C. Elegans Newsletter

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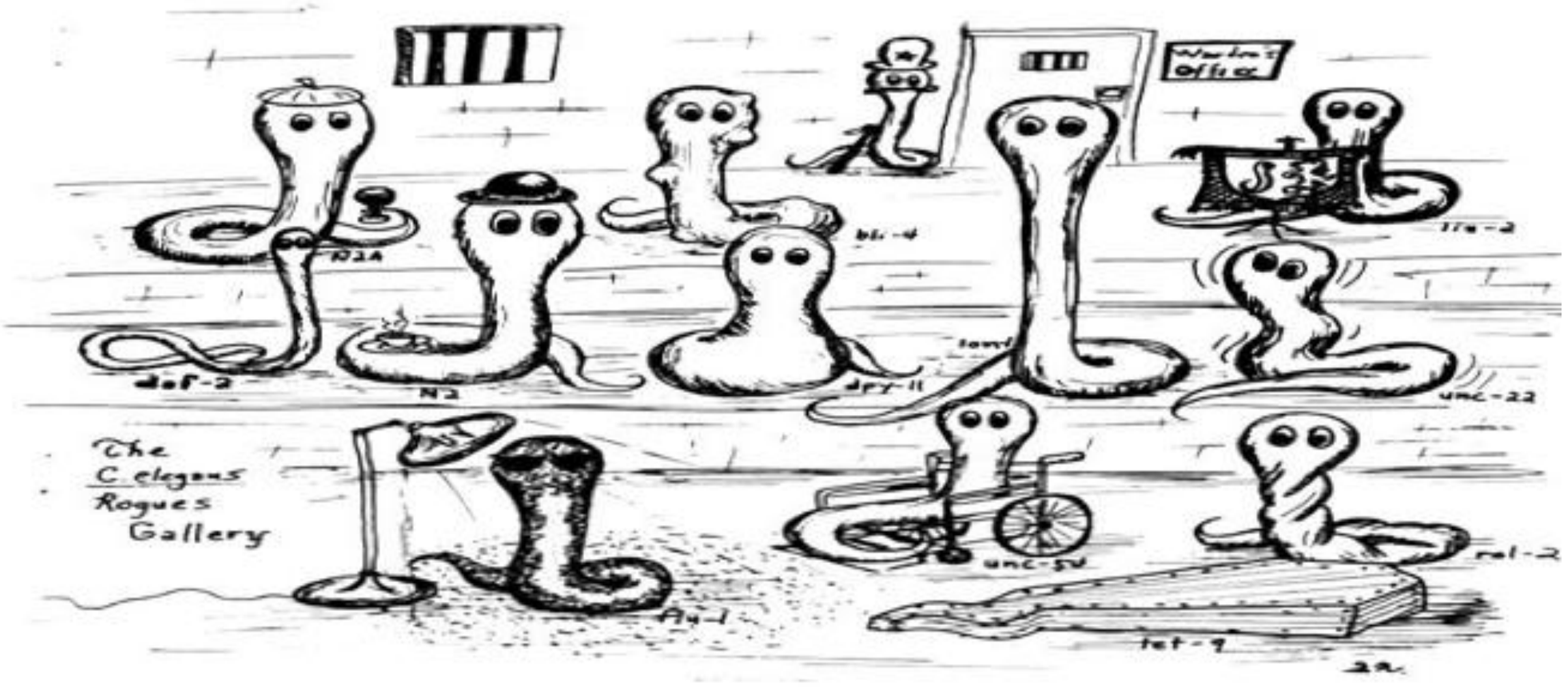

THE GENETICS OF *CAENORHABDITIS ELEGANS*

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Manuscript received December 10, 1973

