that their closeness and inverse relationship are fortuitous. In this case the differences would be attributed to differences in the solubilities of the members in the two extraction systems. For example, one could assume that protein 24 is well extracted by the Mg-acetic acid procedure and protein 26 is barely soluble. In the HCl-acetone procedure, the solubility of protein 24 would be decreased and that of protein 26 would be correspondingly increased. Similar explanations could be offered for spots 5 and 10. The solubility hypothesis seems more contrived to me than the artifact hypothesis, but obviously neither theory is proved by the observations. As discussed later, artifacts can be detected more readily by column chromatography and chemical analyses than by gel electrophoresis.

Solubility differences, however, may be a more reasonable explantion for spots 6 and 17 than artifact formation. In the HCl-acetone gel, spot 6 was absent and spot 17 stained strongly (Fig. 7a) but when the Mg-acetic acid method of protein extraction was used, spot 6 was prominent and spot 17 was faint. Again an inverse

relationship is evident and conversion of one unique protein to two forms could be invoked. But the two spots are located in relatively distant areas of the gels. Displacements of this extent were not observed with mutant ribosomal proteins of bacteria. Unless one assumes that a drastic chemical change occurred, conversion seems an unlikely explanation for spots 6 and 17. The presence of two different polypeptide chains with quite different migration properties and solubilities seems to be a more plausible theory. Again the hypothesis cannot be proved by gel electrophoresis alone.

Three major differences were apparent in gels solubilized by the two methods but the overall patterns for the majority of spots were remarkably similar (Fig. 6a to 7c). The Mg-acetic acid method removes about 80% of the protein from rat liver ribosomes (Welfle, et al., 1971). No evaluation of the HCl-acetone method was found in the literature. The similarity suggests that the HCl-acetone and Mg-acetic acid methods extract about the same number and kinds of proteins from Drosophila ribosomes. The three differences in the HCl-acetone and Mg-acetic acid gels, however, illustrate how 2-dimensional gel electrophoresis can reveal differences in protein samples.

Number of Drosophila ribosomal proteins. Proteins that remained at the origin in the first dimension and migrated directly toward the cathode in the second dimension, have

generally not been represented in the schematic diagram, except for spots 4, 9 and 65 (Fig. 5). Such spots could be due to proteins with isoelectric points equal to 8.6 and hence fail to migrate in the first dimension. Or a protein may exhibit low solubility at pH 8.6 in the first dimension and not move from the origin. It may then exhibit increased solubility at pH 4.3 in the second dimension and move away from the origin. The latter interpretation seems more appropiate than the former, especially for spots 4 and 9. They are connected to spots 5 and 10 by streaks which are indicative of insolubility. This type of artifact has been observed previously in 2-dimensional gel electrophoresis (Sherton and Wool, 1972). Spot 65 at the tip of the central axis, however, may be a unique protein with an isoelectric point close to pH 8.6 as it is not represented by a corresponding spot to one side of the central axis in the second dimension (Fig. 9 and 10).

In estimating the number of unique proteins present in the Drosophila ribosomal preparations, the previously discussed pairs of spots (24 and 26, 5 and 10, 6 and 17) can be regarded as 6 unique proteins or 3 unique proteins and 3 artifacts. The three spots 52, 55 and 59 have been counted as one protein because they may be artifacts (Fig. 5 and 7b). A series arrangement like this in the gels of rat liver ribosomal proteins was replaced by one spot after a change in pretreatment of the ribosomes

(Sherton and Wool, 1972). In the HCl-acetone preparations of Drosophila ribosomal proteins, there were 42 - 45 major and 16 minor proteins with isoelectric points greater than 8.6, 1 protein with an isoelectric point close to 8.6, and possible 10 minor proteins with isoelectric points less than 8.6 (Fig. 5). A total of 69 - 72 protein components were present.

Comparison of 2-dimensional gels of ribosomal proteins from males and females. The gel patterns for Oregon-R males and C(1)RM/Y females were quite similar to one another and also to those for mixed samples from Oregon-R males and females. Differences, however, in the degree of staining of some spots were observed. Previous studies of Drosophila ribosomal proteins by 1-dimensional gel electrophoresis also indicated that males and females produced similar banding patterns although quantitative differences were observed (Kiefer and Gross, 1969; M. Gould Somero, personal communication).

Such quantitative differences are probably due to variation within the procedure and do not represent real differences in the samples. 2-dimensional gel electrophoresis is not intended to be a quantitative method. Staining conditions are not necessarily optimal and the amount of residue at the origin or along the axes varies.

The fact that the patterns for males and females were alike suggests that the isolation method produces ribosomes

that are reasonably free of extraneous proteins. Sex differences can create problems in isolating ribosomes. Egg proteins in female crickets, for example, were found to adsorb to the ribosomes and prevent their isolation by customary methods (Kavlenas, 1970). When females were freed of eggs, ribosomes could be isolated by the same method used for males.

2-dimensional gel electrophoresis of ribosomal proteins from cold-sensitive mutants. For each sample, one preparation of ribosomes was isolated and at least two gels were done for each preparation. In the 2-dimensional gels of the Drosophila mutants, 5^{cs}, 15^{cs}, 16^{cs}, 26^{cs} and 27^{cs}, no new spots or displaced ones were detected. In the gels of mutants 5^{cs} and 27^{cs} some of the faintly staining spots (58, 52, 55, and 59) were absent (Fig. 10 and 11) but were present in the gels of the mixed sample of Oregon-R males and females, Oregon-R males alone, C(1)RM females, and the three other mutants (Fig. 9 and 12).

The absence of the faintly staining spots is probably due to variability in the methods. They may be contaminants which are frequently but not always present in the isolated ribosomes. Or they may be ribosomal proteins occurring in low concentrations and may sometimes be lost during protein extraction or gel electrophoresis. Though a constant amount of sample was applied to the gels, the effective concentration of the various proteins which

migrate onto a gel may vary due to precipitation at the origin or along the axes.

An altered ribosomal protein from an E. coli mutant is detected by its displacement from the normal position found in gels of the wild type. A decreased amount of single protein is generally not observed. However, one E. coli "revertant" from streptomycin dependence had a displaced S4 spot which stained very faintly in 2-dimensional gels (Deusser, et al., 1970; Funatsu, et al., 1972). This protein normally produces a darkly staining spot. Other "revertants" had displaced S4 spots but they were not reduced in amount. The decreased staining of S4 from this particular mutant was attributed to a change in solubility of the protein and loss during gel electrophoresis. Protein S4 of the mutant was later isolated by column chromatography and found to be altered in end group, tryptic digest fingerprints, and molecular weight (Funatsu,, et al., 1972). It was concluded that the mutant protein was shorter by 9% compared to the wild-type protein.

It is unlikely that the quantitative differences observed in the gels of 5^{cs} and 27^{cs} are similar to the above example for the following reasons: (1) Several decreases in spots were noted. A single change in a protein would be expected to result from a mutation in the structural gene of a ribosomal protein. (2) Though the

pattern of protein migration in the gels was found to be constant, the degree of staining of spots was variable. (3) The degree of purity of the ribosomal preparations is uncertain. (4) Though mutants 5^{cs} and 27^{cs} have abnormal abdomen phenotypes, they are not related genetically. The mutations map to different areas of the X chromosome and the trans heterozygotes of 5^{cs} and 27^{cs} look like wild type.

DISCUSSION

Chemical analysis of ribosomes. Much effort has gone into the chemical analysis of E. coli ribosomes (Kurland, 1972). An accurate accounting of ribosomal constituents is a necessary preliminary to understanding how each component of the ribosomes functions in protein synthesis. On the basis of 2-dimensional gel electrophoresis, column chromatography, and reconstitution studies, it has been concluded that the small subunit contains 20-21 proteins (Wittmann, et al., 1971). The number of ribosomal proteins in the large subunit is still uncertain and may be as low as 28 or as high as 34 (Mora, et al., 1971; Dzionora, et al., 1970; Kaltschmidt and Wittmann, 1970b).

The main difficulty in such studies has been to distinguish ribosomal proteins from contaminants and artifacts. By ammonium sulfate precipitation of ribosomes, diethylaminoethyl chromatography and ammonium chloride washing of ribosomes, it was found that much extraneous protein could be removed from ribosomes without impairing their biological activity (Kurland, et al., 1966: Nomura, 1970). The problem of distinguishing unique proteins from artifacts was solved by isolating and analyzing each component of purified, biologically active ribosomal preparations. Two-dimensional gel electrophoresis of purified E. coli ribosomes, for example, indicated that 21 protein components were present in the small subunit

and 34 in the large subunit (Kaltschmidt and Wittmann, 1970). As some of the protein components were faintly staining they could be ribosomal proteins present in fewer copies than the major components or artifacts created by secondary modification of proteins. Minor protein fractions were also obtained when ribosomal proteins were separated by column chromatography. The advantage of this method, however, is that the fractions can be analyzed.

End group analysis, tryptic digest fingerprints, molecular weight determinations and immunological tests have shown that many protein components of the ribosome have specific characteristics and therefore are unique proteins (Mora, et al., 1971; Stoffler and Wittmann, 1971). On the other hand, some protein components are probably artifacts because their tryptic fingerprints etc. are similar to those of other fractions (Mora, et al., 1971). As already indicated, ribosomal mutants and the in vitro reconstitution of subunits are also important methods of establishing the identity of ribosomal proteins.

The analytical studies have indicated that isolated ribosomes of E. coli are heterogeneous (Kurland, 1972). Twelve of the proteins of the 30S subunits, for example, are present in approximately one copy per ribosome while eight "fractional" proteins are present in 0.5 or fewer copies per ribosome (Voynow and Kurland, 1971). Obviously isolated ribosomes cannot be uniform in composition.

One protein of the 50S subunit is unusual in that it appears to be present in 2 or 3 copies per ribosome (Moller, et al., 1972; Terhorst, et al., 1972). A number of interpretations of the heterogeneity have been advanced. One is that heterogeneity is an artifact and does not occur in vivo. A more interesting theory is that heterogeneity represents different functional states that the ribosome undergoes during protein synthesis (Kurland, 1972). The "fractional" ribosomal proteins, like the supernatant factors, may shuttle to and fro among the ribosomes during initiation, propagation, and termination of protein synthesis.

Proteins of Drosophila ribosomes. About 10 proteins with isoelectric points lower than pH 8.6 were observed in the present study of Drosophila ribosomes. Though these proteins occur in low concentration, they should not be ignored as contaminants until this is proved. Like the lightly staining spots on the cationic side of the gel, they may be fractional ribosomal proteins. To help distinguish between the two possibilities, Drosophila ribosomes should be purified by the methods previously discussed. The effectiveness of the procedures in removing extraneous proteins while maintaining the biological activity of the ribosomes should be established. Unfortunately the suitability of many E. coli procedures has not been established for Drosophila.

Separation of ribosomal subunits may also help to remove extraneous proteins. In 2-dimensional gels of purified rat liver "80S" ribosomes, for example, a maximum of 6 proteins was observed on the anionic side of the gel. When the ribosomal subunits were examined, 3 of the anionic spots, but no other spots were absent. As the procedure produced subunits that still functioned normally *in vitro*, only the 3 anionic proteins remaining are by definition, ribosomal proteins. Since 11 ribosomal proteins from *E. coli* subunits occur on the anionic side of 2-dimensional gels, it is thought that these proteins may occur more frequently in prokaryotic ribosomes than in eukaryotic ones.

The present study indicated that the number of proteins in *Drosophila* ribosomes is 69 to 72. In one-dimensional gels developed at acidic pH values, 22-30 protein bands are evident (Kiefer and Gross, 969; Lambertsson, *et al.*, 1970: M. Gould Somero, personal communication). Though two-dimensional gel electrophoresis resolved the *Drosophila* proteins to a greater extent than one-dimensional gels, it is obvious from the previous discussion that the number 69-72 is still only an approximation. The isolation procedure produces ribonucleoprotein particles of unknown purity and these were not tested for biological activity. Moreover, though one can make reasonable assumptions about artifacts in 2-dimensional gels, these cannot be proved except by

chemical analysis.

Despite some variation in estimates, it is generally found that "80S" eukaryotic ribosomes contain more proteins than 70S E. coli ribosomes. Purified preparations of rat liver ribosomes, for example, contained 68-72 protein components in 2-dimensional gels (Sherton and Wool, 1972). Estimates for other eukaryotes by this method vary from 70 to 76 ribosomal proteins. Hence the present estimate of 69-72 proteins for 82S Drosophila ribosomes is reasonable (Lambertsson, et al., 1970).

In fact, the number of Drosophila ribosomal proteins may have been slightly underestimated due to incomplete resolution of the proteins in a system designed for E. coli. Three pairs of proteins from the "80S" ribosomes of rat liver, for example, were not separated until the gel concentration and pH in the first dimension were changed from the standard conditions used for E. coli (Sherton and Wool, 1972). One pair of proteins was separated only when the subunits were examined.

Examination of ribosomes from cold-sensitive mutants of Drosophila. The advantage of 2-dimensional gel electrophoresis is that it requires a small sample and resolves most ribosomal proteins in one, relatively quick, procedure. The method should detect changes in the charge or size of ribosomal proteins if these occur in the cold-sensitive mutants. However, five sex-linked, cold-sensitive mutants

examined by the method, exhibited no displaced proteins. Quantitative differences observed in gels of mutants $\underline{5^{cs}}$ and $\underline{27^{cs}}$ were attributed to variability of the method. It was concluded that there was no detectable change in adult ribosomal proteins caused by mutations $\underline{5^{cs}}$, $\underline{15^{cs}}$, $\underline{16^{cs}}$, $\underline{26^{cs}}$ and $\underline{27^{cs}}$.

As a precaution, mutants $\underline{5^{cs}}$ and $\underline{27^{cs}}$ should be retested, preferably by an independent method such as sedimentation of ribosomal particles produced at low temperature. If the quantitative changes are due to contaminants or variability of the electrophoresis method, the sedimentation profiles for the mutants should be like that of wild-type ribosomal particles.

Ribosomal proteins from numerous cold-sensitive mutants were examined by 1-dimensional gel electrophoresis (M. Gould Somero, personal communication). These included 8 other recessive, cold-sensitive lethals of the X chromosomes; 25 dominant, cold-sensitive lethals of the 2nd chromosomes; 11 dominant, cold-sensitive developmentally retarded mutants of the 2nd chromosomes; and 3 recessive, cold-sensitive lethals of the 3rd chromosomes. The dominant retarded mutations are not lethals but prolong development from 5 to 10 days at 17^{o} C and 1-3 days at 22^{o} C (Rosenbluth, et al., 1972). Heterozygous females of the tested dominant, retarded mutants were sterile or poorly viable at 22^{o} C. Although irregularities were sometimes noted in the gels of some of the mutants, none were

reproducible (M. Gould Somero, personal communication). It was concluded that there was no evidence of change in the ribosomal proteins from the mutants.

