A COLLECTION OF VISIBLE MUTANTS IN THE FREE-LIVING NEMATODE, *PANAGRELLUS REDIVIVUS*

by

Rock A. Pulak

A thesis

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CONTENTS

LIST OF TABLES

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ABSTRACT

A library of visible mutants in the nematode *Panagrellus redivivus* was collected. Ethyl methanesulfonate, proflavin, proton irradiation and UV light were used to induce the mutations. The protocol used involved screening progeny of isolated brother-sister matings of the F_2 generation. Such a protocol increases the chances of finding autosomal recessive mutations.

The types of mutations isolated were similar to those isolated by Brenner (1974). A total of 136 mutant lines have been established in *P. redivivus*. Methods for manipulating the worms, isolating mutants and storing mutants for further analysis are described. Some preliminary genetic analysis - complementation and linkage analysis - is presented in appendices.

INTRODUCTION

Genetic techniques provide one of the most powerful experimental tools in biology. The relationships and interactions of biological processes can be described in terms of discrete genetically controlled steps. Such a discription begins with the isolation of a set of mutants. Analysis of mutants perturbed at each of these steps exposes integrated assemblages controlling the biological process. The mutants may be spontaneous mutations recovered from the wild-type population or may be induced by various physical or chemical agents. Such mutants have been successfully used in determining the underlying mechanism of many biological processes.

The stepwise elucidation of various pathways has been accomplished by isolating mutants expressing similar phenotypes and combining biochemical and genetical analysis (Goldberger, 1979). Hartwell (1974) collected yeast mutants defective in the cell division cycle and determined the temporal order of the genes for the normal division cycle. The genes involved in the pathways leading to lysogenic and lytic growth of the bacteriophage lambda has been predominantly determined using genetic analysis (Ptashne *et al.*, 1980). Some of the steps of the photosynthetic pathway were determined using genetic analysis as well as much of our present knowledge of the proton-translocating ATPase system. By collecting mutants

defective in energy generation and analyzing them biochemically and genetically, the underlying pathways were revealed.

Among the many unanswered questions in biology, those involving development and behavior are the most numerous. Various organisms have been exploited in an attempt to determine how development progresses and behavior is manifested. Some simple developmental switches have been successfully investigated but little is known of the control of development at the multicellular level.

One of the developmental switches studied is phase variation in *Salmonella*. This bacterium is capable of expressing two antigenicly distinct flagellar proteins. A single bacterium will express only one of the two types. However, bacteria expressing the other of the flagellar proteins will arise in the population at a frequency of 10^{-3}. to 10^{-5}. (Simon *et al.*, 1980; Silverman and Simon, 1980). Another system well studied is mating type in yeast which led to the formulation of the "cassette model" for the control of gene expression (Hicks *et al.*, 1977). The study of B-lymphocyte maturation and the immunoglobulin switch during maturation is being extensively studied at the molecular level (Tonegawa, 1983). A somewhat different problem is being addressed by *Drosophila* researchers working with homoeotic mutants. The developmental fate of the segments appears to be controlled by a couple of gene complexes (Lawrence and Morata,

1983). Much of the work being done is at the molecular level using the powerful tools of molecular genetics and molecular biology. However, classical genetics played a major part in identifying the underlying genetic control and in an initial description of the phenomenon.

The nematode, *Panagrellus redivivus* is a suitable multicellular organism for investigating development and behavior. It has a short life cycle, can be easily cultivated and is small enough to be handled in large numbers. It has relatively few cells and developmental pattern is invariant allowing for cell lineage analysis. The complete post embryonic cell lineage is known (Sternberg and Horvitz, 1981,1982) as well as much of the early embryonic lineage (Denich, unpublished data). It has a DNA C-value twenty times less than *Drosophila* (Sin and Pasternak, 1970). Previous studies on *P. redivivus* include the use of chemical and physical "insults" to perturb normal development in an attempt to elucidate the underlying biological control. Stage specific post-embryonic events were focussed on; growth and ecdysis (Samoiloff and Pasternak, 1968; Samoiloff and Pasternak, 1969; Samoiloff, 1970; Pasternak and Samoiloff, 1970; Samoiloff 1973a, 1973b), gonad development (Boroditsky and Samoiloff, 1973), mating behavior (Cheng and Samoiloff, 1971, 1972; Samoiloff *et al.*, 1973; Samoiloff *et al.*, 1974; Balakanich and Samoiloff, 1974), chemotaxis and osmotic tolerance (Pollock and

Samoiloff, 1976), the nutritional requirements of development (Abdulrahman and Samoiloff, 1975). The worm has a limited behavioral repertoire, although the small physical size of *P. redivivus* makes physiological analysis of behavioral processes difficult.

One of the objectives of this present work was to determine how amenable *P. redivivus* is to genetic analysis. *Panagrellus redivivus* has a haploid set of 5 chromosomes (Hechler, 1970). Sex determination is by an XO mechanism with the males being the heterogametic sex. *Panagrellus* is dioecious with males being necessary for fertilization. It is ovoviviparous and females will produce 200-250 juveniles over a ten day period, while males can sire up to 5000 progeny (Samoiloff,1980b). Burke (1978) isolated a set of autosomal and sex-linked visible mutations in *P. redivivus*, but most of these could not be maintained. Eleven autosomal mutants were isolated but only six would successfully mate. Three of these were discarded because of variable expressivity. The remaining three have since been lost. It became apparent, therefore, that some refinements in manipulating the nematodes would be required for further isolating and maintaining mutant lines.

The present genetic analysis of the free-living nematode *Panagrellus redivivus* stems from an interest in using this organism to study behavior and development. In the mid-sixties Sydney Brenner began to use *Caenorhabditis elegans*, a free-

living, hermaphroditic soil nematode, as a model for the study of the genetic control of behaviour and development in multicellular organisms. The first major publication (Brenner, 1974) in this effort presented the genetics of the nematode and reported a set of 350 morphological and behavioural mutants. Many studies on *C. elegans* followed, including the determination of the complete cell-lineage of the organism (Sulston *et al.*, 1983). Until recently, most of the study on development of *C. elegans* has centered on embryonic events. On the other hand, *Panagrellus* studies (primarily by Samoiloff and co-workers) have focussed on post-embryonic events. If *P. redivivus* is to be used to study the developmental pattern of post-embryonic events, it is essential to begin a more thorough genetic analysis of the organism.

Earlier studies on the development of *P. redivivus* applied target theory to determine the number of genes required for postembryonic maturation (Denich 1980, Samoiloff 1980a). The involved using irradiation to perturb the completion of successive molts through one generation. The results suggested that bursts of gene activity were required at specific times in the stepwise postembryonic development of the nematode. These bursts of gene activity coincide with the molting steps that occur in the development from first juvenile stage to adult. Cell lineage studies of postembryonic development in *P. redivivus* (Sternberg and Horvitz, 1982) suggests that some cell

divisions appear to be tightly coupled to ecdysis, while other cell divisions appear to be independent program of ecdysis. Such stepwise development can be further investigated by isolation of conditional mutations that block development at specific stages. The selection of such mutations can be more easily performed by using visible mutations as genetic markers; mutagenizing marked chromosomes, and performing crosses that permit the recognition of F_2 individuals homozygous for the marked chromosome which can be tested for the conditional phenotype.

The major objectives of the work reported here were:

1) establishing a protocol for isolating visible mutants in *P. redivivus* with particular emphasis on autosomal recessives.
2) to isolate a library of such mutants.
3) to establish a method of storing mutant lines for future use and analysis.