Post-embryonic Cell Lineages of the Nematode, *Caenorhabditis elegans*

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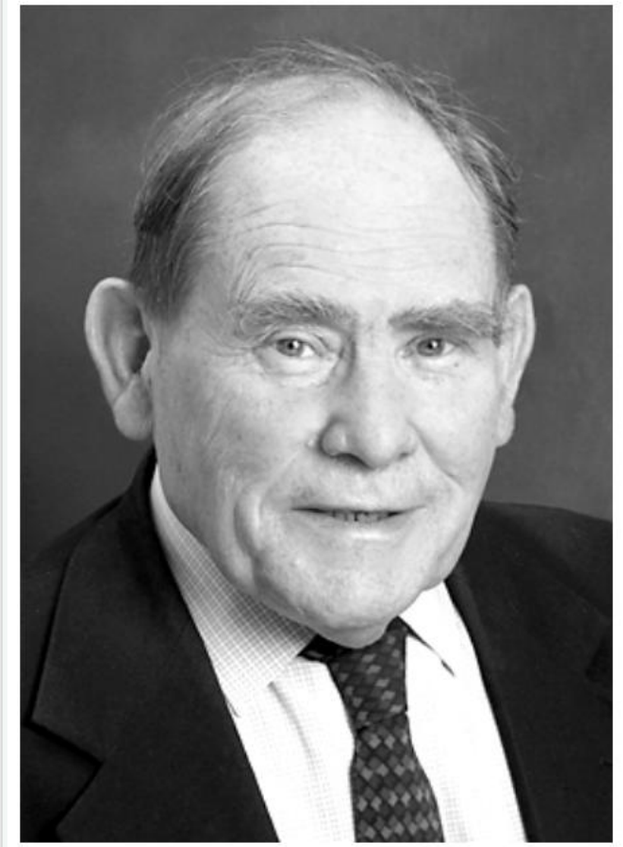
Received August 23, 1976; accepted November 4, 1976