The sedimentation preperties of ribosomal particles from a few of the cold-sensitive mutants were also studied (M. Gould Somero, personal communication). These included 5 recessive, cold-sensitive lethals of the X chromosomes which had been examined by 1-dimensional gel electrophoresis. Adult males were fed overnight at 17° C H^3 uridine with Drosophila food. Ribosomal preparations were sedimented in sucrose gradients. All the flies incorporated label into ribosomes at the low temperature. There was no apparent difference between the sedimentation profiles of ribosomes from the mutants and wild-type.

Limitations of the study and some recommendations. Do some cold-sensitive mutants of Drosophila have altered ribosomal proteins? No direct evidence to support the hypothesis was obtained. On the other hand, the negative results are not conclusive for the following reasons.

(1) A cold-sensitive mutant may have an altered amino acid sequence in a ribosomal protein but the electrophoretic properties of the protein may not be changed. Slight alterations in charge or size would be particularly difficult to detect in 1-dimensional gels. A study of streptomycin independence mutants of E. coli illustrates the problem (Deusser, et al., 1970). Out of 13 mutants,

4 exhibited changes in protein S4 in 2-dimensional gels. Three other mutants had S4 proteins which were indistinguishable from wild-type in 2-dimensional gels but were clearly different in immunological properties. If cold-sensitive ribosomal mutants of Drosophila occur in low frequency, the limitations of the method would be critical.

(2) A serious problem in the biochemical testing of the dominant, cold-sensitive mutants was the necessity of examining them in the heterozygous condition. Homozygotes of the dominant, cold-sensitive lethals are inviable even at the permissive temperature. And homozygotes of the dominant, retarded mutants could not be obtained because of the sterility or low fertility of the heterozygous females. If a dominant, cold-sensitive mutant had an altered ribosomal protein, the change may not have been detected in 1-dimensional gels because of the production of the normal ribosomal protein from the wild-type allele.

Altered ribosomal proteins in heterozygotes may be difficult to detect in other tests. I am uncertain whether abnormal subparticles would be detected in sedimentation profiles if normal ribosomes and subunits were produced at the same time. A change in the electrophoretic properties of a major ribosomal protein, however, would probably be detectable in heterozygotes by 2-dimensional gel electrophoresis. Both the normal and altered allelic ribosomal proteins of an E. coli mutant,

for example, were distinguishable in 2-dimensional gels of ribosomal proteins from the heterozygous merodiploid (Bollen, et al., 1973).

(3) Another limitation in the testing of the cold-sensitive mutants is that only adults were examined. If ribosomal proteins in the various developmental stages differ, then mutants with defects in larval or pupal ribosomes would have been overlooked.

There is good evidence that some components of the protein synthesizing system change during insect development. Numerous quantitative changes in the isoaccepting forms of t-RNAs were observed in 1st and 3rd instar larvae and adults of Drosophila (White, et al., 1973). It has also been shown that pupae of Tenebrio moletor produce a new species of t-RNA and a new synthetase (Ilan, 1969; Ilan, et al., 1970). In addition, stage-specific initiation factors for protein synthesis were found in this insect (Ilan and Ilan, 1971).

Two types of experiments suggest that the protein constituents of Drosophila ribosomes change during development. The ribosomal proteins from 2 larval and 2 pupal stages, as well as from adults, were compared by 2-dimensional gel electrophoresis (Lambertsson, 1972). Numerous differences in the protein patterns were apparent. Some spots increased in staining intensity from one stage to another while others faded. The observations suggest that some ribosomal proteins may be stage specific. The

evidence is not conclusive, however, because the observed differences may be due to (1) contaminants unrelated to protein synthesis, (2) supernatant factors such as initators of protein synthesis which become attached to ribosomes momentarily and (3) secondary changes in ribosomal proteins produced by proteolytic enzymes in the different developmental stages. The other experiments suggesting that ribosomes of larvae and adults differ, were discussed in the introduction. Larvae, but not adults, of the scarlet eye stock were susceptible to streptomycin (Duke and Glassman, 1968). And ribosomes from streptomycin sensitive larvae bound more streptomycin than those from streptomycin resistant larvae (Lambertsson and Rasmuson, 1971).

(4) An obvious omission are the cold-sensitive mutants still remaining to be tested. These include 14 recessive, cold-sensitive lethals on the X chromosomes and 29 recessive, cold-sensitive lethals of the 3rd chromosomes (Tasaka and Suzuki, 1973).

(5) More worrisome are the cold-sensitive mutants that may have been missed in the X chromosome screen. The present screen was designed on the basis of the genetic characteristics of the bacterial ribosomal mutants, i.e. the Sad mutations are cold-sensitive lethals and dominant to the normal allele. By observing the effects of low temperature in Drosophila with the genotype X/Y, where the X chromosome has been exposed to mutagen, the following

types of cold-sensitive mutants were expected: (a) Dominant conditional lethals in genes that are present on both the X and Y chromosomes. (b) Dominant conditional lethals in genes present on the X chromosome only. (c) Recessive conditional lethals in genes present only on the X chromosome and hence revealed in the hemizygous condition. As well as providing for three types of possible mutants, the screen involved less work than schemes which introduce the mutagenized chromosome into heterozygous females.

In fact, the screen gave only (c) type cold-sensitive mutants, i.e. recessive lethals detectable in the hemizygous condition. It is now known that almost all dominant heat- and cold-sensitive mutations of Drosophila are lethal when homozygous at the permissive temperature (Suzuki and Procunier, 1969; Rosenbluth, et al., 1972; Holden and Suzuki, 1973). Thus screening via hemizygosity may select against dominant mutations. Also, dominant mutations of types (a) and (b) may not have been isolated because they occur infrequently and insufficient numbers of chromosomes were tested.

A fourth type of possible mutant, i.e. recessive lethals in genes present on both the X and Y, could not be detected by our screen. Such mutants may be important if the characteristics of the ribosomal protein genes are like those of the ribosomal RNA genes. The bobbed

mutations, which are thought to be deletions in the redundant regions coding for ribosomal RNA, are recessive and map on the X and Y chromosomes (Ritossa, et al., 1966; Lindsley and Grell, 1967). Although the genes for ribosomal proteins occur in single copies in E. coli, this situation is not necessarily true for Drosophila (Davies and Nomura, 1972). Eukaryotes, for example, have more repetitive DNA than bacteria (Britten and Kohn, 1968). Furthermore a recent study of ribosomes from D. melanogaster - D. simulans hybrids by 1-dimensional gels suggests that genes for ribosomal proteins are located on the X chromosome near bb (Steffensen, 1973).

It may, therefore, be worthwhile to rescreen for cold-sensitive mutations on the X chromosomes by another selection scheme. All four types of mutants should be obtainable in a traditional screen where the mutagenized X chromosome is introduced into a heterozygous female and then tested in both the homozygous and heterozygous condition (Suzuki, et al., 1967; Tasaka and Suzuki, 1973).

(6) Biochemical examination of the mutants was almost exclusively by gel electrophoresis of ribosomal proteins. It is possible that the sedimentation of ribosomal particles from cold-treated Drosophila could have detected differences where electrophoresis cannot. Although subparticles which do not greatly differ in size from normal subunits are difficult to detect by this method, it was successfully used to identify several Sad mutants of

bacteria (Tai, et al., 1969; Nomura, 1970).

None of the Drosophila mutants seemed to be susceptible to the lethal effect of cold as adults (Rosenbluth, et al., 1972, Tasaka, personal communication). Hence the effect of cold on subunit assembly can be readily studied in adults. To apply this approach to pupae and larvae, however, would require preliminary study of the development of the mutants. The lethal phase and temperature-sensitive period of each mutant should be determined. The lethal phase is the developmental stage at which the organism dies after continuous exposure to the restrictive temperature (Suzuki, 1970). The temperature-sensitive period precedes the lethal phase and is considered to be the interval when the gene product is produced, activated or first needed for a critical developmental process. Hence if a mutant is kept at the non-permissive temperature during the temperature-sensitive period, it will later die even if shifted to the permissive temperature. The temperature-sensitive period of the cold-sensitive mutants may be the developmental stage when a defect in subunit assembly is most evident. Establishment of the temperature-sensitive period and lethal phase would also be a useful means of comparing cold-sensitive mutants and the previously studied heat-sensitive mutants.

Conclusion. The hypothesis that ribosome assembly defects should occur in cold-sensitive mutants is based on chemical

principles. It should, therefore, apply to the ribosomes of other organisms as well as those of *E. coli*: Although considerable work was done in testing the theory on *Drosophila*, no evidence for or against the hypothesis was found. Because of the greater complexity of *Drosophila*, the theory has still been incompletely tested.

BIBLIOGRAPHY

Apirion, D. and Schlessinger, D. 1967. The loss of phenotypic suppression in streptomycin-resistant mutants. Proc. Nat. Acad. Sc. U.S. 58: 206-12.

Atwood, K.C. 1969. Some aspects of the bobbed problem in Drosophila. Genetics, Supplement 61: 319-327.

Baillie, D., Suzuki, D.T., and Tarasoff, M. 1968. Temperature-sensitive mutations in Drosophila melanogaster. 11. Frequency among second chromosome recessive lethals induced by ethyl methanesulfonate. Can. J. Genet. Cytol. 10: 412-420.

Bayliss, F.T. and Vinopal, R.T. 1971. Selection of ribosomal mutants by antibiotic suppression in yeast. Science 174: 1339-1341.

Birge, E.A., Craven, G.R., Hardy, S.J.S., Kurland, C.G., Voynow, P. 1969. Structure determinant of a ribosomal protein: K. locus. Science 164: 1285-86.

Birge, K.A. and Kurland, C.G. 1969. Altered ribosomal protein in streptomycin-dependent Escherichia coli. Science 166: 1282-1284.

Birge, E.A. and Kurland, C.G. 1970. Reversion of a streptomycin dependent strain of Escherichia coli. Mol. Gen. Genet. 109: 356-69.

Birnie, G.D. (ed.) 1972. "Subcellular components, preparation and fractionation." London, Butterworths. p206-211.

Bollen, A., Davies, J., Ozaki, M., and Mizushima, S. 1969. Ribosomal protein conferring sensitivity to the antibiotic spectinomycin in Escherichia coli. Nature 165: 85-86.

Bollen, A. and Herzog, A. 1970. The ribosomal protein altered in spectinomycin resistant Escherichia coli. FEBS Letters 6: 69-72.

Bollen, A., et al. 1973. The structural gene for the ribosomal protein S18 in Escherichia coli. 1. Genetic studies on a mutant having an alteration in the protein S18. J. Mol. Biol. 76: 463-472.

Boshes, R.A. 1970. Drosophila ribosomes. The characterization of two populations by cell fractionation and isotopic labeling with nucleic acid and protein precursors. J. Cell Biology 46: 477-490.

Breckenridge, L. and Gorini, L. 1969. The dominance of streptomycin sensitivity re-examined. Proc. Nat. Acad. Sc. U.S.A. 62: 979-85.

Britten, R.J. and Kohne, D.E. 1968. Repeated sequences in DNA. Science 161: 529-540.

Brown, D.D., and Dawid, I.B. 1968. Specific gene amplification in oocytes. Science 160: 272-280.

Cooper, K.W. 1959. Cytogenetic analysis of major heterochromatic elements (especially Xh and Y) in Drosophila melanogaster and the theory of Heterochromatin. Chromosoma 10: 535-588.

Counce, S.J. 1956a. Studies on female-sterility genes in Drosophila melanogaster. 1. The effects of the gene deep orange on embryonic development. Z. indukt. Abstamm. Vererbungs. 87: 443-461.

Counce, S.J. 1956b. Studies on female-sterility genes in Drosophila melanogaster. 11. The effects of the gene fused on embryonic development. Z. indukt. Abstamm. Vererbungs. 87: 462-481.

Counce, S.J. 1956c. Studies on female-sterility genes in Drosophila melanogaster. 111. The effects of the gene rudimentary on embryonic development. Z. indukt. Abstamm. Vererbungs. 87: 482-492.

Cox, J.H. and Strack, H.B. 1971. Cold-sensitive mutants of bacteriophage λ. Genetics 67: 5-17.

Craven, G.R., Voynow, P., Hardy, S.J.S., and Kurland, C.G. 1969. The ribosomal proteins of Escherichia coli. 11. Chemical and physical characterization of the 30S ribosomal proteins. Biochem. 8: 2906-2915.

Davies, J. 1964. Studies on the ribosomes of streptomycin-sensitive and resistant strains of Escherichia coli. Proc. Nat. Acad. Sci. U.S.A. 51: 659-64.

Davies, J. 1966. Streptomycin and the genetic code. Cold Spring Harbor Symp. Quant. Biol. 31: 665-669.

Davies, J. and Nomura, M. 1972. The genetics of bacterial ribosomes. Annual Review of Genetics 6: 203-234.

Dekio, S. and Takato, R. 1969. Genetic studies of the ribosomal proteins of Escherichia coli. II. Altered 30S ribosomal protein component specific to spectinomycin resistant mutants. Mol. Gen. Genetics. 105: 219-24.

Dekio, S., Takata, R., and Osawa, S. 1970. Genetic studies of ribosomal proteins in Escherichia coli. VI. Determination of chromosomal loci for several ribosomal protein components using a hybrid strain between Escherichia coli and Salmonella typhimurium. Mol. Gen. Genet. 109: 131-41.

Deusser, E., Stoffler, G., Wittmann, H.G., and Apirion, D. 1970. Ribosomal proteins, XVI. Altered S4 proteins in Escherichia coli revertants from streptomycin dependence to independence. Mol. Gen. Genet. 109: 298-302.

Dowell, C. 1967. Cold-sensitive mutants of ϕX174. 1. A mutant blocked in the eclipse function at low temperature. Proc. Nat. Acad. Sci. U.S.A. 58: 958-961.

Dudick, M. and Wright, T.R.F. A temperature sensitive lethal allele of suppressor of forked in Drosophilia. (In Press).

Duke, E.J. and Glossman, E. 1968. Drug effects in Drosophila: Streptomycin sensitive strains and fluorourogil resistant strains. Nature 220: 588-9.

Dzionara, M., Kaltschmidt, E., and Wittman, H.G. 1970. Ribosomal proteins, XIII. Molecular weights of isolated ribosomal proteins of Escherichia coli. Proc. Natl. Acad. Sc. U.S. 67: 1909-13.

Edgar, R.S., and Lielausis, I. 1964. Temperature-sensitive mutation in T4D: their isolation and genetic characterization. Genetics 49: 649-662.