The first phase of such an undertaking was to develop the methods of manipulating *Panagrellus* under controlled lab conditions. The techniques for growing the worms, handling them, isolating mutants and storing mutants for further analysis are prerequisites to any long term project on development and behavior using a genetical approach. The techniques for growing and manipulating *Panagrellus* are also described in this report. Initial studies on the genetic

analysis of some of the mutant lines are described in the appendices. Complementation of a subset of mutants and the strategy for linkage analysis with the ultimate objective of marking each chromosome with several visible mutant phenotypes are described in detail.

MATERIALS AND METHODS

GROWTH MEDIA FOR *Panagrellus*

Panagrellus redivivus is grown under very simple limited nutritional conditions. Water-agar plates supplemented with a steroid provides the substrate upon which stocks are maintained. A liquid growth medium of autoclaved yeast is used as food for these particle feeding nematodes. Contamination of stocks is minimal under these conditions and seldom presents a problem. A more nutritionally rich culture mediium had been used in the past for *Panagrellus* but proved unsatisfactory because of periodic problems with extreme contamination.

The following formulations of media were used:

Water-agar plates:

17 grams agar (Fisher Biological Grade)

1 mL cholesterol solution

glass distilled water to make 1.0 liter solution

Water-agar medium is mixed in glass Erlenmyer flasks with screw-caps. Agar is suspended into solution by shaking and autoclaved for 20 minutes at 15 p.s.i.

M9 buffer:

6 g Na_2HPO_4

3 g KH_2PO_4

5 g NaCl

0.25 g $MgSO_4$

Glass distilled water to make 1.0 liter solution.

Buffer is autoclaved at 15 psi for 20 minutes.

M9-Y Liquid Growth Medium (M9-Y LGM):

50 mg dried baker's yeast

1 liter M9 buffer

1 mL cholesterol solution

Autoclave for 20 minutes at 15 psi. The growth medium contains a final concentration of 0.05 mg/ml of yeast.

Cholesterol solution:

5 mg cholesterol/ml ethanol

MAINTAINING STOCKS

The wild-type strain of *Panagrellus redivivus* used in this study, designated C15, was generated by two rounds of 15 generation sib-sib matings, from a stock originally sent by Dr. A. Coomans from Ghent, Belgium.

Stock cultures were maintained on 100 X 15 mm water-agar plates. Sub-culturing was required every two weeks. Approximately 5 mL of fresh M9-Y LGM was poured onto the old

plate, and the plate is gently swirled to suspend worms in the liquid. The suspended worms are then distributed to fresh plates. Stock cultures are maintained at room temperature.

FREEZING STOCKS

The basic strategy for freezing stocks was derived from the techniques developed for *C. elegans*. Alterations in the *C. elegans* freezing protocol were adopted because *P. redivivus* is grown under conditions different from those for *C. elegans*.

Media for freezing:

1) B medium

3.0 g NaCl

5.7 g KH_2PO_4

Glycerol 300 ml

Distilled H_2O 644 ml

Adjust to pH 6.0, autoclave

add 0.3 ml of 1 M $MgSO_4$ (sterile)

2) S medium + glycerol

20 ml 1M NaCl

10 ml 1M KH_2PO_4

adjust to pH 6.0

60 ml glycerol

110 ml distilled water - autoclave.

Worm stocks for freezing are grown on 100 by 15 mm plates. Conditions were controlled in such a way as to ensure the presence of large numbers of early juvenile-stage worms in a partially starved state. The final sub-culturing prior to freezing the stocks was done using M9 buffer instead of food. The only food available to the stock was whatever was carried over from the previous stock plate. Secondly, worms collected in autoanalyzer cups for freezing were washed three times in M9 buffer and held twenty-four hours in the buffer prior to freezing. Semi-starved early stage juveniles survive the freezing and subsequent thawing better than satiated juveniles and considerably better than either starved or fed later stage juveniles or adults.

Stocks that were to be frozen were initially well fed in order to increase the number of worms available for freezing. Once the stock plate had a sufficient number of worms it was subcultured one last time using M9 buffer instead of M9-Y LBM. Three to four days later the worms were collected in autoanalyzer cups. By flooding the stock plate with M9 buffer and tilting the plates lightly, so that all the worms fell to the bottom, they could be easily collected with a micropipet. Several hundred worms (500-1000) were placed in each of five autoanalyzer cups. The cups were flooded with M9 buffer and the worms were allowed to settle to the bottom. All but 0.5 mL of the M9 buffer was removed from the autoanalyzer cup using

a pasteur pipet. The cups were then filled with M9 buffer a second and a third time to wash as much of the food away as possible. After the third washing the worms were held in 0.5 mL of M9 buffer for twenty-four hours. 0.5 mL of either B medium or S medium + glycerol was then added. The final volume was 1.0 mL and the final glycerol concentration was about 15% by volume. The cups were then immediately capped and the autoanalyzer cup holding-tray was placed in a styrofoam freezing box. Each tray holds 100 cups so that twenty stocks were frozen at a time. The freezing box was put into an ultralow temperature freezer (Revco) set at -80 degrees Celsius. The styrofoam box causes the temperature to drop slowly, a condition required for successful recovery of worms upon thawing. A minimum of 48 hours was allowed to pass before any further manipulation of the frozen worms. Once frozen, autoanalyzer trays can be stored in the Revco on the shelf. The freezing box can now be used for another group of stocks.

The autoanalyzer cup can be thawed at room temperature in about 10 minutes. Contents were poured onto a water-agar plate. It was found that the majority of the worms remain in the last few drops of freezing solution in the autoanalyzer cup. To recover these worms the cup was washed with M9-Y LBM. Survivability was noted and food was added to the plate. Following the suggestion of the *Caenorhabditis* Genetics Stock Center, Columbia, Missouri, once the penultimate autoanalyzer

cup is thawed, the stock is refrozen. This leaves one cup as a master cup for those "just-in-case" situations when something unforseeable happens.

MANIPULATING STOCKS

Isolating virgins - *Panagrellus redivivus* females mate upon completion of the last molt (Duggal 1978c). In order to secure virgins for crosses, older juveniles are collected and allowed to mature in isolation. It is not possible to distinguish females from males at the late juvenile stage using a dissecting microscope. To insure that enough virgin females were available for subsequent crosses the number of juveniles isolated was always greater than twice the number of virgin females required. Juveniles were isolated in 2.0 ml autoanalyzer cups containing 0.5 ml M9-Y LGM. The cups are capped and juveniles are allowed to complete their final molts over a two day period. The sex of the mature adults is determined by screening the individual worms using a dissecting microscope. Males have a distinct crescent-shaped spicule near the posterior end of the worm. Also noticeable in adult males is the *vas deferens*, observed as a clear area in the posterior third of the worm. Males curl their tail in a distinctive fashion when swimming in liquid. Females are slightly larger and have a more tapered tail than males. The ovary with

maturing eggs can be seen as a dense granular region in older adult females.

Worms can be individually isolated and transferred by either of two methods. A sharpened wooden applicator stick, sterilized in alcohol or boiling water, can be used to pick up individual worms from a dry plate. A culture plate is referred to as being dry to distinguish it from a culture plate that has been flooded with M9 buffer or M9-Y LGM. A culture plate that has been flooded is referred to as a wet plate. Transferring individual worms via a sharpened applicator stick is best accomplished if the transfer is being made to a drop of liquid (M9 buffer or M9-Y LGM) on a fresh agar plate or liquid in an autoanalyzer cup. The worms from a dry plate adhere to the stick but will swim off of it when submerged in liquid. All sizes of worms can be transferred this way. However, early juvenile stage worms are the most difficult to manipulate because of their small size.