Epler, J.L. 1966. Ethyl methanesulfonate-induced lethals in Drosophila-frequency-dose relations and multiple mosaicism. Genetics 54: 31-36.

Epstein, R.H., Bolle, A., Steinberg, C.M., Kellenberger, E., Boy de la Tour, E., Chevalley, R., Edgar, R.S., Susman, S., Denhardt, G., and Lielausis, A. 1963. Physiological studies of conditional lethal mutations of bacteriophage T4D. Cold Spring Harb. Symp. Quant. Biol. 28: 375-392.

Funatsu, G., Puls, W., Schilts, E., Reinbolt, J. and Wittmann, H.G. 1972. Ribosomal proteins XXXI. Comparative studies on altered proteins S4 of six Escherichia coli revertants from streptomycin dependence. Molec. Gen. Genetics 115: 131-139.

Gall, J.G. 1969. The genes for ribosomal RNA during oogenesis. Genetics 61: 121-132, Suppl. 1.

Gorini, L., Jacoby, G.A., and Breckenridge, L. 1966. Ribosomal ambiguity. Cold Spring Harbor Symp. Quant. Biol. 31: 657-64.

Gorini, L. 1971. Ribosomal discrimination of t-RNAs. Nature New Biol. 234: 261-64.

Gunter, B. and Sabatini, D. 1971. Dissociation of mammalian polyribosomes into subunits by puromycin. Proc. Nat. Acad. Sc. 68: 390-394.

Guthrie, C., Nashimoto, H., and Nomura, M. 1969a. Structure and function of E. coli ribosomes. VIII. Cold-sensitive mutants defective in ribosome assembly. Proc. Nat. Acad. Sci. U.S.A. 63: 384-391.

Guthrie, C., Nashimoto, H., and Nomura, M. 1969b. Studies on the assembly of ribosomes in vivo. Cold Spring Harbor Symp. Quant. Biol. 34: 69-75.

Hardy, S.J.S., Kurland, C.G., Voynow, P., and Mora, G. 1969. The ribosomal proteins of Escherichia coli. I. Purification of the 30S ribosomal proteins. Biochem. 8: 2897-2905.

Hartwell, L.H. 1967. Macromolecule synthesis in temperature-sensitive mutants of yeast. J. Bact. 93: 1662-1670.

Hashimoto, K. 1960. Streptomycin resistance in Escherichia coli analyzed by transduction. Genetics 45: 49-62.

Holden, J., and Suzuki, D.T. 1973. Temperature-sensitive mutations in Drosophila melanogaster. XII. The genetic and developmental characteristics of dominant lethals on chromosome 3. Genetics, in press.

Igarashi, S. 1966. Temperature-sensitive mutation in Paramecium. 1. Induction and inheritance. Mut Res. 3: 13-24.

Ilan, J. 1969. The role of t-RNA in translational control of specific mRNA during insect metamorphosis. Cold Spring Harbor. Symp. Quant. Biol. 34: 789-791.

Ilan, J., Ilan, J., and Patel, N. 1970. Mechanism of gene expression in Tenebrio molitor. Juvenile hormone determination of translation control through transfer ribonucleic acid and enzyme. J. Biol. Chem. 245: 1275-1281.

Ilan, J. and Ilan, J. 1971. Stage-specific initiation factors for protein synthesis during insect development. Devel. Biol. 25: 280-292.

Jacobson, K.B. 1971. Role of an isoacceptor transfer ribonucleic acid as an enzyme inhibitor: Effect on trypotphane synthetase. Nature New Biol. 231: 17-19.

Jenkins, J.B. 1967. Mutagenesis at a complex locus in Drosophila with the mono-functional alkylating agent, EMS. Genetics 57: 783-793.

Kahan, L., Zengel, J., Nomura, M., Bollen, A., and Herzog, A. 1973. The structural gene for the ribosomal protein S18 in Escherichia coli. II. Chemical studies on the protein S18 having an altered electropharetic mobility. J. Mol. Biol. 76: 473-483.

Kaji, H. and Tanaka, Y. 1968. Binding of dihydrostreptomycin to ribosomal subunits. J. Mol. Biol. 32: 221-30.

Kaltschmidt, E., and Wittman, H.G. Ribosomal proteins. 1970a. VII. Two-dimensional polyacrylamide gel electrophoresis for fingerprinting of ribosomal proteins. Analytical Biochemistry. 36: 401-412.

Kaltschmidt, E., and Wittmann, H.G. 1970b. Ribosomal proteins. XII. Number of proteins in small and large ribosomal subunits of Escherichia coli as determined by two-dimensional gel electrophoresis. Proc. Nat. Acad. Sci. U.S.A. 67: 1276-1282.

Kaltschmidt, E., Stoffler, G., Dzionara, M., and Wittmann, H.G. 1970c. Ribosomal proteins. XVII. Comparative studies on ribosomal proteins of four strains of Escherichia coli. Molec. gen. Genet. 109: 303-308.

Kaulenas, M.S. 1970. Factors affecting ribosome isolation from adult crickets, Acheta domesticus J. Insect Physiol. 16: 813-824.

Kiefer, B.I. 1969. Dosage regulation of ribosomal DNA in Drosophila melanogaster. Proc. Nat. Acad. Sc. 61: 85-89.

Kiefer, B.I. and Gross, K. 1969. Isolation and characterization of the ribosomal proteins of Drosophila melanogaster. Genetics 61, supplement 1969, No. 2, part 2, pS22.

King, R.C. 1970. "Ovarian development in Drosophila melanogaster." N.Y., Academic Press. 227p.

Kreider, G. and Brownstein, B.L. 1972. Ribosomal proteins involved in the suppression of streptomycin dependence in Escherichia coli. J. Bacterial. 109: 780-83.

Kurland, C.G. 1966. The requirements for specific binding by ribosomes. J. Mol. Biol. 18: 90-108.

Kurland, C.G., Voynow, P., Hardy, S.J.S., Randall, L., and Lutter, L. 1969. Physical and functional heterogeneity of E. coli ribosomes. Cold Spring Harbor Symp. Quant. Biol. 34: 17-24.

Kurland, C.G. 1972. Structure and function of the bacterial ribosome. Annual Review of Biochemistry 41: 377-408.

Lambertsson, A.B., Rosmuson, S.B. and Bloom, G.D. 1970. The ribosomal proteins of Drosophila melanogaster. I. Characterization in polyacrylamide gel of proteins from larval, adult, and NH_4 Cl - treated ribosomes. Molec. Gen. Genet. 108: 349-357.

Lambertsson, A.G. and Rosmuson, S.B. 1971. Studies on the ribosomes and ribosomal proteins of Drosophila melanogaster. Hereditas (Lund) 69: 299 (1971).

Lambertsson, A.G. 1972. The ribosomal proteins of Drosophila melanogaster. II. Comparison of protein patterns of ribosomes from larvae, pupae, and adult flies by two-dimensional gel electrophoresis. Molec. Gen. Genet. 118: 215-222.

Leboy, P.S., Cox, E.C., and Flaks, J.L. 1964. The chromosomal site specifying a ribosomal protein in Escherichia coli. Proc. Nat. Acad. Sci. U.S.A. 52: 1367-74.

Lewandowski, L.J. and Brownstein, B.L. 1969. Characterization of a 43 S ribonucleoprotein component of a mutant of Escherichia coli. J. Mol. Biol. 41: 277-90.

Lewis, E.B., and Bacher, F. 1968. Method of feeding ethyl methanesulfonate (EMS) to Drosophila males. Dros. Inform. Serv. 43: 193.

Lindsley, D.L., and Grell, E.H. 1968. Genetic variations of Drosophila melanogaster. Carnegie Inst. Wash. Publ. 627.

Martin, R.G. 1963. The first enzyme in histidine biosynthesis: The nature of feedback inhibition by histidine. J. Biol. Chem. 238: 257-268.

Mathias, A.P. and Williamson R. 1964. Studies on the Protein of Rabbit Reticulocyte Ribosomes. J. Mol. Biol. 9: 498-502.

Mayoh, H. and Suzuki, D.T. 1973. Temperature-sensitive mutations in Drosophila melanogaster. XVI. The genetic properties of sex-linked recessive cold-sensitive mutants. Can. J. Genet. Cytol. 15: 237-254.

Mayuga, C., Meier, D., and Wang, T. 1968. Escherichia coli: The K12 ribosomal protein and the streptomycin region of the chromosome. Biochem. Biophys. Res. Commun. 33: 203-06.

Mizushima, S. and Nomura, M. 1970. Assembly mapping of 30S ribosomal proteins from E. coli. Nature 226: 1214-18.

Mohan, J., and Ritossa, F.M. 1970. Regulation of ribosomal RNA synthesis and its bearing on the bobbed phenotype in Drosophila melanogaster. Dev. Biol. 22: 495-512.

Moller, W., Groene, A., Terhorst, C., Amons, R. 1972. 50S ribosomal proteins. Purification and partial characterization of two acidic proteins, A1 and A2, isolated from 50S ribosomes of Escherichia coli. Eur. J. Biochem. 25: 5-12.

Mora, G., Donner, D., Thammana, P., Lutter, L., Kurland, C.G., and Craven, G.R. 1971. Purification and characterization of 50S ribosomal proteins of Escherichia coli. Mol. Gen. Genet. 112: 229-242.

Nashimoto, H. and Nomura, M. 1970. Structure and function of bacterial ribosomes. XI. Dependence of 50S ribosomal assembly on simultaneous assembly of 30S subunits. Proc. Nat. Acad. Sci. U.S. 67: 1440-47.

Nashimoto, H., Held, W., Kaltschmidt, E., and Nomura, M. 1971. Structure and function of bacterial ribosome. XII. Accumulation of 21S particles by some cold-sensitive mutants of Escherichia coli. J. Mol. Biol. 62: 121-138.

Nomura, M. 1970. Bacterial ribosome. Bacteriological Reviews. 34: 228-277.

Nomura, M. and Erdmann, V.A. 1970. Reconstitution of 50S ribosomal subunits from dissociated molecular components. Nature. 228: 744-48.

Nomura, M. and Engback, F., 1972. Expression of ribosomal protein genes as analyzed by bacteriophage Mu-induced mutations. Proc. Nat. Acad. Sc. U.S. 69: 1526-1530.

Nomura, M. 1972b. Assembly of bacterial ribosomes. Fed. Proc. 31: 18-20.

O'Donovan, G.A., Kearney, C.L., and Ingraham, J.L. 1965a. Mutants of Escherichia coli with high minimal temperatures of growth. J. Bact. 90: 611-616.

O'Donovan, G.A. and Ingraham, J.L. 1965b. Cold-sensitive mutants of Escherichia coli resulting from increased feedback inhibition. Proc. Nat. Acad.Sc. U.S. 54: 451-457.

Ozaki, M., Mizushima, S., Nomura, M. 1969. Identification and functional characterization of the protein controlled by the streptomycin-resistant locus in E. coli. Nature 222: 333-39.

O'Neil, D.M., Baron, L.S., Sypherd, P.S. 1969. Chromosomal location of ribosomal protein cistrons determined by intergeneric bacterial mating. J. Bacteriol. 99: 242-47.

Redfield, H. 1926. The maternal inheritance of a sex-limited lethal effect in Drosophila melanogaster. Genetics 11: 482-502.

Reid, P. 1971. Isolation of cold-sensitive rifampicin resistant RNA polymerase mutants of E. coli. Biochem. Biophys. Research Communications 44: 737 (1971).

Ritossa, F.M., Atwood, K.C., Lindsley, D.L., and Spiegelman, S. 1965. On the chromosomal distribution of DNA complementary to ribosomal and soluble RNA. Nat. Cancer Inst. Mono. 23: 449-472.

Ritossa, F.M., Atwood, K.C., and Spieglman, S. 1966. A molecular explanation of the bobbed mutants of Drosophila as partial deficiencies of ribosomal RNA. Genetics 54: 819-34.

Roberts, L.M. and Reeve, E.C.R. 1970. Two mutations giving low-level streptomycin resistance in Escherichia coli K12. Genet. Res. Comb. 16: 359-65.

Rosenbluth, R., Ezell, D., and Suzuki, D.T. 1972. Temperature-sensitive mutations in Drosophila melanogaster. IX. Dominant cold-sensitive lethals on the autosomes. Genetics 70: 75-86.

Rosset, R. and Gorini, L. 1969. A ribosomal ambiguity mutation. J. Mol. Biol. 39: 95-112.

Rosset, R., Vola, C., Feunteun, J., and Monier, R. 1971. A thermosensitive mutant defective in ribosomal 30S subunit assembly. FEBS Letters 18: 127-129.

Schalet, A., and Finnerty,V. 1968a. New mutants. Dros. Inform. Serv. 43: 65-66.

Schalet, A., and Finnerty, V. 1968b. The arrangements of genes in the proximal region of the X chromosome of Drosophila melanogaster. Dros. Inform. Serv. 43: 128-129.

Schalet, A. 1973. The suppressor of forked locus: seven characteristics in search of a moleculor author. Third European Drosophila Res. Conf. Abstracts. In press.

Scotti, P.D. 1968. A new class of temperature conditional lethal mutants of bacteriophage T4D. Mut. Res. 6: 1-14.

Leaf 99 omitted in page numbering.

Page 99 does not exist

Sheppard, D.E. 1964. Mutants of Salmonella typhimurium resistant to feedback inhibition by L-histidine. Genetics. 50: 611-623

Sherton, C.C. and Wool, I.G. 1972. Determination of the number of proteins in liver ribosomes and ribosomal subunits by two-dimensional polyacrylamide gel electrophoresis. J. Biol. Chem. 247: 4460-4467.

Smith, J.D. 1972. Genetics of transfer RNA. Annual Review Genetics 6: 235-256.

Smith, R.H. 1968. Unstable temperature-sensitive mutations in Habobracon. Genetics 60: 227 (Abst.)

Spofford, J.B. 1961. Parental control of position-effect variegation. II. Effect of sex of parent contributing white-mottled rearrangement in Drosophila melanogaster. Genetics 46: 1151-1167.

Steffensen, D.M. 1973. Mapping genes for the ribosomal protein of Drosophila. Nature New Biology 244: 231-234.

Stoffler, G. and Wittmann, H.G. 1971. Sequence differences of Escherichia coli 30S ribosomal proteins as determined by immunochemical methods. Proc. Natl. Acad. Sc. U.S. 68: 2283-87.

Stoffler, G., Deusser, E., Wittmann, H.G., Apirion, D. 1971. Ribosomal proteins. XIX. Altered S5 ribosomal protein in an Escherichia coli revertant from streptomycin dependence to independence. Mol. Gen. Genet. 111: 334-41.

Strigini, P. and Gorini, L. 1970. Ribosomal mutations affecting efficiency of amber suppression. J. Mol. Biol. 47: 517-30.

Suzuki, D.T. 1970. Temperature-sensitive mutations in Drosophila melanogaster. Science 170: 695-706.

Suzuki, D.T., and Piternick, L.K. 1972. Temperature-sensitive mutations in Drosophila melanogaster. XVI. Genetic positions and viability indices of sex-linked recessive lethals. Submitted for publication.

Suzuki, D.T., Piternick, L.K., Hayashi, S., Tarasoff, M., Baillie, D., and Erasmus, U. 1967. Temperature-sensitive mutations in Drosophila melanogaster. I. Relative frequencies among δ-ray and chemically induced sex-linked recessive lethals and semilethals. Proc. Nat. Acad. Sci. U.S.A. 57: 907-912.

Suzuki, D.T., and Procunier, D. 1969. Temperature-sensitive mutations in Drosophila melanogaster. III. Dominant lethals and semilethals on chromosome 2. Proc. Nat. Acad. Sci. U.S.A. 62: 369-376.

Sypherd, P.S., O'Neil, D.M., and Taylor, M.M. 1969. The chemical and genetic structure of bacterial ribosomes. Cold Spring Harbor Symp. Quant. Biol. 34: 77-84.

Tai, P.C., Kessler, D.P., and Ingraham, J. 1969. Cold-sensitive mutations in Salmonella typhimurium which affect ribosome synthesis. J. Bact. 97: 1298-1304.

Tarasoff, M., and Suzuki, D.T. 1970. Temperature-sensitive mutations in Drosophila melanogaster. VI. Temperature effects on development of sex-linked recessive lethals. Dev. Biol. 23: 492-509.

Tartof, K.D. and Perry, R.P. 1970. The 5S RNA genes of Drosophila melanogaster. J. Mol. Biol. 51: 171-183.

Tasaka, S.E. and Suzuki, D.T. 1973. Temperature-sensitive mutations in Drosophila melanogaster. XVII. The genetic properties of heat- and cold-sensitive lethals on chromosome 3. Genetics (in press).

Terhorst, C., Wittmann-Liebold, B., and Moller, W. 1972. 50S ribosomal proteins. Peptide studies on two acidic proteins, A1 and A2, isolated from 50S ribosomes of Escherichia coli. Eur. J. Biochem. 25: 13-19.

Thompson, D.H. 1921. A new type of sex-linked lethal in Drosophila. Anat. Rec. 20: 215 (Abst.).

Traub, P. and Nomura, M. 1968. Streptomycin resistance mutation in Escherichia coli: altered ribosomal protein. Science 160: 198-99.

Traub, P. and Nomura, M. 1969. Structure and function of Escherichia coli ribosomes. VI. Mechanism of assembly of 30S ribosomes studied in vitro. J. Mol. Biol. 40: 391-413.

Twardzik, D.R., Grell, E.H. and Jacobson, K.B. 1971. Mechanism of suppression in Drosophila: a change in tyrosine transfer RNA. J. Mol. Biol. 57: 231-245.

Valentine, R.C., Wrigley, N.G., Scrutton, M.C., Irias, J.J., and Merton, F.U. 1966. Pyrurate carboxylase. VIII. The subunit structure as examined by electron microscopy. Biochemistry. 5: 3111-3116.

Van Holde, K.E. 1966. The molecular architecture of multichain proteins in "Molecular Architecture and Cell Physiology" Hayashi, T. and Szent Gyorgyi, A.G. ed. Englewood Cliffs, N.J.: Prentice-Hall: 1966.

Voynow, P. and Kurland, C.G. 1971. Stoichiometry of the 30S ribosomal proteins of Escherichia coli. Biochem. 10: 517-24.

Welfle, H., Stahl, J. and Bielka, H. Studies on proteins of animal ribosomes. VIII. Two-dimensional polyacrylamide gel elextrophoresis of ribosomal proteins of rat liver. Biochimica et Biophysica Acta 243: 416-419 (1971).

White, B.N., Tener, G.M., Holden, J., and D.T. Suzuki. 1973a. Activity of a tRNA modifying enzyme during the development of Drosophila and its relationship to the su(s) locus. J. Mol. Biol. 74: 635-651.

White, B.N., Holden, J., Suzuki, D.T. and Tener, G.M. 1973b. Analysis of tRNA during the development of Drosophila. Devel. Biol. 33: 185-195.

Wimber, D.E. and Steffensen, D.M. 1970. Localization of 5S RNA genes on Drosophila chromosomes by RNA-DNA hybridization. Science 170: 639-41.

Wittmann, H.G., et al. 1971. Correlation of 30S ribosomal proteins of Escherichia coli isolated in different laboratories. Mol. Gen. Genet. III: 327-33.

Wright, T. 1973. The recovery, penetrance, and pleiotropy of X-linked, cold-sensitive mutants in Drosophila. Molec. Gen. Genet. 122: 101-118.

Yamada, T. and Davies, J. 1971. A genetic and biochemical study of streptomycin- and spectinomycin-resistance in Salmonella typhimurium. Mol. Gen. Genet. 110: 197-210.

Zimmermann, R.A., Garnin, R.T., Gorini, L. 1971. Alteration of a 30S ribosomal protein accompanying the ram mutation in Escherichia coli. Proc. Nat. Acad. Sc. U.S. 68: 2263-67.

Appendix

Calculation of the frequency of cs lethals as a percentage of single hits.

$\hat{XXY}$ x X/Y in bottles

no treatment, $\sigma^{\uparrow}/\varphi = \frac{469}{463} = 1.01$

$\sigma^{\uparrow}\sigma^{\uparrow}$ treated with 0.005 M EMS, $\sigma^{\uparrow}/\varphi = \frac{332}{517} = 0.64$

To calculate the frequency of single hits

$$P_n = \frac{X^n e^{-X}}{n!}$$

where n = number of hits and X = frequency of hit.

When n = 0, i.e. no hit,

$$P_n = .64 = \frac{X^0 e^{-X}}{0!}$$

$$e^{-X} = .64$$

$$X = .46$$

Frequency of single hits

$$P_1 = \frac{.46^1 (.64)}{1!} = .2904$$

Frequency of cs lethals = $\frac{18}{2,597}$ x .64 = .004436

where no. of tested $\sigma^{\uparrow}\sigma^{\uparrow}$ = 2,597, no. of cs lethals = 18, and frequency of no hit $\sigma^{\uparrow}\sigma^{\uparrow}$ = .64

% single hit sex-linked lethals that are cold-sensitive = $\frac{.44}{29}$ x 100 = 1.5% on the average.

In a single experiment, 9 cs lethals were found out of 672 tested chromosomes. The maximum % single hit sex-linked lethals that were cold-sensitive = $\frac{9}{672}$ x .64 x $\frac{100}{.29}$ = 3%.

TABLE I

The effect of temperature on the viabilities of mutant males, relative to the Oregon-R control, from the cross, $\ell(1)^{cs}/Y$ ♂ x C(1)RM or C(1)DX♀.

Stock* ♂♂	Stock* ♀♀	Test	22°C Viability (%)	22°C Total Flies	17°C Viability (%)	17°C Total Flies
OR		1	100	1252	100	1095
1^{cs}	RM	1	81.8	795	0	347
		2	72	192	0	63
		3	71.8	375	1	232
	RA	1	69.6	340	0.5	288
2^{cs}	RM	1	42.0	249	0.5	163
	RA	1	34	187	0.9	86
3^{cs}	RM	1	35	118	0	42
		2	37.7	341	1	148
		3	41	190	0.6	263
		4	102	252	0	110
	RA	1	43	208	0.4	180
4^{cs}	RM	1	49.9	421	3.3	340
		2	16	268	2	259
		3	14	283	3	140
		4	42	166	2	296
	RA	1	49.4	265	3	428
7^{cs}	RM	1	78.1	275	6.1	267
		2	87.6	303	6.2	253
		3	89.1	277	11	352
8^{cs}	RM	1	47	167	2	98
		2	46	87	3	104
	RA	1	56.6	284	0.3	368
9^{cs}	RM	1	61	171	0	225
		2	60	106	11	121
10^{cs}	RM	1	31	147	1	129
		2	22	177	0	156
		3	56.0	212	3	146
11^{cs}	RM	1	61.2	305	2	322
		2	78.1	261	8.6	117
		3	70.9	286	3	263
		4	58	196	23	177
	RA	1	60.6	366	3.3	388
12^{cs}	RM	1	50.1	253	0	319
		2	28	134	0.3	266
		3	48	183	0	51
		4	36	116	0	76

Table I (cont'd)

13^{cs}	RM	1	66.5	449	0.2	362
	RA	1	34.1	336	1	300
		2	5	68	0	62
14^{cs}	RM	1	33.3	259	7.3	226
		2	36.7	299	1	225
		3	45.0	244	9.3	146
		4	45.4	292	12	245
		5	31.1	489	2	292
16^{cs}	RM	1	92.0	307	0	226
		2	47	135	0	38
		3	10	135	1	366
	RA	1	68.6	225	0.5	160
17^{cs}	RM	1	45	74	0	83
		2	57.3	266	2.4	322
		3	62.4	308	0.8	99
	RA	1	87	140	9.4	163
18^{cs}	RM	1	76.6	951	12.5	746
	RA	1	79.6	333	12	330
		2	76.6	958	15.1	794
19^{cs}	RM	1	52.6	222	0	103
		2	52.8	355	4	143
		3	80	94	15	122
	RA	1	85.4	351	18	340
20^{cs}	RM	1	47	131	0	40
		2	71.4	637	0	326
	RA	1	67.5	1007	0	486
21^{cs}	RM	1	69	124	7	24
		2	98.5	1167	1	545
	RA	1	76.6	1362	0.3	601
22^{cs}	RM	1	100	155	2	103
		2	82.5	370	13	373
	RA	1	65.7	228	4.8	266
23^{cs}	RM	1	29	249	0	59
		2	100	340	1	230
	RA	1	63.4	381	0.3	266
24^{cs}	RM	1	48.9	379	2	88
		2	52.1	372	4.7	323
		3	60.3	347	2.4	414
	RA	1	85.4	284	3	229
25^{cs}	RM	1	21	261	0	63
		2	75.2	393	12	496
	RA	1	50.5	653	17.1	704

Viability at 22°C = (mutant ♂♂)//(C(1)RM/Y ♀♀) x 100/1.37
Viability at 17°C = (mutant ♂♂)//(C(1)RM/Y ♀♀) x 100/1.32
RM = C(1)RM/Y females RA = C(1)DX, y f/Y females OR = Oregon-R
*Mutants 1 to 18 were derived from 2,597 X chromosomes treated with 0.005M EMS; mutants 19 to 27, from 1,322 chromosomes treated with 0.0125M EMS.

TABLE II

The effect of temperature on the frequencies of the abnormal abdomen phenotype and relative viabilities of mutant males from the cross, $l(1)^{cs}$/Y♂ X C(1)RM/Y or C(1)DX/Y♀.

Stock ♂♂	Stock ♀♀	Test	22°C %V*	22°C Total Flies	22°C % aa	17°C %V**	17°C Total Flies	17°C % aa
OR		1	100	1252	0	100	1095	0
5^{cs}	RM	1	66.8	249	-	0	306	-
		2	67.4	273	-	2	165	-
		3	55.5	310	49	3.4	304	100
	RA	1	64	133	40	11	71	78
		2	67.4	458	33	9.8	359	54
6^{cs}	RM	1	54.7	357	-	5.1	316	-
		2	70	159	-	6.5	227	-
		3	69.3	341	56	6.8	327	70
	RA	1	72.5	291	-	21	268	46
		2	83	147	65	6	55	100
		3	78.8	540	28	18	400	61
15^{cs}	RM	1	70.9	339	-	1	166	-
		2	52	210	-	0.9	83	-
		3	51	121	-	7.4	135	-
		4	75.9	270	77	12	235	81
	RA	1	65.0	225	-	21	183	70
		2	58.8	251	37	6	88	50
		3	60.9	457	37	18	476	55
26^{cs}	RM	1	73	170	-	63	68	-
		2	95.6	629	-	52.9	489	49
	RA	1	78.8	521	29	55.8	224	86
27^{cs}	RM	1	83.9	308	-	69	126	67
		2	75.2	209	28	41.4	385	48
		3	81.0	430	12	71.6	667	14
		4	72.2	350	44	52	137	61
	RA	1	81.8	529	6.8	84.8	560	18
		2	84	159	42	57	121	60

%V* = relative viability at 22°C = (mutant♂♂)//(C(1)RM/Y ♀♀) x 100/1.37
%V**= relative viability at 17°C = (mutant♂♂)//(C(1)RM/Y ♀♀) x 100/1.32
RM = C(1)RM/Y females
RA = C(1)DX, y f/Y females
OR = Oregon-R
%aa = percentage of ♂♂ showing abnormal abdomen traits

TABLE III

Male progeny at 22°C and 17°C of females heterozygous for y cv v f car and mutations which have visible phenotypes.