A second method of transferring individual worms involves using a micropipet, made from a disposable Pasteur pipet. A micropipet with a small bore size can be created for collecting juveniles. A larger one can be produced for collecting the larger later stage juveniles and adults. The ease of isolating and transferring individual worms is often dependent on the use of an appropriately sized micropipet. Micropipets are used for

collecting worms from wet plates, or autoanalyzer cups. The worms usually swim when immersed is M9 buffer or M9-Y LGM.

Stock plates from which virgins are to be isolated are sub-cultured on a weekly basis. A 5-7 day old stock plate will have healthy well-feed worms from which to select virgins. Many of the mutants expressing an uncoordinated phenotype (Unc) are best identified on wet plates. Wild type (C15) worms swim in liquid with a distinct sinusoidal movement. The Unc phenotypes vary from subtle movement differences to no movement at all. All other phenotypes are identifiable on dry plates.

MUTAGENESIS

Mutants were isolated from wild-type C15 worms that were subjected to various mutagenic agents; ethyl methanesulfonate (EMS), proflavin, proton irradiation, and ultraviolet light(UV). The EMS mutagenesis protocol was similar to that used by Brenner (1974) - a concentration of 0.05 M EMS for four hours followed by three 20 minute washings in M9 buffer. For the proflavin mutagenesis, *Panagrellus* was grown in a 0.0001% solution for four days. The nematodes were then washed in M9 buffer and plated. Proton irradiation of *Panagrellus* at the Cyclotron Laboratory, Department of Physics, University of Manitoba, was performed by Darlene Agar. A dose of 126 greys (30 Mev) was used. An ultraviolet irradiation of approximately 4 grey was used in an experiment conducted by Darlene Agar on

the sensitivity of C15 to UV light and some of these worms were screened for mutants.

In each case a population of several hundred C15 worms was subjected to the mutagen and, after mutagenesis, early juvenile stage worms were removed,leaving a population of late stage juveniles and adult mutagenized worms. Mutagenized worms were left on a 100 by 15 mm agar plate overnight. Those early stage juveniles appearing the following morning were removed, thereby eliminating worms harboring somatic mutations. The F_1 were isolated in pairs in minibeakers (autoanalyzer cups) with 0.5 ml M9-Y LGM. Each F_1 pair represents an isolate of chromosomes. Four days after isolation the juveniles reach sexual maturity. The minibeakers were then checked to determine if each contained a mating pair. If a minibeaker contained two worms of the same sex, one was moved and the appropriate sex for mating was added. All minibeakers with one male-one female were plated on 60 mm by 15 mm water-agar plates. Worms mating on water-agar plates did so more successfully and produced a larger brood than those mating in minibeakers.

Brother-sister matings in the F_2 allowed for segregation of identical homologous chromosomes. Screening the F_2 permits identification of dominant and X-linked mutations only while screening the F_3 allowed autosomal recessives to be found (see Discussion). All of the paired matings were screened at the F_3

generation to increase the chances of isolating autosomal recessive mutations.

SCREENING FOR VISIBLE MUTANTS

The mutant hunt was carried out visually using a Wild dissecting microscope with substage illumination. The plates being screened were flooded with M9-Y LGM, to insure that both movement and morphological mutants could be detected. The initial screen identifies putative mutant lines. If considerably less than 1/16 of the F_3 population of worms on the plate did not express a scorable phenotype, no mutation was present and the colony was discarded (see Results). At the time of screening for putative mutants, the individual isolates or colonies started from a pair of F_1's had a mixed population of F_2's and F_3's on the plate. Because of the large number of pairs screened (over 2800), it was not possible to tend to each culture to the point of narrowing the age distribution of the F_2 generation so it would not overlap the F_3 generation. However, recessive autosomal mutations were still found despite the background cluttering of the F_2 generation, which would all be expressing a wild-type phenotype. A subculture of mutant worms was established from all plates carrying putative mutants. To make a mutant line homozygous usually required one more subculturing of larvae expressing the phenotype.

GENETIC NOMENCLATURE

The naming system used for mutants of free-living nematodes has been discussed by Burke (1978) and is the one agreed upon by *C. elegans* workers (Horovitz *et al.*, 1979). All mutant lines of *P. redivivus* have been isolated from strain C15, which has been designated wild-type. Gene names refer to a broad phenotypic descriptive characteristic. The name consists of three italicized or underlined lower case letters. Different genes expressing the same phenotype are distinguished by different italicized Arabic numbers, separated from the general name by a hyphen. Mutation names consist of one lower case italicized or underlined letter followed by an italicized or underlined Arabic number. Every independently isolated mutation is given a unique designation.

Each strain or mutant line is given an individual name. The laboratory uses a single uppercase non-italicized letter followed by an Arabic number (eg., C15, N2). The letter used is the same letter as the mutation name (eg., R13, D7). When describing phenotypic characteristics a non-italicized abbreviation corresponding to the gene name and having the first letter capitalized is used, eg. Dpy animals have difficulty mating in minibeakers.

Gene names used in this study were:

bli, blistered;

dpy, dumpy;

lon, long;

rol, roller;

sho, short,

ski, skinny;

sma, small;

sup, suppressor;

unc, uncoordinated.

RESULTS

MUTANT SCREEN

The protocol followed for isolating mutants was based on obtaining homozygotes for each mutagenized chromosome in order to identify autosomal recessive visible mutations. X-linked and dominant visible mutations were also found using this protocol. An individual F_1 worm of treated parents is, obviously, the product of two independently mutagenized gametes. By establishing a colony from two F_1 and allowing brother-sister matings in the F_2, 1/16 of the F_3 population will be homozygous for any particular chromosome. If a dominant autosomal visible mutation occurs, not only will the F_1 parent express the mutation, but so will 1/2 of the F_2 progeny. If an X-linked recessive mutation is carried by an F_1 female, the phenotype will be expressed in 1/2 the male offspring of the F_2 generation. If an X-linked recessive mutation is carried by the F_1 male, the mutant phenotype will disappear for one generation only to reappear in 1/2 of the males of the F_3 population and a few females of the F_3 if a father-daughter mating occurs.

Screening of both the F_2 and F_3 generations of 2814 different F_1 colonies established after mutagenesis with EMS, proflavin, and proton irradiation led to the isolation of 118 different mutant lines. These mutant lines are designated the r-set and in subsequent discussions will carry the prefix r.

The four UV light generated mutants (part of the d-set) were isolated during an experiment on the sensitivity *P. redivivus* to UV light conducted by Darlene Agar. Data on the number of colonies screened were not recorded since a comprehensive mutant hunt was not part of her experiment.

A subsequent screening of 250 colonies from an EMS mutagenesis resulted in the isolation of 10 more mutant lines, designated the m-set of mutants.

PHENOTYPES OF MUTANTS

Table 1 presents list of the seven mutant lines maintained in the laboratory before 1980. A listing of the mutant lines with phenotype designation isolated for this study is presented in Table 2. The mutant types are:

1. Unc: Unc is the phenotypic designation for animals expressing uncoordinated movement. The Unc phenotype can range from extreme reduction in all movement to slight variation in the wild-type swimming pattern. Brief description of each mutant line is meant only as a guide when viewing the phenotype. Uncs were screened on flooded plates (either with M9 buffer or M9-Y LBM) and phenotypes are most distinct when viewed on such plates.
2. Dpy: Dpy is the designation for mutant lines where animals are shorter and fatter than wild-type. Some Dpy animals are not significantly shorter but are distinctly fatter than wild-type suggesting that Fat be a more appropriate designation. Introducing this new phenotype name was avoided.
3. Sma: Sma animals are small worms that are shorter than wild-type but do not appear fatter than wild-type.

4. Ski: These worms are skinnier than wild-type but generally are neither longer or shorter. The phenotype is not easy to identify and is most distinct in juveniles and males.
5. Lon: Lon animals are of two types - those that are longer and skinnier than wild-type and those that are longer and of the same thickness as wild-type.
6. Bli: one mutant line (r96) segregates worms expressing a blister either 1/3 from the head-end or 1/3 from the tail end. There are pleiotropic effects with considerable juvenile lethality occurring.

Table 1. List of mutant lines before 1980

The mutant lines listed in this table have been collected before 1980. TYPE refers to the mutant phenotype and are the same as those described by Brenner (1974) and Horovitz *et al.*, 1979. Linkage refers to the linkage group but only with reference to the X chromosome (X) or to the autosomes (A).

	TYPE	LINKAGE	COMMENT
c30	*dpy*	A	
c31	*dpy*	A	
s1	*sup*	X	a suppressor of crossing over for a large part of the X-chromosome.
j1	*lon*	X	cold-shock induced mutation, allele of B8 and D7.
j1c64	*dbl*	X	
c64	*unc*	X	touch insensitive at the anterior end, distinctly slow movement.
b7	*unc*	X	coils in liquid, reduced movement

Table 2: A list of visible mutants

The mutant lines listed in this table have been collected since 1980. TYPE refers to the mutant phenotype and is the same as described by Brenner (1974) and Horovitz *et al.*, 1979. LG refers to the linkage group but only with reference to the X chromosome (X) or to the autosomes (A). If this has not been determined for the mutant line, an "nd" is found in the LG column. The abbreviations for the mutagens used are: EMS, ethyl methanesulfonate; PFN, proflavin; PTN, proton irradiation; and UV, ultraviolet light.

	TYP	LG	MUTAGEN	COMMENT
r1	*unc*	X	EMS	limited, jerky movement when swimming.
r2	*unc*	nd	EMS	not as severe as r1, jerky swimmer, possibly a Sma mutant tightly linked.
r3	*sho*	nd	EMS	some larval lethality.
r4	*sho*	nd	EMS	
r5	*lon*	nd	EMS	not as distinct as j1.
r6	*dpy*	A	EMS	
r7	*dpy*	A	EMS	reduced fecundity.
r8	*unc*	X	EMS	reduced movement.
r9	*dpy*	A	EMS	reduced fecundity.
r10	*unc*	A	EMS	exaggerated coiling but considerable movement.
r11	*unc*	A	EMS	

Table 2 (continued)

r13	*dpy*	A	EMS	referred to as rice-crispy, severe dumpy, females reduced fecundity, few males(<2.5%)
r17	*sho*	X	EMS	
r18	*dpy*	A	EMS	
r19	*unc*	X	EMS	greatly reduced movement.
r20	*ski*	nd	EMS	most noticeable in larva and adult males.
r21	*ski*	nd	EMS	most noticeable in larvae and adult males.
r22	*dpy*	A	PTN	
r23	*dpy*	A	EMS	reduced fecundity.
r24	*unc*	X	EMS	greatly reduced movement.
r26	*dpy*	X	EMS	
r33	*unc*	A	EMS	reduced movement, jerky swimming.
r34	*unc*	X	EMS	coiling when swimming.
r35	*unc*	A	PTN	greatly reduced movement.
r36	*dpy*	X	PTN	
r37	*unc*	nd	PTN	shaky swimming movement, overexaggerated head swing (curl).

Table 2 (continued)

r38	*unc*	nd	PTN	shaky swimming movement, overexaggerated head swing (curl).
r39	*unc*	nd	PTN	shaky swimming movement, overexaggerated head swing (curl).
r40	*unc*	nd	PTN	shaky swimming movement, overexaggerated head swing (curl).
r41	*sho*	A	EMS	reduced fertility, variable length, not homozygoused.
r42	*sho*	A	EMS	reduced fecundity, variable length, not homozygoused.
r43	*unc*	A	EMS	reduced movement.
r45	*sho*	A	EMS	
r46	*unc*	X	EMS	reduced movement, exaggerated swimming motion (c64-like).
r48	*unc*	X	EMS	reduced movement, not as active as C15.
r49	*unc*	A	EMS	reduced movement, exaggerated swimming motion (c64-like).
r50	*sho*	X	EMS	
r51	*sho*	A	EMS	

Table 2 (continued)

r52	*dpy*	A	EMS	
r54	*sho*	A	EMS	
r56	*unc*	A	PFN	reduced movement, coiling when swimming, slight twitching.
r57	*sho*	A	PFN	
r59	*sho*	A	PFN	
r60	*unc*	X	PFN	reduced movement, smooth sinusoidal movement is disrupted.
r61	*lon*	nd	PFN	not as long as j1 or d7.
r62	*unc*	A	PFN	
r63	*unc*	X	PFN	
r64	*unc*	A	PFN	
r65	*unc*	A	PFN	
r66	*unc*	A	PFN	
r67	*unc*	A	PFN	
r68	*sho*	A	PFN	
r69	*unc*	A	PFN	
r71	*unc*	X	EMS	
r72	*unc*	A	EMS	slightly reduced activity head curls and pauses.

Table 2 (continued)

r73	*unc*	A	PFN	
r80	*sma*	A	PTN	
r81	*sho*	A	PTN	some juvenile lethality.
r82	*unc*	A	PTN	shaky swimming movement (unc-22 like??).
r83	*ski*	nd	PTN	most noticeable in juveniles and adult males.
r84	*dpy*	X	PTN	
r85	*sma*	X	PTN	
r86	*unc*	A	PTN	reduced movement, very distinct phenotype.
r87	*sma*	X	PTN	
r88	*sma*	nd	PTN	
r89	*sma*	X	PTN	
r90	*sma*	nd	PTN	
r91	*dpy*	X	PTN	
r92	*dpy*	X	PTN	
r93	*unc*	A	PTN	reduced movement, slight coiling.
r94	*unc*	X	PTN	reduced movement, slight shake in swimming, uncoordinated swimming.

Table 2 (continued)

r95	*unc*	A	PTN	
r96	*bli*	nd	PTN	blister forms 1/3 from head end or tail end. Many larvae very sick and some lethality.
r97	*unc*	A	PTN	slight twitch in swimming movement, partial reduced movement, exaggerated head swing.
r98	*sma*	nd	PTN	
r99	*sma*	A	PTN	
r100	*ski*	nd	PTN	most apparent in juveniles and adult males.
r101	*unc*	A	PTN	
r102	*unc*	A	PTN	partial reduced movement, curls when swimming.
r103	*unc*	A	PTN	
r104	*unc*	A	PTN	reduced movement, slight twitching.
r105	*unc*	A	PTN	reduced movement, slight twitching.
r106	*unc*	A	PTN	reduced movement, slight coiling.
r107	*sho*	nd	PTN	
r109	*ski*	nd	PTN	most noticeable in larvae and adult males.