Crossover Classes (C.O.)	C.O. Region	Oregon-R	Mutant 5cs 17°C MP*	Mutant 5cs 17°C +	Mutant 5cs 22°C MP	Mutant 5cs 22°C +	6cs 17°C MP	6cs 17°C +	6cs 22°C MP	6cs 22°C +	8cs 17°C MP	8cs 17°C +	8cs 22°C MP	8cs 22°C +	15cs 17°C MP	15cs 17°C +	15cs 22°C MP	15cs 22°C +	16cs 17°C +	16cs 22°C MP	16cs 22°C +	20cs 17°C +	20cs 22°C MP	20cs 22°C +	26cs 17°C MP	26cs 17°C +	27cs 17°C MP	27cs 17°C +
Parental																												
y cv v f car	0	172	0	72	1	189	0	82	0	244	0	129	0	151	0	128	0	190	137	0	288	299	1	107	7	427	0	186
+ + + + +		165	1	1	85	108	13	0	95	189	55	0	152	0	3	3	58	157	0	301	0	1	97	0	349	366	36	131
Single																												
+ cv v f car	1	41	0	10	1	42	0	17	1	51	2	28	0	21	0	21	0	35	40	0	59	21	0	18	5	41	17	30
y + + + +		33	1	0	15	21	2	0	8	43	2	0	12	0	2	0	1	39	0	60	0	1	26	0	13	75	0	45
+ + v f car	2	69	0	27	1	72	0	35	0	114	4	29	16	21	0	41	0	78	62	0	109	149	0	50	67	93	21	53
y cv + + +		51	0	0	32	31	0	0	28	70	1	24	5	24	1	0	9	29	0	74	0	0	12	0	1	116	0	65
+ + + f car	3	75	0	47	1	90	0	60	1	116	9	0	56	0	0	64	0	101	35	5	41	242	1	39	86	175	10	74
y cv v + +		63	0	0	42	15	0	0	46	48	0	54	1	72	0	0	16	44	29	31	67	0	26	0	2	144	0	97
+ + + + car	4	32	0	10	2	25	0	13	0	26	1	0	9	0	0	8	0	16	0	99	0	0	6	0	36	45	5	27
y cv v f +		16	0	0	9	4	0	0	9	8	0	7	0	15	0	0	3	6	10	0	30	34	0	3	0	45	0	17
Multiple																												
+ cv	1**	11	0	1			1	0			1	22			0	0			12			2			6	15	6	19
y +		17	0	11			0	19			2	1			0	17			10			26			3	65	0	38
+ v	2	24	0	4			0	3			1	15	4	3	1	0			16			9			27	32	4	12
cv +		16	0	4			0	6			1	20	3	10	0	13			3			8			5	35	0	19
+ f	3	26	0	6			0	18			0	5			0	19			12	5	10	33	0	22	8	67	0	27
v +		24	0	2			0	0			0	27			1	2			18	9	25	0	8	0	26	28	6	16
+ car	4	9	0	7	0	11	0	4	0	13	2	9			0	12	0	11	0			1	5	1	2	28	0	31
f +		7	0	0	4	2	1	0	7	4	1	2			0	0	3	7	9			13	0	8	8	13	4	7
TOTAL ♂♂		828	186		856		248		1199		388		609		303		857		353		1253	791		428	2277		904	

* MP mutant phenotype

** Multiple c.o. class, region 1, includes all ♂ offspring with a c.o. in region 1 and a 2nd c.o. in any other region.

TABLE IV

Survival of progeny in tests of $\ell(1)\ 5^{cs}$, 6^{cs}, 15^{cs}, and 16^{cs} with duplications and deficiencies of proximal heterochromatin of the X chromosome.

Mutant	°C	(a) mal^6 deficiency and mal^{106} duplication					(b) mal^{12} deficiency				(c) sc^4sc^8 deficiency				
		$\ell(1)^{cs}$ / mal^6Df	FM6 / mal^6Df	$\ell(1)^{cs}$ / $Ymal^{106}Dp$ MP*	$\ell(1)^{cs}$ / $Ymal^{106}Dp$ +	FM6 / $Ymal^{106}Dp$	$\ell(1)^{cs}$ / $mal^{12}Df$	$\ell(1)^{cs}$ / $In49mal^2$	$In49mal^2$ / Y	$mal^{12}Df$ / Y	$\ell(1)^{cs}$ / $sc^4L_{sc}{}^8R_{Df}$	FM6 / $sc^4L_{sc}{}^8R_{Df}$	FM6 / Y	$\ell(1)^{cs}$ / Y MP*	$\ell(1)^{cs}$ / Y +
1^{cs}	22	134	160	88	12	108	24	41	23	0	22	20	13	8	13
	17	190	208	0	0	90	21	36	0	0	30	21	17	19	0
6^{cs}	22	180	150	82	4	130	28	38	28	0	135	108	90	49	84
	17	263	238	0	0	141	45	50	16	0	109	82	72	13	4
15^{cs}	22	205	219	118	2	145	53	59	40	0	76	77	60	62	31
	17	270	294	0	0	105	56	56	14	0	119	69	86	7	1
16^{cs}	22	179	155	76	52	136	63	61	48	0	113	116	82	19	36+
	17	266	270	0	0	138	45	55	18	0	206	211	65	0	0
Oregon-R	22	166	194	0	149	130	66	79	64	0	46	46	48	0	42
	17	213	227	0	176	109	74	75	4	0	49	35	16	0	44

cross: (a) ♀♀ $\ell(1)^{cs}/FM6$ X ♂♂ $In49B^{M1}$ v mal^6Df/y^+Y $mal^{106}Dp$

(b) ♀♀ $Df(1)mal^{12}sc^8B/In49$ v $sn^{x2}mal^2$ X ♂♂ $\ell(1)^{cs}/Y$

(c) ♀♀ $\ell(1)^{cs}/FM6$ X ♂♂ $In(1)sc^{4L}sc^{8R}$, y cv v f/Y

MP* = mutant phenotype

\+ = wild type phenotype

TABLE V

Viabilities and phenotypes of trans heterozygous and homozygous females in complementation tests of cold-sensitive mutations

$l(1)^{csx}/FM6$ ♀♀		$l(1)^{csy}/Y$ ♂♂							
		Ore-R	26	5	15	16	6	27	21
Ore-R	VR 22°C	1.02(842)	0.905(282)	0.993(287)	1.18(305)	0.892(210)	0.829(267)	0.938(312)	1.02(333)
	VR 17°C	1.03(947)	0.960(343)	0.899(338)	1.01(428)	0.820(344)	1.00 (364)	1.18 (533)	1.00(456)
	% aa	0 (426)	0.8 (134)	0 (143)	0 (165)	0 (99)	0 (121)	0 (151)	0.6 (166)
26	VR 22°C	0.947(296)	1.16(197)	1.09 (192)	1.04(188)	1.16 (158)	1.01 (229)	0.778(176)	0.917(232)
	VR 17°C	0.792(95)	0.774(110)	0.678(97)	0.667(95)	0.792(86)	0.931(253)	1.36(125)	0.814(156)
	% aa	1 (144)	39 (106)	2 (100)	3 (96)	4 (85)	2 (115)	4 (77)	3 (111)
5	VR 22°C	0.862(229)	1.11(268)	1.05(302)	0.617(228)	1.12(269)	0.783(246)	1.05(170)	0.973(219)
	VR 17°C	0.792(172)	0.752(198)	0.0441(71)	0.0364(114)	0.96(179)	0.0513(82)	1.09(138)	1.00(126)
	% aa	0 (106)	0.7 (141)	52 (155)	45 (87)	0.7 (147)	43 (108)	0 (87)	0 (108)
15	VR 22°C	0.754(328)	1.00(176)	0.722(303)	0.915(316)	1.18(308)	0.849(233)	1.17(419)	0.866(209)
	VR 17°C	1.01(310)	0.725(226)	0.141(194)	0.0460(182)	1.04(421)	0.236(131)	1.39(421)	1.21(199)
	% aa	0.7 (141)	0 (88)	52 (127)	62 (151)	0.6 (167)	56 (107)	0 (226)	0 (97)
16	VR 22°C	1.24(273)				1.18(203)	0.933(290)	0.839(206)	1.02(266)
	VR 17°C	1.04(411)				0 (162)	0.919(378)	0.958(417)	1.10(369)
	% aa	0 (151)				72 (110)	0.7 (140)	0 (94)	0 (134)
6	VR 22°C			0.933(290)	0.783(321)		0.950(156)	1.22(307)	1.07(290)
	VR 17°C			0.106(238)	0.161(332)		0.164(170)	0.975(480)	1.02(503)
	% aa			60 (140)	25 (141)		28 (76)	0 (169)	0 (150)
27	VR 22°C							1.13(207)	1.06(107)
	VR 17°C							0.814(312)	0.949(308)
	% aa							0 (110)	2 (55)
21	VR 22°C	1.00(134)	0.824(166)	0.973(148)	1.27(204)		1.24(224)		1.33(119)
	VR 17°C	1.02(107)	0.690(147)	0.547(147)	0.969(250)		0.913(241)		0 (77)
	% aa	0 (67)	0 (75)	0 (73)	0 (114)		0 (124)		0 (68)

VR (viability ratio) = $\frac{l(1)^{csx}/l(1)^{csy}}{♀♀}$ // $\frac{l(1)^{csy}/FM6}{♀♀}$

% aa = percent of $\frac{l(1)^{csx}/l(1)^{csy}}{♀♀}$ showing the abnormal abdomen trait at 22°C

TABLE VI

The effect of temperature on the viabilities of mutant males and homozygous females from the cross, $\ell(1)^{cs}$/FM6 ♀♀ x $\ell(1)^{cs}$/Y ♂♂, as compared to the Oregon-R control.

	Relative Viabilities			
	22°C		17°C	
Chr*	♂	♀	♂	♀
OR**	100% (867)	100% (842)	100% (889)	100% (947)
1^{cs}	111 (565)	101 (569)	0 (159)	0 (159)
2^{cs}	85 (174)	186 (279)	9 (97)	117 (201)
3^{cs}	36 (101)	22 (93)	0 (88)	0 (88)
4^{cs}	75.5 (672)	70.1 (681)	5 (408)	5 (413)
5^{cs}	86.3 (456)	102 (493)	4 (270)	2 (266)
6^{cs}	99.3 (262)	74.4 (241)	6 (183)	4 (180)
8^{cs}	89 (213)	78 (210)	3 (158)	2 (156)
9^{cs}	111 (305)	88.9 (288)	0 (98)	0 (98)
10^{cs}	68.3 (340)	54.9 (326)	6 (204)	4 (201)
11^{cs}	94.0 (339)	103 (374)	7 (219)	9.1 (226)
12^{cs}	100 (593)	34.4 (417)	0 (264)	0 (264)
13^{cs}	23 (213)	51 (267)	12 (106)	16 (111)
14^{cs}	49.0 (522)	36.5 (494)	12 (379)	11 (381)
15^{cs}	45 (244)	44 (250)	0.8 (281)	1 (283)
16^{cs}	44 (265)	30 (248)	0 (279)	0.4 (280)
17^{cs}	49 (294)	57.0 (321)	0 (123)	0 (123)
18^{cs}	62.4 (494)	75.3 (555)	12 (205)	44.9 (269)
19^{cs}	87.6 (285)	109 (333)	37 (134)	43 (144)
20^{cs}	73.6 (439)	40.0 (369)	0 (114)	0 (114)
21^{cs}	98 (196)	111 (219)	0 (77)	0 (77)
22^{cs}	87.3 (353)	106 (407)	11 (226)	75 (363)
23^{cs}	113 (274)	76.9 (239)	0 (161)	0 (161)
24^{cs}	91.3 (261)	98.0 (284)	64 (128)	35 (110)
25^{cs}	89 (122)	94 (131)	28 (111)	6 (94)
26^{cs}	109 (182)	118 (197)	73 (103)	75 (110)
27^{cs}	99.1 (185)	108 (207)	76.2 (291)	79.0 (312)

Relative Viability of:
♂♂ at 22°C = $(\ell(1)^{cs}/Y)//(FM6/\ell(1)^{cs})$ x 100/0.918
♂♂ at 17°C = $(\ell(1)^{cs}/Y)//(FM6/\ell(1)^{cs})$ x 100/0.908
♀♀ at 22°C = $(\ell(1)^{cs}/\ell(1)^{cs})//(FM6/\ell(1)^{cs})$ x 100/1.02
♀♀ at 17°C = $(\ell(1)^{cs}/\ell(1)^{cs})//(FM6/\ell(1)^{cs})$ x 100/1.03
Chr* = Chromosome
OR** = Oregon-R

TABLE VII

Fertility at 22°C of females homozygous for cold-sensitive lethals.

♀	Progeny/♀ when mated to: FM6/Y	FM6/Y + ω^{cs}/Y	ω^{cs}/Y	Fertility*
OR	86 (6)		64 (6)	F
1^{cs}		75 (30)		F
2^{cs}	95 (6)		76 (10)	F
3^{cs}	14 (12)		1.7 (12)	Ss
4^{cs}	39 (4)		66 (4)	F
5^{cs}		0 (27)		St
6^{cs}	11 (18)		7 (18)	Ss
8^{cs}	35 (18)		15 (18)	Ss
9^{cs}		63 (21)		F
10^{cs}	0 (8)		0 (8)	St
11^{cs}	0.9 (12)		3.5 (12)	Ss
12^{cs}	0 (7)		0 (8)	St
13^{cs}		0 (24)		St
14^{cs}			51 (6)	F
15^{cs}	0 (18)		0 (18)	St
16^{cs}	15 (18)		2 (18)	Ss
17^{cs}	0 (12)		0 (12)	St
18^{cs}		69 (30)		F
19^{cs}	52 (3)		28 (3)	F
20^{cs}	4 (18)		3 (21)	Ss
21^{cs}		18 (18)		Ss
22^{cs}		14 (18)		Ss
23^{cs}		7** (21)		Ss
24^{cs}		13 (26)		Ss
25^{cs}		11 (24)		Ss
26^{cs}		0 (36)		St
27^{cs}		70 (15)		F

* F = fertile; Ss = semi-sterile; St = sterile, i.e. produces no viable adult offspring.

** only FM6/ω^{cs} offspring recovered

OR = Oregon-R

TABLE VIII

Male progeny of females heterozygous for y cv v f car and mutant chromosomes at 17°C.

Crossover (C.O.) Classes	C.O. Region	Oregon-R	$\ell(1)^{cs}$ Mutation Number 1	2	3	4	7	9	11	12	13	14	18	19**	21	22	23≠	25
Parental																		
y cv v f car		172	69	78	66	97	122	179	166	90	90	83	65	204/63	72	152	145	59
+ + + + +		165	0	1	0	3	2	5	4	0	0	3	15	0/174	4	10	0	8
Single																		
+ cv v f car	1	41	1	19	15	14	2	0	0	0	4	17	19	24/7	15	28	0	16
y + + + +		33	33	0	1	0	44	55	44	24	27	0	8	0/46	4	1	0	1
+ + v f car	2	69	0	29	35	37	0	1	3	0	0	32	28	52/11	36	34	49	27
y cv+ + +		51	26	0	0	1	46	59	47	24	45	0	0	0/35	0	47	0	0
+ + + f car	3	75	0	41	1	48	0	0	0	0	0	10	25	46/22	1	1	46	17
y cv v + +		63	33	1	29	4	58	82	59	29	35	3	0	13/33	34	69	28	10
+ + + + car	4	32	0	1	0	0	0	0	0	0	0	0	7	0/19	0	1	0	0
y cv v f +		16	6	8	7	4	17	15	23	7	15	14	4	17/3	13	13	8	8
Multiple																		
+ cv	1*	11	1	2	8	0	0	0	0	0	1	2	2	4/9	10	14	0	3
y +		17	10	14	3	6	24	55	9	18	18	3	8	10/7	3	0	0	1
+ v	2	24	2	5	14	8	0	5	2	2	4	3	2	4/7	11	6	10	0
cv +		16	10	5	1	3	6	21	8	6	8	1	9	7/3	0	12	5	3
+ f	3	26	14	15	1	9	22	29	12	15	12	2	11	17/6	0	6	6	4
v +		24	1	1	18	1	2	3	2	0	2	1	2	2/14	16	14	5	2
+ car	4	9	4	1	2	0	10	29	2	7	10	1	5	0/3	0	4	0	1
f +		7	0	5	3	7	0	0	0	1	0	3	1	6/1	3	3	6	2
TOTAL ♂♂		828	189	202	178	224	323	458	365	198	244	170	194	819	200	385	292	149

* multiple c.o. class, region 1 includes all offspring with a c.o. in region 1 and a 2nd c.o.(s) in any other region(s).