Table 2 (continued)

r110	*unc*	nd	PTN	lacks the smooth motion of wild-type, jerky motion.
r111	*ski*	nd	PTN	most noticeable in larvae and adult males.
r112	*dpy*	X	PTN	much larval lethality.
r113	*sho*	nd	PTN	subtle difference in length, nearly wild-type, some larval lethality.
r114	*ski*	nd	PTN	most apparent in juveniles and adult males.
r115	*sho*	X	PTN	
r116	*unc*	A	PTN	reduced movement, shakes when submerged in food or buffer (r125-like).
r117	*sho*	X	PTN	
r118	*unc*	A	PTN	greatly reduced activity
r120	*unc*	A	PTN	
r121	*unc*	nd	PTN	
r122	*unc*	nd	PTN	
r123	*unc*	Xd	PTN	
r124	*sho*	nd	PTN	also slightly uncoordinated, possibly a double mutant.
r125	*unc*	A	PTN	shakes while swimming

Table 2 (continued)

r126	*unc*	nd	PTN	
r127	*unc*	nd	PTN	
r128	*dpy*	A	EMS	
r129	*dpy*	A	EMS	
r130	*unc*	nd	EMS	
r131	*unc*	nd	EMS	
r132	*unc*	nd	EMS	
r133	*unc*	nd	EMS	
r134	*unc*	nd	EMS	
r135	*sho*	nd	EMS	
r136	*sho*	nd	EMS	
r137	*unc*	nd	EMS	twitchy movement in liquid.
r138	*unc*	nd	EMS	
r139	*sho*	nd	EMS	
r140	*unc*	nd		from Chris Link, Boulder, Colo., reduced movement, bending rather than smooth motion.
r141	*unc*	nd	EMS	
r142	*unc*	nd	EMS	

Table 2 (continued)

r143	*unc*	nd	EMS	
m1	*sho*	X	EMS	many dead F_1 juveniles in cross to C15 males.
m2	*unc*	nd	EMS	
m3	*dpy*	X	EMS	dead F_1 juveniles in cross to C15 males.
m4	*unc*	nd	EMS	
m6	*unc*	nd	EMS	
m9	*unc*	nd	EMS	
m10	*sma*	nd	EMS	
m11	*sho*	nd	EMS	
m12	*unc*	nd	EMS	
m13	*unc*	nd	EMS	
q1	*dpy*	A	EMS	Allele of c30.
d1	*dpy*	A	PTN	
d2	*sma*	X	PTN	
d3	*dpy*	A	UV	
d4	*dpy*	A	UV	
d5	*dpy*	nd	UV	greatly reduced fecundity.
d6	*dpy*	nd	UV	tail appears truncated in both males and females.
d7	*lon*	X	PTN	an allele of j1.

MUTANT CHARACTERIZATION

The amount of time required to characterize completely 136 mutant lines only became evident as the work was being attempted. Appendices 1, 2, 3, and 4 discuss genetic characterization that has been done on a subset of the mutant lines. Mutant lines were backcrossed to wild-type males. The progeny were scored for the expression of the mutant phenotype. If mutant expression in the F_1 was restricted to males, the mutant phentotype was considered to be X-linked. If the phenotype disappeared for one generation and reappeared in both male and female F_2 progeny, the mutant phenotype was considered to be autosomal recessive. When both male and female F_1 progeny express the phenotype (or partially express the phenotype), F_2 progeny were paired and colonies no longer generating wild-type progeny were considered the homozygous mutant line. These mutants were designated as dominant or semi-dominant depending upon the degree of penetrance. All mutant lines were kept, even those showing incomplete penetrance and variable expressivity.

Results on complementation analysis, linkage group analysis and recombination mapping within a linkage group are presented in appendices 2, 3, and 4 respectively.

Preliminary work on the morphometrics of a small subset of the morphological mutants is presented in Table 3. Measurements were taken of worms from seven day old cultures.

Heat-killed worms were stained with lactophenol cotton blue and 28X magnified prints of the worms were made on a Canon Microfiche printer 370. Measurements were made on a Hewlett Packard 9111A Graphics Tablet coupled to a Hewlett Packard 9835A Desk Top Computer. The software was written by Dr. M. Samoiloff. The data demonstrate the distinct difference in length and width of mutants compared to wild-type. Table 4 presents relative values comparing the length and width of the mutants to wild-type (C15). There appears to be some degree of sex-specific expression on the mutant phenotype, since male and female mutants only occasionally vary the same degree, relative to their wild-type counterparts.

There are three definite classes of worms smaller than wild-type. Dpy (dumpy) worms were both shorter and fatter; Sma (small) worms were both shorter and thinner; Sho (short) worms were the same width as wild-typebut were shorter. Brenner (1974) does not distinguish the Sho phenotype in his analysis of the mutants but includes these with the Dpy's.

Table 3: Size determination of a subset of the morphological mutants.

The sizes are given in micrometers.

		MALES				FEMALES			
STOCK	TYPE	LENGTH	(SD)	WIDTH	(SD)	LENGTH	(SD)	WIDTH	(SD)
C15	WILD	753	77	43	10	1011	132	64	12
j1	*lon*	1039	99	37	8	1264	166	51	11
c30	*dpy*	654	45	59	12	746	84	77	17
q1	*dpy*	648	71	48	9	689	80	75	12
r112	*sho*	579	46	44	11	635	67	62	13
r84	*sho*	605	45	44	9	741	54	66	17
r85	*sma*	537	60	39	7	612	61	51	11
r89	*dpy*	643	60	57	15	736	60	76	14
r22	*dpy*	611	48	54	7	801	65	73	8
r92	*dpy*	610	63	49	10	719	51	73	14
r80	*sma*	630	57	41	8	722	103	57	10
r87	*sma*	570	64	45	9	651	61	59	13
r99	*sho*	676	78	45	11	845	67	66	13
d1	*sho*	606	50	46	7	703	68	67	11
d2	*sma*	537	39	41	7	667	53	56	12
r91	*sho*	591	57	45	9	714	61	65	9
c31	*dpy*	634	60	55	11	818	101	74	11

Table 4. Ratio of mutant length and width relative to wild type.

STOCK		MALE LENGTH	MALE WIDTH	FEMALE LENGTH	FEMALE WIDTH
j1	*lon*	1.38	0.86	1.25	0.80
c30	*dpy*	0.87	1.37	0.74	1.20
q1	*dpy*	0.86	1.12	0.68	1.17
r112	*sho*	0.77	1.02	0.63	0.97
r84	*sho*	0.80	1.02	0.73	1.03
r85	*sma*	0.71	0.91	0.61	0.80
r89	*dpy*	0.85	1.33	0.73	1.19
r22	*dpy*	0.81	1.26	0.79	1.14
r92	*dpy*	0.81	1.14	0.71	1.14
r80	*sma*	0.84	0.95	0.71	0.89
r87	*sma*	0.76	1.05	0.64	0.92
r99	*sho*	0.90	1.05	0.84	1.03
d1	*sho*	0.80	1.07	0.70	1.05
d2	*sma*	0.71	0.95	0.66	0.88
r91	*sho*	0.78	1.05	0.71	1.02
c31	*dpy*	0.84	1.29	0.81	1.16

The complementation analysis only included a small subset of the mutant lines. The theory behind complementation analysis and some representative data are included in Appendix 2. One of the more interesting observations in complementation testing was that r60, an X-linked Unc mutant epistatic to *unc-1* (b7), is itself recessive to wild-type. However, crosses to *unc-1* produced an r60 Unc phenotype. Table 5 shows the results of crosses between r60 and b7 mutant worms.

Complementation analysis of some of the autosomal unc mutations produces conflicting results. For example, r62, r64, and r69 are autosomal recessive mutations producing Unc phenotypes. Both r64 and r69 worms were non-complementing with r62 animals, suggesting that the three mutants are independent mutations at a single locus. Yet r69 females crossed to r64 males produced 4 non-Unc out of 156 progeny. Although not enough work has been done on this set of mutants to say with any degree of certainty what is happening, it is possible that some form of suppression is occurring in these autosomal recessive mutations.The frequency or wild-type in this cross is too high for the results to be explained in terms of back-mutation. If the gene defined by r64 and r69 is a complex locus and these results are the consequence of intracistronic recombination, the gene complex would have to be large. At this point none of these possibilities is excluded.

Table 5. Complementation results for B7 and R60, an X-linked recessive Unc showing epistasis to *unc-1*.