** late emerger, not lethal - 1st 9 days/last 11 days

≠ all offspring y because mothers were y cv v f car/y + + $\ell(1)^{cs}$ + +

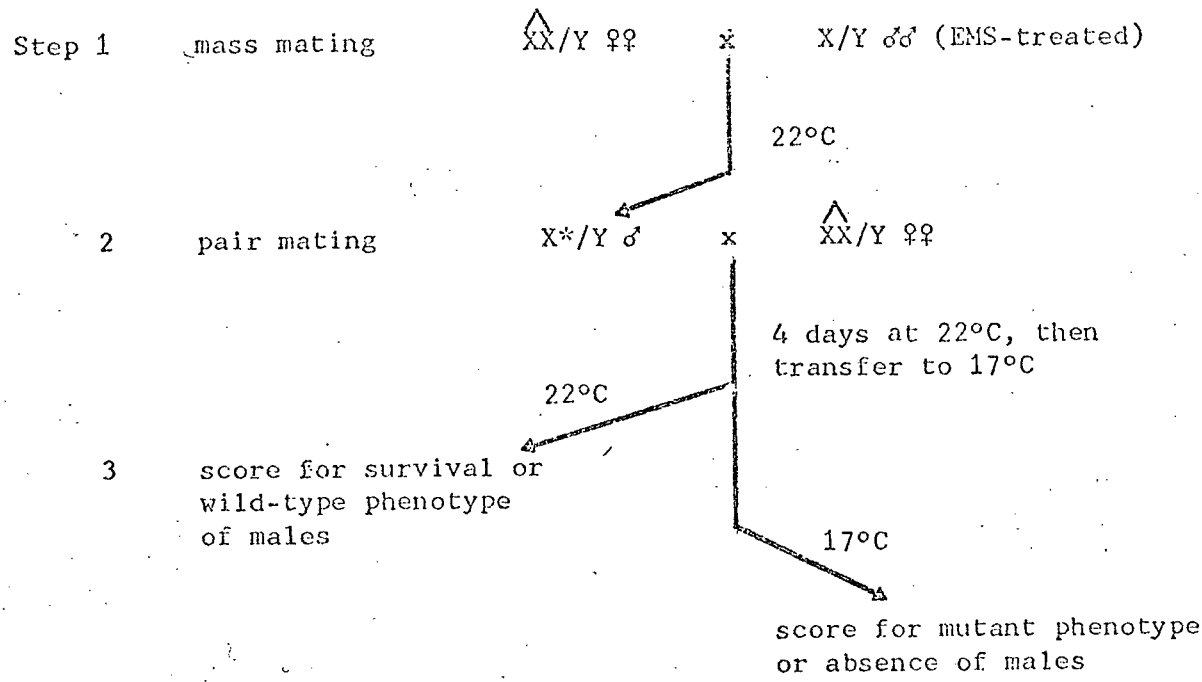
TABLE IX

Characteristics of cold-sensitive mutants of the X chromosome.

Mutant	♂ RV 22°C	♂ RV 17°C	Fertility of $\ell(1)^{cs}/\ell(1)^{cs}$ ♀	Visible phenotype of mutation	Genetic Position
1	82	0	F	None	0.6
2	42	0.5	F	None	57
3	35	0	Low F	None	34
4	50	3	F	None	65
5	67	0	S	Reduced sternites and etched or disturbed tergites, extra wing veins	at centromere or between car & 134
6	55	5	Low F	Like 5 but penetrance lower than 5	allele of 5 & 15
7	78	6	-	None	0.4
8	47	2	F	Upheld wings	22-25
9	61	0	F	None	0
10	31	1	S	None	-
11	61	2	Low F	None	0
12	50	0	S	None	0
13	67	0.2	S	None	1.4
14	33	7	F	None	51
15	71	1	S	Like 5	allele of 5 & 6
16	92	0	Low F	Short, fine bristles and etched tergites	46-49
17	45	0	S	None	-
18	77	13	F	None	61
19	53	0	F	None	52**
20	47	0	Low F	Like 16	57
21	69	7	Low F	None	34
22	100	2	Low F	None	22
23	29	0	Low F*	None	48
24	49	2	Low F	None	-
25	21	0	Low F	None	45
26	73	63	S	Abnormal abdomen traits like 5	5≠
27	84	69	F	Abnormal abdomen traits like 5 low penetrance	0

* only $\ell(1)^{cs}$/FM6 progeny recovered
** late emerger; not lethal when mapped
≠ another mutation present in this stock
♂RV= relative viability of males, see Table I and II
♀ fertility: see Table VII
Genetic locations: see Tables III and IV.

FIGURE 1



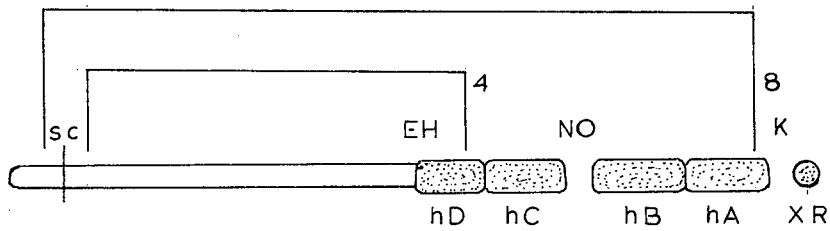
* denotes EMS-treated chromosome.

Figure 1. Screening procedure for the detection and recovery of sex-linked recessive cold-sensitive mutations.

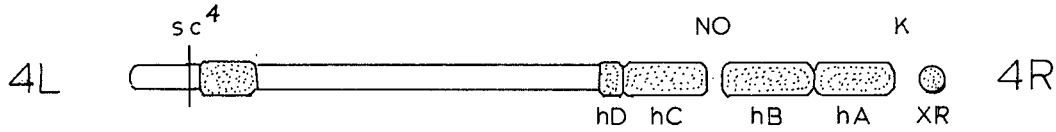
Figure 2. Cytological representation of X chromosome scute inversions. (adapted from Cooper, 1959).

EH = euchromatic - heterochromatic junction with heterochromatin shaded and euchromatin open.
NO = nucleolus organizer
K = centromere
XR = right arm of X
hA, hB, hC, hD = main heterochromatic segments of left arm of X.
In (1)sc^{4L} sc^{8R} originates from a crossover between In(1)sc^{4} and In(1)sc^{8}

Figure 2

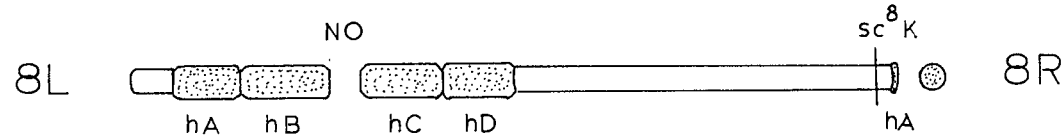


Normal X



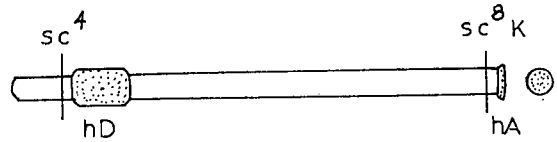
In (1) sc^4

X



In (1) sc^8

↓



In (1) $sc^{4L}sc^{8R}$

Figure 3. Relative break points of deficiencies and duplications of the Ma-1 region (Schalet and Finnerty, 1968b).

NO = nucleolar organizer
EH = euchromatic - heterochromatic junction

Figure 3

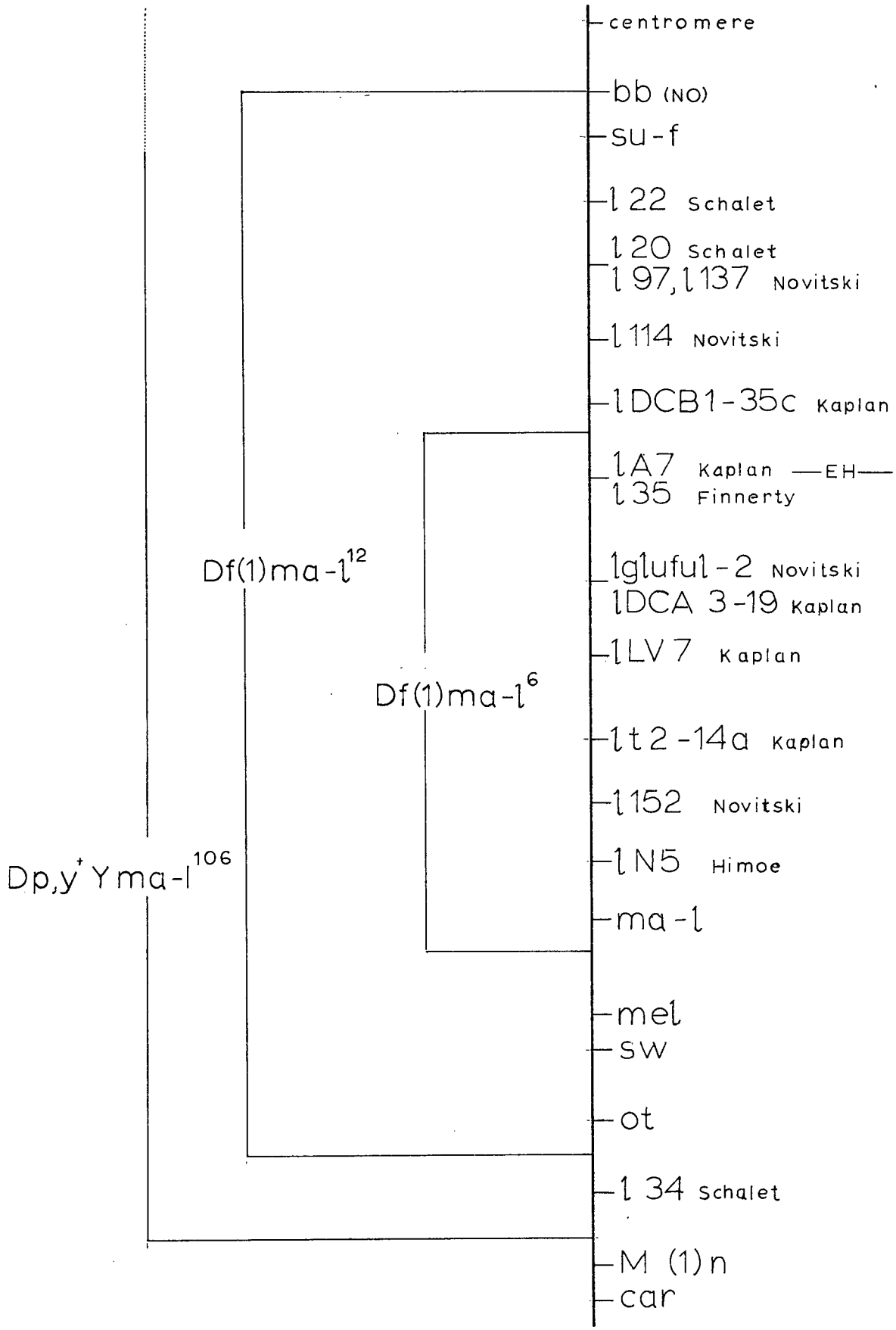

centromere
bb (NO)
su-f
l 22 Schalet
l 20 Schalet
l 97, l 137 Novitski
l 114 Novitski
l DCB1-35c Kaplan
l A7 Kaplan —EH—
l 35 Finnerty
lglufu1-2 Novitski
l DCA 3-19 Kaplan
l LV 7 Kaplan
l t 2-14a Kaplan
l 152 Novitski
l N5 Himoe
ma-l
mel
sw
ot
l 34 Schalet
M (1) n
car
Df(1)ma-l12
Df(1)ma-l6
Dp,y+Yma-l106

Figure 4. Genetic positions of cold-sensitive, heat-sensitive and non-conditional lethal mutations on the X chromosome.

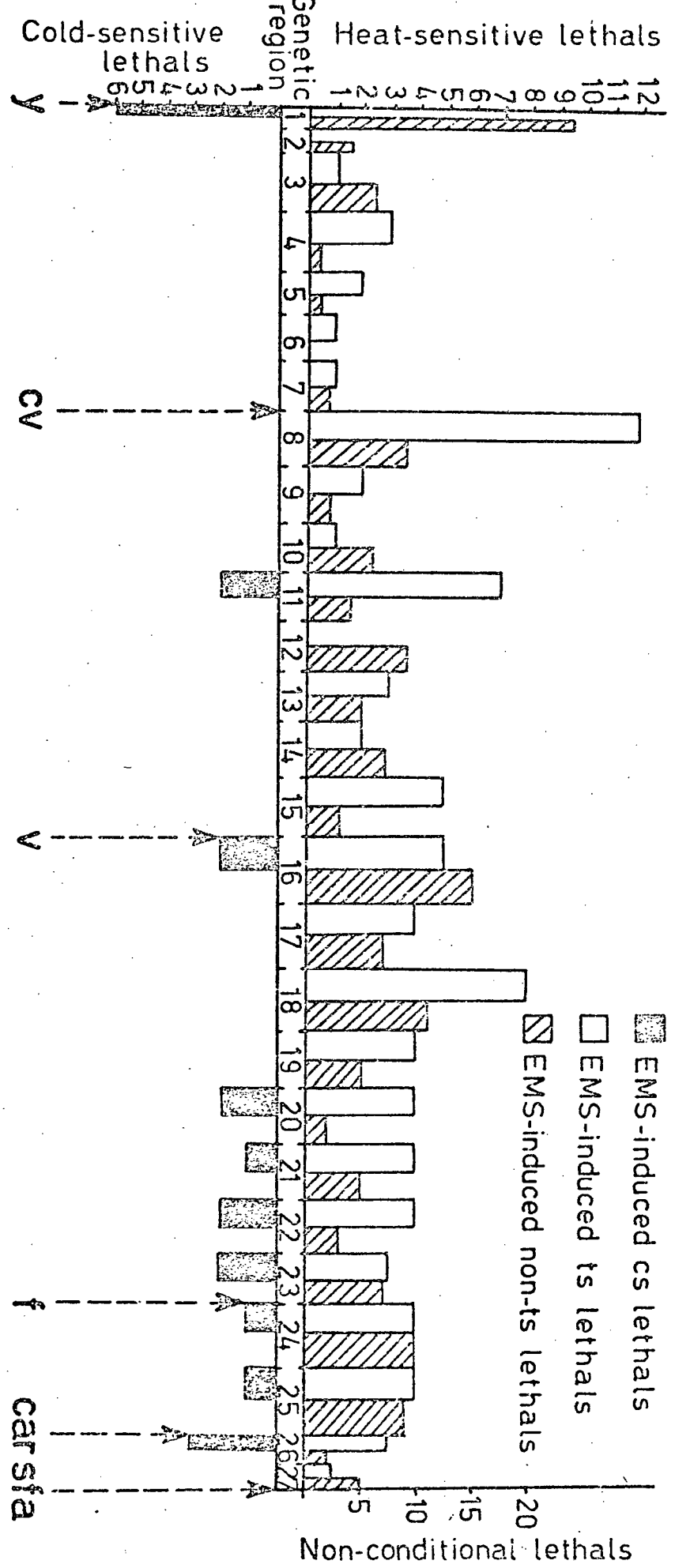

Cold-sensitive lethals
Genetic region
Heat-sensitive lethals
y
cv
v
f
car sta
EMS-induced cs lethals
EMS-induced ts lethals
EMS-induced non-ts lethals
Non-conditional lethals

FIGURE 4

Figure 5. Composite diagram of two-dimensional electrophorograms of the ribosomal proteins of D. melanogaster

Adult males and females. The solid spots are always seen. The cross-hatched spots are faintly staining and may sometimes be absent. The open spots indicate differences between HCl-acetone and Mg-acetic acid preparations of ribosomal proteins.

A. represents a long run to resolve the slow moving spots. The fast moving spots have migrated off the gel into the electrode buffer. See Figures 6a and 7a for example.

B. represents a short run. The slow moving spots are not fully resolved but the fast moving spots are retained on the gel slab. Only the fast moving spots are shown in the diagram along with neighbouring spots so that A and B may be related. See Figures 6c, 7c, and 9 for examples.

Figure 5

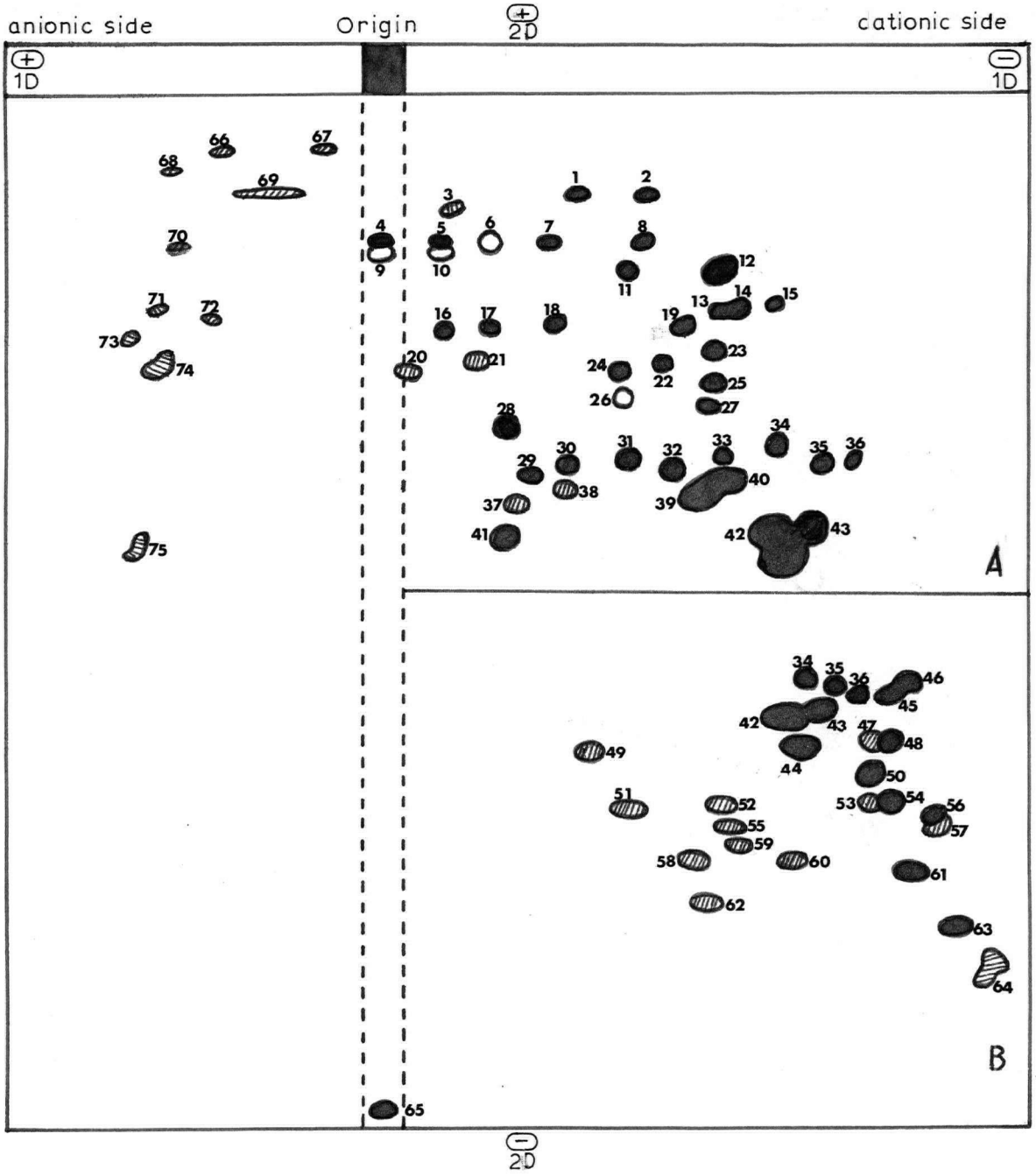


Figure 6a. Two-dimensional gel electrophoresis of Mg-HAc preparation of Drosophila ribosomal proteins.

Long Run

3 mg protein, Oregon-R males and females. First dimension: 23 hr. at 12 mamp/4 gels and 110 - 195 volt. Second dimension: 20 hr. at 200 mamp/4 gels and 65 - 140 volt.

Figure 6b. Two-dimensional gel electrophoresis of Mg-HAc preparation of Drosophila ribosomal proteins.

Intermediate Run

3 mg protein, Oregon-R males and females. First dimension: 14.5 hr. at 12 mamp/4 gels and 150-310 volt. Second dimension:
16.5 hr. at 200 mamp/4 gels and 60-100 volt.

Figure 6a

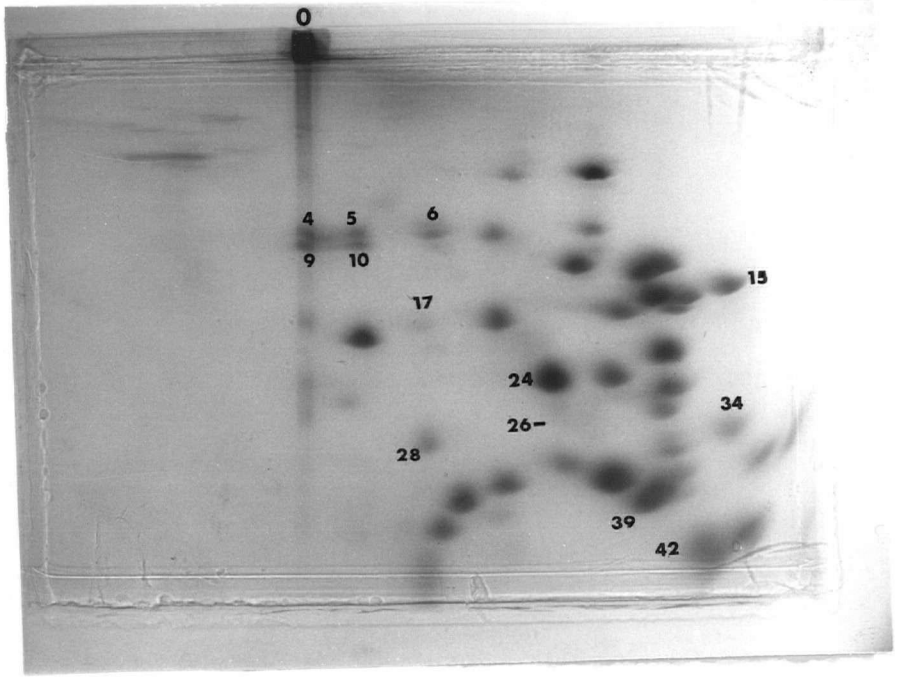


Figure 6b

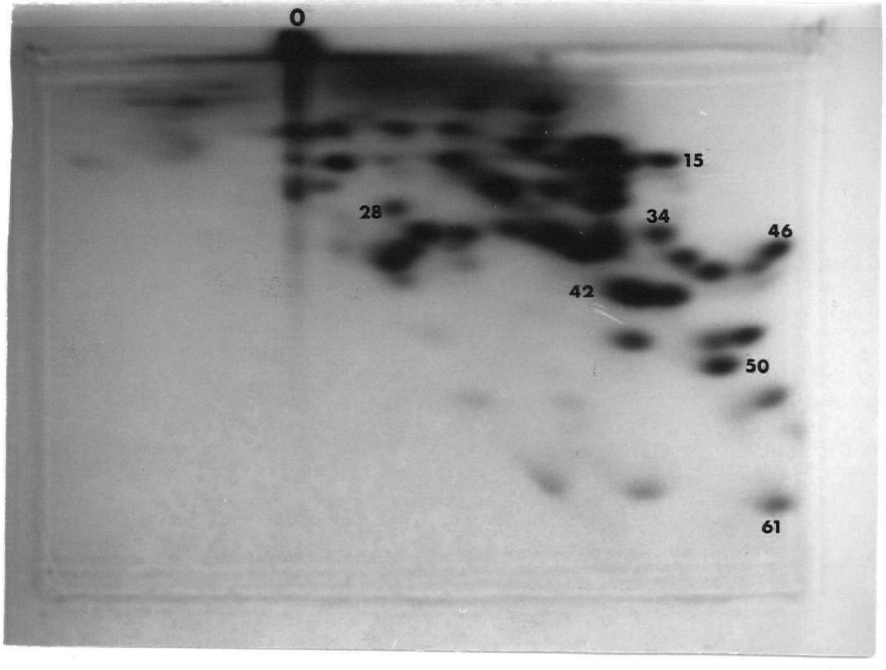


Figure 6c. Two-dimensional gel electrophoresis of Mg-HAc preparation of Drosophila ribosomal proteins.

Short Run

3 mg protein, Oregon-R males and females. First Dimension: 10 hr. at 12 mamp/4 gels and 152-185 volt. Second dimension: 15.5 hr. at 200 mamp/4 gels and 65-170 volt.

Figure 7a. Two-dimensional gel electrophoresis of HCl-acetone preparation of Drosophila ribosomal proteins.

Long Run

3 mg protein, Oregon-R males and females. First dimension: 23 hr. at 12 mamp/4 gels and 110 - 195 volt. Second Dimension: 20 hr. at 200 mamp/4 gels and 65 - 140 volt.

Figure 6c

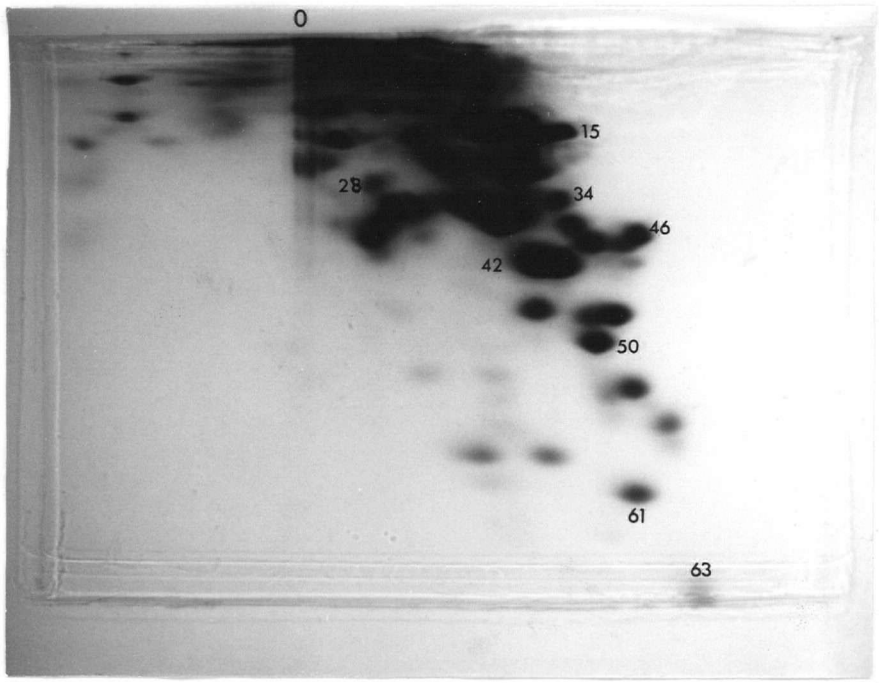


Figure 7a

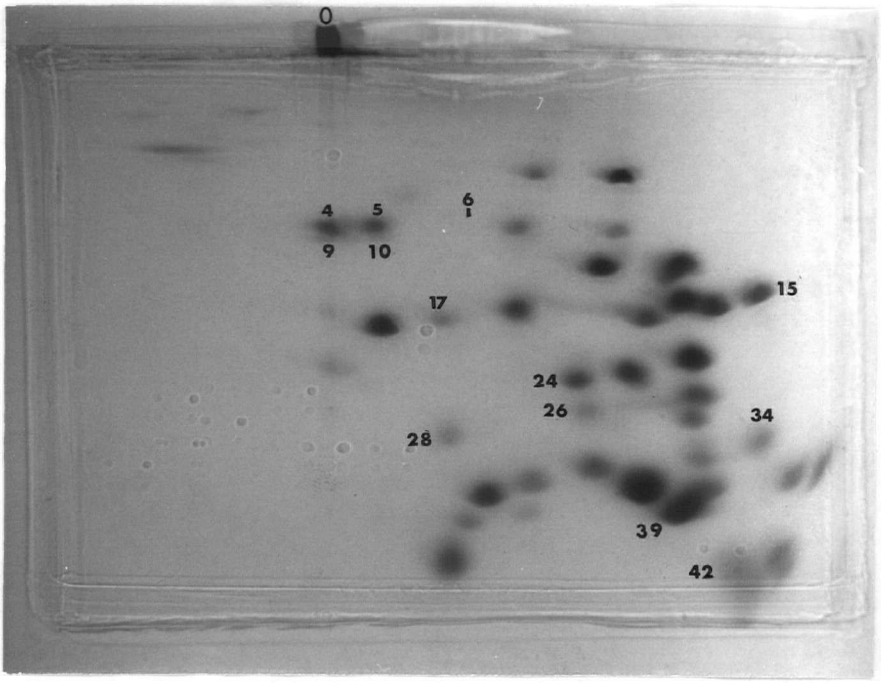


Figure 7b. Two-dimensional gel electrophoresis of HCl-acetone preparation of Drosophila ribosomal proteins.