PARENT		PROGENY	
female	male	daughters	sons
C15	r60	wild-type	wild-type
r60	C15	wild-type	r60-like Unc
b7	r60	r60-like Unc	b7-like Unc
r60	b7	r60-like Unc	r60-like Unc

DISCUSSION

In his 1974 paper Brenner describes the eight year project of developing the genetics of *Caenorhabditis elegans*. *C. elegans* is a self-fertilizing hermaphrodite, permitting easy isolation of recessive autosomal mutations as well as isolatiion of mutants severly afflicted in their ability to move. The adult hermaphrodite first makes about 50 sperm which are stored in the spermatheca. The hermaphrodite then starts making eggs which are fertilized by the sperm. One half of the sperm cells and one half of the mature eggs of a worm heterozygous for a particular mutation will carry the mutant gene. Therefore, one quarter of the self-progeny will be homozygous for the mutation. By isolating F_1 progeny of a mutagenized hermaphrodite and scoring the F_2 (self-progeny) of each hermaphrodite for a mutation, many visible mutants can be found. Only a few F_1 progeny of each mutagenized parent need to be screened in this manner to ensure independent origin of each mutation. Because *C. elegans* is self-fertilizing, severe mutations in movement such as *unc-54* can be isolated. *Unc-54* is a mutation in the gene coding for the major heavy myosin making up the musculature of the body wall. The worm can still feed and reproduce despite its inability to move around on the plate.

In *C. elegans*, males arise spontaneously by non-disjunction of the X-chromosome at a frequency of about 1 in

700. Non-disjunction of the X-chromosome can be induced at a greater frequency (10-20%) by treatment with a heat pulse. Cross-fertilization occurs and the sperm from a male is used preferentially to the self-produced hermaphrodite sperm. However, an out-crossed hermaphrodite will still self-fertilize once the sperm from the cross is used up. This makes genetics with *C. elegans* somewhat more cumbersome than with a dioecious species such as *P. redivivus*.

Brenner isolated 300 ethyl methane sulfonate (EMS) induced visible mutant lines in *C. elegans* which defined 77 genes by complementation analysis.

P. redivivus is a dioecious species requiring males for fertilization. Autosomal recessive mutations are most easily found by screening the F_2 progeny of brother-sister matings as described in Materials and Methods and Results. This protocol is no different than screening for autosomal recessives in any dioecious species. Although more work is required to isolate mutants of *P. redivivus* than for *C. elegans*, mutants of *P. redivivus* are readily found. The severe uncoordinated phenotypes found in *C. elegans* cannot be easily maintained in *P. redivivus*, because in the dioecious species males and females have to be able to move well enough to find each other, and be coordinated enough to mate. Most of the phenotypes found by Brenner in *C. elegans* were also found in *P. redivivus* (roller mutants were found but stocks of these were not

established). A limited number of visible phenotypes is expected as nematodes possess few cosmetic features that can be altered without drastically decreasing viability or reproduction.

One novel phenotype found in *P. redivivus* is a severe dumpy (r13). This autosomal recessive mutation is more extreme than any of the dumpys found in *Caenorhabditis*. The homozygote is only 1/3 as long as wild-type and 1 1/2 times as wide. The phenotype is referred to as "rice-crispy" because of the resemblance of the homozygotes to the breakfast cereal (although they do not go snap-crackle-n' pop, as do their commercial namesakes). Females homozygous for r13 will mature sexually, and when mated to wild-type males will produce a limited number of offspring (anywhere from 10 to 40). The heterozygotes are fatter and shorter than wild-type and when crossed to each other, produce wild-type, dumpy heterozygotes, and rice-crispy in the expected 1:2:1 ratio. Only 2 of 80 r13 adults showed mature male features. However, as 1/4 of the progeny of r13/+ females crossed to r13/+ male express the "rice-crispy" phenotype it is reasonable to conclude that "rice-crispy" males are present but rarely mature. The r13 mutant is maintained as a heterozygote, and it is necessary to remove wild-type animals from the stock plate at regular intervals.

Among the mutant lines generated, some carried new X-linked recessive mutations. Burke (1978) isolated X-linked mutants of *P. redivivus*. He did not find any X-linked dumpy or small mutants and could only maintain six X-linked mutant lines. Since then five of these have been lost. Before this study was initiated only three X-linked visible mutant strains of *P. redivivus* were cultured. One of these mutants was an *unc* from Burke's study (b7). The second X-linked visible mutant (j1) was a *lon* that was found by Yvonne Jordan and appears to have spontaneously arisen or possibly was cold-shock induced. A second *unc* (c64) was isolated by Dr. M. Samoiloff. This mutant is touch insensitive - when gently touched on the head wild-type worms will reverse their movement temporarily, then start forward again. The c64 mutant worm when touched gently on the head stops moving, attempts a reverse movement but seems incapable of backing up. The swimming behavior of c64 in liquid is slower than wild-type and the anterior end of the worm exhibits an exaggerated swinging movement.

A series of crosses to determine the type of linkage (sex-linked or autosomal) and type of expression (dominant or recessive) were performed with each mutant stock. Virgin mutant females are crossed with C15 males to determine if the mutation is X-linked. Twenty nine of the mutant strains that have been backcrossed are X-linked, but only a small number of these X-linked mutants have been complemented. However,

preliminary results indicate there are two distinct *dpy* loci on the X chromosome. Four complementing X-linked *unc* mutations have also been found in the early analysis. *C. elegans* has 105 different *unc* mutants of which 19 are X-linked, 26 different *dpy* mutants of which 7 are X-linked, and 5 *sma* mutants of which one is X-linked. There is one X-linked *lon* in *C. elegans*, which can be used as a landmark for the chromosomal comparison with the *P. redivivus* X-chromosome. The recombination data from Burke & Samoiloff (1981) can be used to determine if any of the mutants isolated in the present study are alleles of mutants previously isolated that have been subsequently lost.

In carrying out mutant hunts for visible phenotypes such as movement and morphological variants, it became apparent that direct screening the F_1 for putative mutations is too time intensive. Often a worm may appear to be a mutant but produces normal F_2 and F_3 progeny. Such a failure in "fixation" of the mutation cuold be due to several causes. The "mutant" phenotype could be due to mutation in the somatic tissue of the embryo. The mutation is, therefore, expressed in that generation but, because it is not present in the germ cells, is not transmitted to successive generations. Alternately, the F_1 worm may be unhealthy, as sometimes occurs if stocks are under partial starvation conditions.

When screening is done in the F_2 and F_3 and a mutation is present in the colony started from a given F_1 pair, the

mutation is easily identified because of the greater number of worms expressing the phenotype. The protocol followed here was successful in that it led to the isolation of many visible mutant lines. Previous difficulties in isolating a set of visible markers may have been due to the protocol followed, focusing on selection of F_1 and F_2 putative mutants. Before this study was undertaken only five autosomal visible mutations had been isolated in *P. redivivus*. From the complementation tests that have been done it appears that at least 5 different autosomal *dpy* and 10 different *unc* mutations have been isolated. It should be noted that only a small subset of the many mutations isolated have been complemented. Only 20 autosomal *dpy* and 19 autosomal *uncs* have been tested extensively in a complementation matrix.

In total, 61 mutant lines were established from 1543 F_1 mated pairs from the EMS mutagenesis, 14 mutant lines were established from 967 F_1 pairs following proflavin mutagenesis, and 56 mutant lines were obtained from the screening of 554 F_1 pairs from the proton mutagenesis. The differences in the frequency of visible mutations isolated from each mutagenesis might reflect the effectiveness of that particular mutagen, but it is more likely that increased facility in recognition of mutant phenotypes as the study progressed resulted in a higher frequency of detection of mutants later in the study. As the person screening the F_2 plates became more familiar with the

wild-type phenotype and more adept at manipulating the organism, fewer plates carrying mutations were overlooked, missed, or discarded.

The need for a method of storing mutant lines for future analysis became apparent while maintaining the 136 mutant lines. Many mutant stocks developed, over several generations, a significant proportion of animals expressing the wild type phenotype. This reversion could be due to either back-mutation or suppression. For these reverting stocks, the mutation often had to be re-isolated. If a second mutation or reversion occurs such that a worm becomes more mobile and, therefore, feed or mate more efficiently, that phenotype will soon predominate the culture. Storing the worms in a way that minimizes the accumulation of revertants is necessary for future work on the mutant lines. Freezing the stocks by a modification of the technique used for *C. elegans* at the Genetics Stock Center allows the mutant lines to be maintained for further use.

The size measurements of some of the morphological mutant lines reveals the range of variations in size. Since all of the post-embryonic cell lineage of *P. redivivus* is known, it is possible that some of the mutations can be correlated to specific lesions in cell lineage. One less round of division of hypodermal cells could produce a short animal. One or more rounds of division for the body wall muscle cells would produce

a dumpy animal. A lesion at the level of the cell size could produce a small phenotype. Electron microscopy could be used to determine if differences in cell size or cellular arrangement have contributed to the altered phenotype.

Previous work on the post-embryonic development of *P. redivivus* has shown that certain events of post-embryonic development can be uncoupled (Samoiloff, 1980a). While growth of older larvae can be blocked with inhibitors of RNA and protein synthesis, sexual maturation still occurs. Work with cell-lineage mutants in *C. elegans* (Albertson *et al.*, 1978) also suggests the independent nature of certain post-embryonic events. A mutation interfering with post-embryonic cellular and nuclear divisions does not affect several other developmental events normally concomitant with these divisions.

Using the mutants isolated in this study, a systematic hunt for conditional mutants affecting post-embryonic development could be performed. Isolating a suite of mutants blocked in post-embryonic development and determining the sequential requirements by complementation and segregation analysis, could reveal the steps leading to normal maturation. By using the power of molecular genetics to isolate the genes affected, such a study could be brought to the molecular level. Many researchers have set these goals and are pursuing them using various biological systems.

P. redivivus satisfies the criteria of a good experimental animal for studying the control of development - its simplicity, ease of handling, amenability to genetic manipulation and a strong background of basic research performed on it has been used as an argument for the exploitation of this organism for extensive study (Samoiloff, 1980b). The addition of this large set of mutant lines strengthens the utility of this organism, while the development of freezing methods for *P. redivivus* ensures that the stocks will be continued.

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APPENDIX 1. BACKCROSS TO C15

Once a mutant line was isolated it was backcrossed to C15, the F_1 progeny crossed, and thier progeny reselected in order to reisolate the mutation in a wild-type background. It is possible for the mutant phenotype to be the result of altered expression of two separate gene loci. If, however, the two mutations are tightly linked, the backcross to C15 might not expose the bi-mutational nature of the phenotype. The backcross also provides information about the linkage group the mutant locus is associated with. If the mutation is an X-linked recessive, then a cross between mutant virgin females and a C15 male will produce mutant male progeny. If the mutation is autosomal recessive, then the same cross produces no mutant F_1 progeny. However, 1/4 of the male and female F_2 progeny will express the phenotype. If it is a dominant X-linked mutation, then all of the F_1 will express the phenotype and 1/2 of the F_2 males will also express the mutation. If it is a dominant autosomal mutation, all of the F_1 will express the phenotype while 1/4 of the F_2 progeny (both male and female) will have a wild-type phenotype. Not every mutant line has been backcrossed. Data in Table 2 (Results section) regarding X-linkage or autosomal linkage are derived from the backcross to wild-type.

APPENDIX 2. COMPLEMENTATION

Complementation testing is a method of determining how many separate loci are represented by a group of mutants expressing a similar phenotype. Complementation in a diploid organism occurs when a wild-type gene at the same locus of the homologous chromosome compensates for the genetic perturbation of a mutated gene. Crossing mutant lines expressing similar phenotypes and scoring the frequency of wild type in the first generation allows one to determine if the two mutant lines represent two different mutant genes or if both mutant lines are genetically perturbed at the same gene locus.

Complementation is not possible with dominant gene mutations. However, scoring the frequency of wild type in the F_2 will indicate if the two mutants represent the same of different loci.

Table 6 and Table 7 describe testing of recessive mutations to determine how many different loci are represented. Table 8 is a matrix for complementation testing of a set of autosomal recessive *dpy* mutants. Table 9 is a matrix for complementation testing of a set of recessive *unc* mutants.

Table 6. Strategy for complementation of autosomal recessives.

parents		progeny phenotype	interpretation
female	male		
mutA	mutB	wild-type	complementing, mutA and mutB are different gene loci
mutA	mutB	mutA and/or mutB	non-complementing complementing mutA and mutB represent the same gene locus

Table 7. Strategy for complementation of X-linked recessives.

parents		progeny	interpretation
female	male		
mutA	mutB	sons= mutA daughters=wild-type	complementing, mutA and mutB are different loci
mutA	mutB	sons, mutA daughters mutA	non-complementing mutA and mutB are same locus

Table 8. Complementation matrix for a set of autosomal recessive Dpy mutants.

Abbreviations:

c complementing
nc non-complementing.
- non-complementing self-cross

	r6	r7	r9	r18	r22	r23	r42	r45	r129
r6	-	c	c	c	c	c	c	c	c
r7	c	-	c	nc	c	nc	nc	nc	c
r9	c	c	-	c	c	c	c	c	c
r18	c	nc	c	-	c	nc	nc	nc	c
r22	c	c	c	c	-	c	c	c	c
r23	c	nc	c	nc	c	-	nc	nc	c
r42	c	nc	c	nc	c	nc	-	nc	c
r45	c	nc	c	nc	c	nc	nc	-	c
r129	c	c	c	c	c	c	c	c	-

Table 9. Complementation of a subset of the autosomal recessive mutations.

Abbreviations:

c	complementing
nc	non-complementing
nd	not determined.
-	non-complementing self cross

	r10	r11	r33	r35	r43	r56	r62	r64	r69	r73	r86	r103	r104	r105
r10	-	nd	nc	nc	nd	nd	nc	c	nd	nd	nc	nd	nd	nd
r11	nd	-	c	c	nd	c	nd	nd	nd	nd	c	c	c	nd
r33	nc	c	-	c	c	c	c	c	c	c	c	c	c	nd
r35	nc	c	c	-	nd	c	c	c	c	c	nc	nc	c	c
r43	nd	nd	c	nd	-	nd	c	c	c	nd	nd	nd	nd	nd
r56	nd	c	c	c	nd	-	c	c	c	nd	c	c	nd	nd
r62	c	c	c	c	c	c	-	nc	nc	nd	c	c	c	c
r64	c	c	c	c	c	c	nc	-	nc	nd	c	c	c	c
r69	c	c	c	c	c	c	nc	nc	-	nd	c	c	c	c
r73	nd	nd	c	c	nd	nd	nd	nd	nd	-	c	c	nd	nd
r86	nc	c	c	nc	nd	c	c	c	c	c	-	nc	c	c
r103	nd	c	c	nc	nd	c	c	c	c	c	nc	-	c	c
r104	nd	c	c	c	nd	nd	c	c	c	nd	c	c	-	nd
r105	nd	nd	nd	c	nd	nd	c	c	c	nd	c	c	nd	-

APPENDIX 3. LINKAGE ANALYSIS

Linkage analysis requires two mutants of dissimilar phenotype. In linkage analysis a double mutant must be obtained, or the coupling frequency between the two mutants must be determined. A double mutant with two mutations of similar phenotype is not normally distinguishable from the single mutant. However, if the two mutants vary in phenotype in such a way that the individual homozygous for the two mutations can be distinguished (such as an *unc* crossed to a *dpy*, *sho*, or *lon*), then the frequency with which both mutations are expressed in a single individual (Unc-Dpy, Unc-Sho, or Unc-Lon) indicates if the genes are linked.