Intermediate Run

3 mg protein, Oregon-R males and females. First dimension: 15.5 hr. at 12 mamp/4 gels and 150-185 volt. Second dimension: 15.5 hr. at 200 mamp/4 gels and 65-170 volt.

Figure 7c. Two-dimensional gel electrophoresis of HCl-acetone preparation of Drosophila ribosomal proteins.

Short Run

3 mg protein, Oregon-R males and females. First dimension: 10 hr. at 12 mamp/4 gels and 152-185 volt. Second dimension: 15.5 hr. at 200 mamp/4 gels and 65-170 volt.

Figure 7b

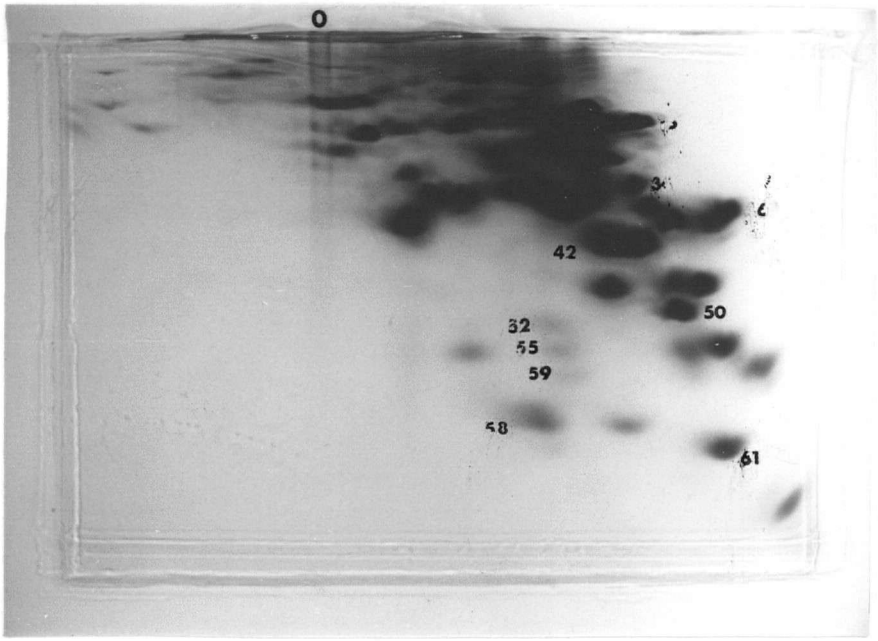


Figure 7c

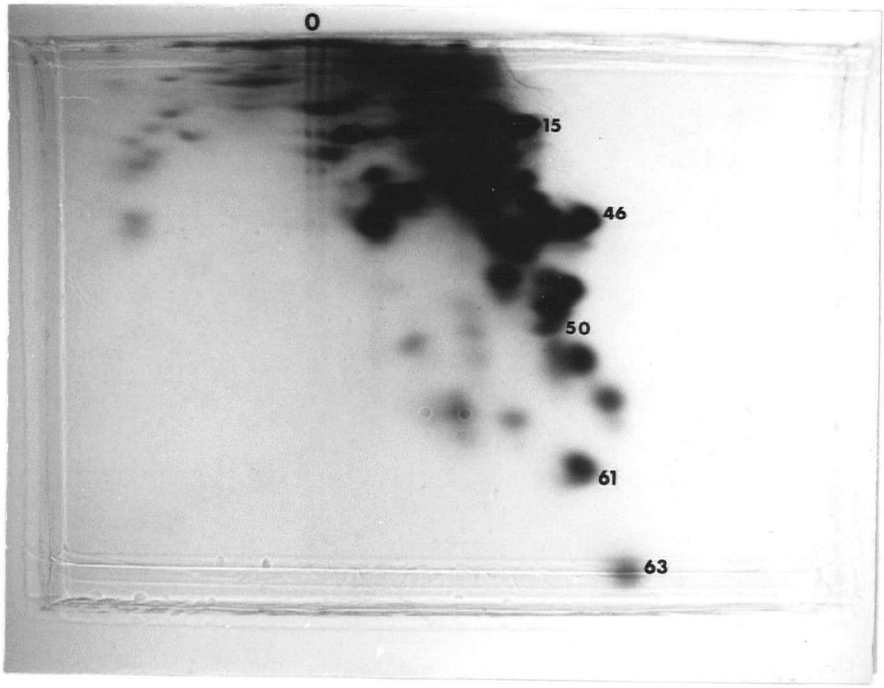


Figure 8

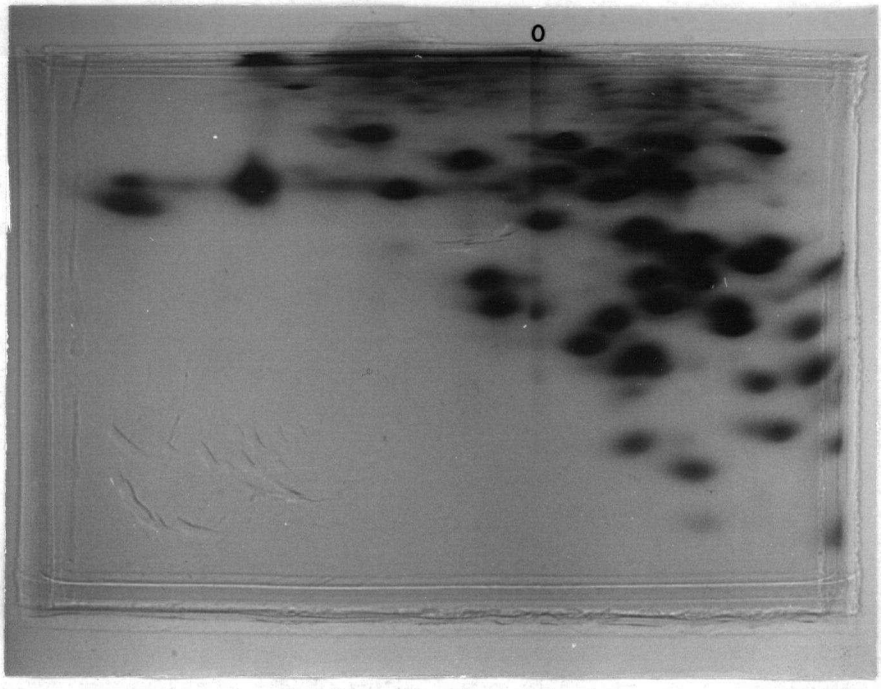


Figure 8. Two-dimensional gel electorphoresis of E. coli ribosomal proteins.

3 mg protein. Mg-HAc preparation. First dimension: 15 hrs., 12 mamps/4 gels and 130-230 volts. Second dimension: 12 hrs., 200 mamps/4gels and 63-145 volts. Some of the proteins on the right side of the gel have started to migrate into the cathode buffer of the first dimension. O = origin, Anionic side of gel on left, cationic side on right.

Figure 9. Two-dimensional gel electrophoresis of ribosomal proteins of mutant $\underline{16^{cs}}$

3 mg protein, HCl-acetone preparation. Mutant males. First dimension: 10.5 hr. at 12 mamp/4 gels and 125-182 volt. Second dimension: 12 hr. at 170-175 mamp/4 gels and 90-180 volt.

Figure 10. Two-dimensional gel electrophoresis of ribosomal proteins of mutant $\underline{5^{cs}}$

3 mg protein, mutant males. HCl-acetone preparation. First dimension: 10.5 hr. at 12 mamp/4 gels and 125-182 volt. Second dimension: 12 hr. at 170-175 mamp/4 gel and 90-180 volt.

Figure 9

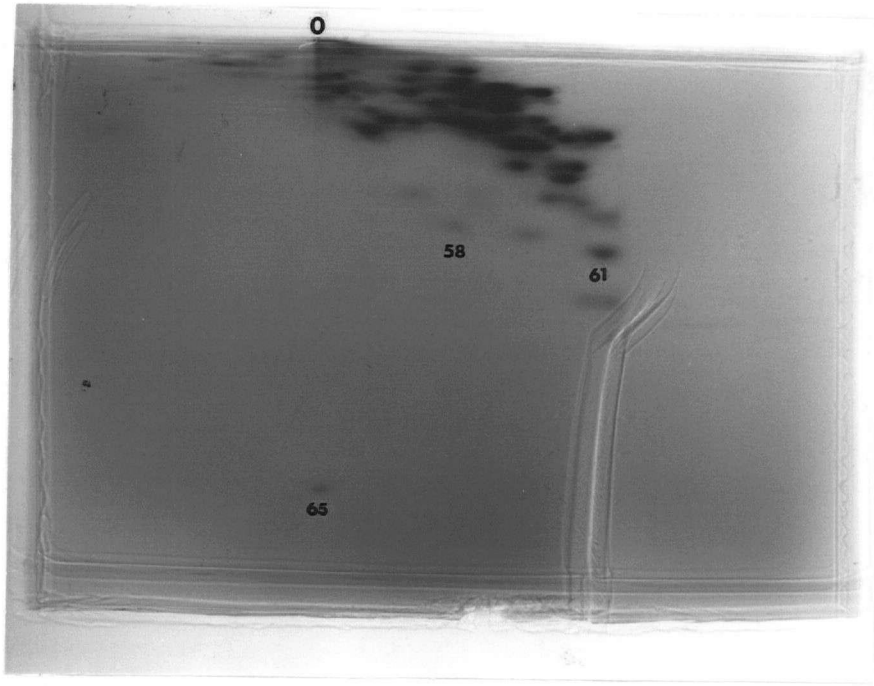


Figure 10

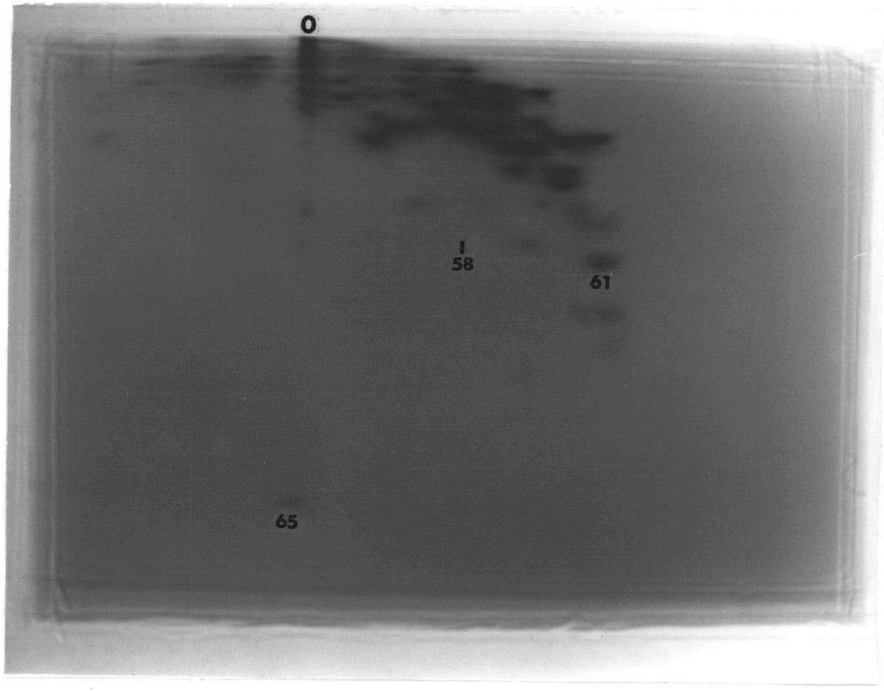


Figure 11. Two-dimensional gel electrophoresis of ribosomal proteins of mutant $\underline{27^{cs}}$

3 mg protein, HCl-acetone preparation. Mutant males. First dimension: 23 hr. at 7-12 mamp/4 gels and 130-200 volt. Second dimension: 21.5 hr. at 200 mamp/4 gels and 100-200 volt.

Figure 12. Two-dimensional gel electrophoresis of ribosomal proteins of Oregon-R males.

3 mg protein, Oregon-R males. HCl-acetone preparation. First dimension: 14 hr. at 12 mamp/4 gel and 125-210 volt. Second dimension: 13 hr. at 200 mamp/4 gels and 75-150 volt.

Figure 11

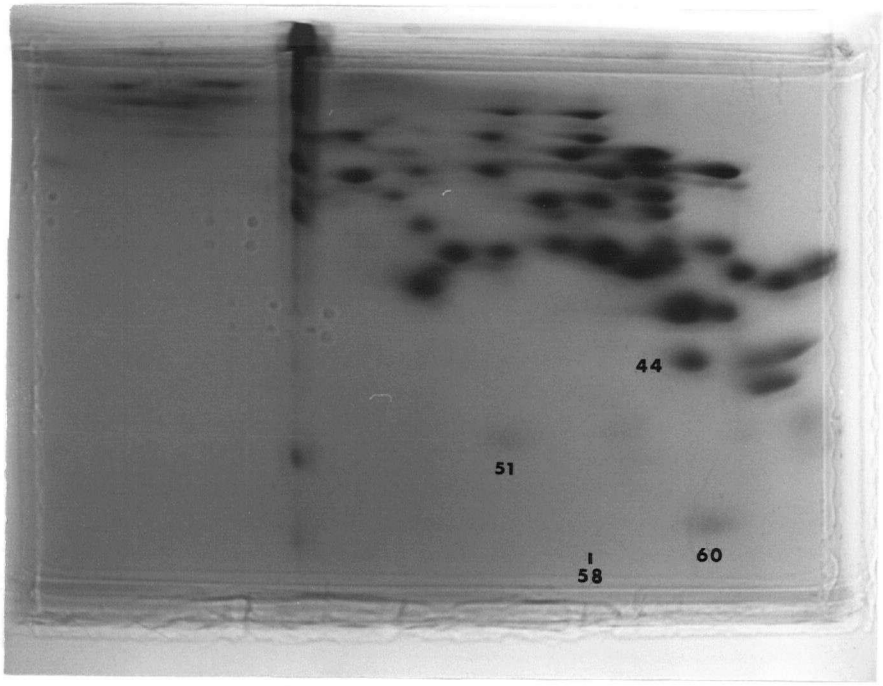


Figure 12