If two autosomal recessive mutants are unlinked, then 1/16 of the F_2 will express the double phenotype. For linked genes, the double will occur at a frequency of $r^2/4$, where r is the recombination frequency. The unit of closeness of linkage is the centimorgan which is the distance producing 1% recombination. If two autosomal recessive mutants are linked and are 50 or more centimorgans apart, the double also appears in about 1/16 of the F_2 progeny. If the mutations are linked and are less than 50 map units apart, then the frequency of the double will be less than 1/16.

The autosomal recessive Dpy mutation C30 was canonically declared linkage group I (LG I). A series of Unc mutant lines were crossed to C30 and the F_1 were allowed to undergo brother-

sister matings. The F_2 were screened and the first Unc not linked to C30 was r86. This mutant line was designated as a member of linkage group II (LG II). At this point other autosomal Dpys were crossed to r86 to find which ones were linked to LG II and which ones were not. A stepwise analysis of the mutants to determine which Unc mutants were linked with which Dpy mutants led to an initial characterization of linkage groups. The strategy for linkage analysis involves crossing a panel of *unc* to a panel of *dpy* mutants and scoring the appearance of doubles in the F_2 for each cross. If the linkage groups are large enough to carry two genes more than 50 map units apart, then these discrepancies will be recognized in the matrix analysis.

It is worth mentioning that only one of the six *C. elegans* linkage groups are larger the 50 map units, although four of the others are approximately 45 map units in length.

Some linkage analysis has been carried out with the *P. redivivus* mutants. However, the data-base is at the early stages and is presented here with that in mind. Table 10 contains data collected for the linkage analysis. Brenner screened a minimum of 1000 progeny (F2 progeny) for each pair of mutations in the linkage analysis of his visible mutants (Brenner 1974). In only two of the crosses in the *P. redivivus* study of linkage analysis had 1000 F2 progeny been screened for doubles. Therefore more data must be collected before linkage

group assignments can be made with a high degree of certainty. The data matrix in Table 10 includes only a small subset of the mutants.

Tentative assignments to linkage groups can be made. r95 and r10 are two *unc* mutants linked to C30 (Dpy) on LG I. The Dpy mutant lines d1, r80, and r7 are linked to r86 on LG II. Because r94 in not linked to either C30 or d1, it is assigned to LG III. Mutation d3 (Dpy) is linked to r94 (Unc) and r97 (Unc) is linked to d3. Therefore these three mutations are found together on LG III. Since r82 and r99 are linked yet do not appear to be linked to any of the above mutants, they are considered to reside on LG IV. These putative designations may have to be altered as more linkage-analysis crosses are performed.

Table 10. Linkage analysis raw data.

	not double	double	sum	expected double	comment
r86/C30	117	11	128	8	unlinked
r94/C30	1013	56	1069	67	unlinked
r95/C30	567	5	572	36	Linked
r97/C30	264	19	283	18	unlinked
r93/C30	336	24	360	23	unlinked
r82/C30	298	18	316	20	unlinked
r104/C30	296	19	315	20	unlinked
r103/C30	310	17	327	20	unlinked
r101/C30	303	20	323	20	unlinked
r49/C30	819	64	883	55	unlinked
r10/C30	1044	35	1979	67	Linked
r64/C30	710	55	765	48	unlinked
r35/C30	900	67	967	60	unlinked
r120/C30	484	23	507	31	unlinked
r125/C30	329	20	349	22	unlinked
r105/C30	281	32	313	20	unlinked
r86/d3	136	8	144	9	unlinked
r86/d1	351	1	352	22	Linked
r86/r99	146	8	154	10	unlinked
r86/r80	362	2	364	23	Linked
r86/r7	230	8	238	15	Linked (maybe)

Table 10 (continued)

	not double	double	sum	expected double	comment
r95/r80	137	10	147	9	unlinked
r104/r80	382	3	385	24	Linked
r104/d1	423	3	426	27	Linked
r104/d3	129	6	135	8	unlinked
r82/d3	117	9	126	8	unlinked
r82/d1	115	6	121	8	unlinked
r94/d3	432	8	440	28	Linked
r94/d1	121	9	130	8	unlinked
r94/r99	142	9	151	9	unlinked
r94/r80	145	10	155	10	unlinked
r95/r99	194	10	204	13	unlinked
r97/r99	123	8	131	8	unlinked
r93/r99	147	11	158	10	unlinked
r82/r99	254	3	257	16	Linked
r97/d1	170	9	179	11	unlinked
r97/d3	186	2	188	12	Linked
r93/d1	387	6	393	25	Linked
r94/c31	503	27	530	33	Linked
r64/c31	770	61	831	52	unlinked
r102/c31	310	6	316	20	Linked
r94/r7	387	24	411	26	unlinked
r64/r7	592	49	641	40	unlinked

r102/r7	291	18	309	19	unlinked
r64/r6	220	8	228	14	unlinked?
r94/r6	571	14	585	36	Linked

MAPPING STRATEGY

If two genes are linked then the frequency with which the double arises in a dihybrid cross can be used to determine how far apart the two genes are from each other in map units. The frequency of coupling will be $r^2/4$ where r is the recombination frequency between the two genes.

Trans-heterozygotes are generated by crossing virgin females of one mutant to males of the other. The F_1 will be heterozygous at both loci. If a recombination event occurs between the two loci the products of that event will be one wild type chromosome and one chromosome carrying both mutant genes. Recombination between the two loci will occur at a constant frequency r. The probability of such an event occurring in both female and male gametes contributing to a single zygote is r^2, but in such cases, the probability of both doubly marked chromosomes forming a double mutant homozygote F_2 is 1/4, while *cis*-heterozygotes, at a frequency of 1/2, and wild-type homozygote, at a frequency of 1/4, will express the wild type phenotype. Therefore, the total probability of a double mutant F_2 occurring is $r^2/4$. Because of the rare occurrence of the coupling product and the possibility of missing the double mutant among the many other F_2 progeny, linkage determined in this fashion is imprecise.

An alternate method of mapping involves scoring the frequency of repulsion. From the dihybrid cross between the

two mutants, the F_2s expressing the double phenotype are collected and a stock culture of these doubles established. Virgin double mutant females are crossed to C15 males to generate *cis*-heterozygotes. The *cis*-heterozygotes are backcrossed to the doubles. The expected F_2 progeny are wild-types and doubles in a 1:1 ratio. Every time a single recombination occurs between the two loci, two recombinant progeny can be identified in the F_2; one expressing one of the two phenotypes and the other expressing the other of the two phenotypes. The frequency of progeny expressing the recombinant chromosome is r, the recombination frequency between the two loci. For two closely linked genes, this method of mapping is more sensitive as a greater number of recombinant products arise. This method reduces the possibility of missing the rare double mutants arising from recombination between two tightly-linked genes. However, this approach is time-intensive, as it requires isolation of the double mutant.

One strategy followed was to collect animals expressing one of the two phenotypes while constantly culling-out those expressing the wild-type and the other phenotype. The recombinant chromosome accumulates and eventually can be found in a stock which has most of the background genetic variability removed. The rare doubles are collected and a stock started from them.