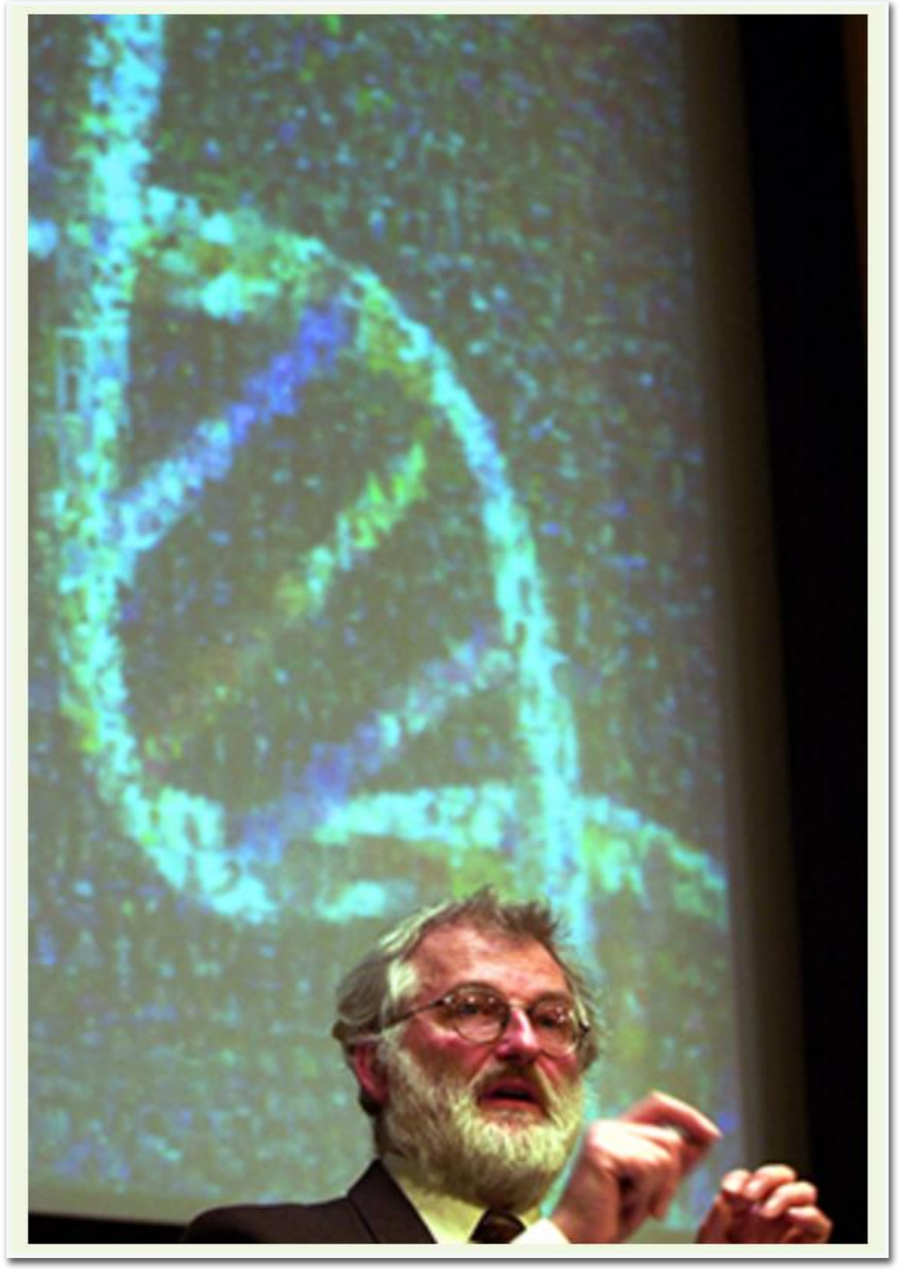
Sydney Brenner: 1927 - Present

- Born in the small town of Germiston, South Africa
- Won a scholarship to the University of Witwatersrand at the age of 15
- Received his PhD from Exeter College, Oxford
- Made several contributions to the emerging field of molecular biology
  - 3 nucleotides code for a single amino acid
  - Helped to prove collinearity between the genetic message and the protein product
  - Helped establish the existence and function of messenger RNA
- Enjoys good wine, traveling, and writes an opinion column



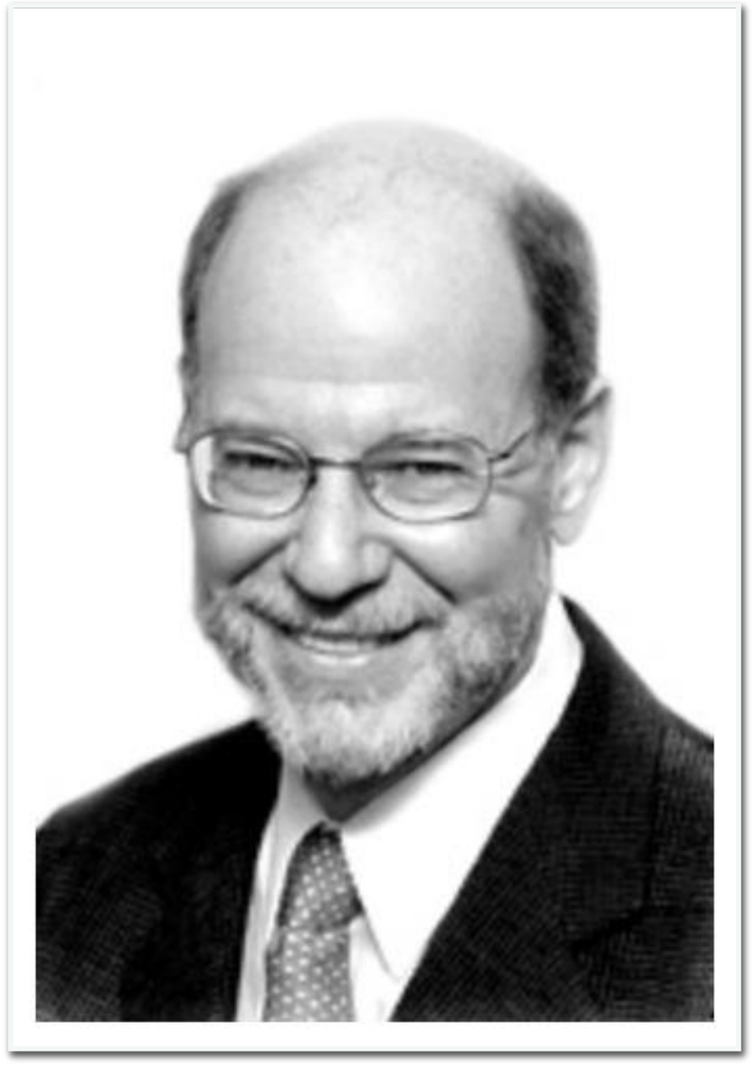
John Sulston: 1942 - Present

- Born in Cambridge, England
- Received his PhD from Cambridge University for work on the chemical synthesis of DNA
- Went to the Medical Research Council in Cambridge to work with Sydney Brenner
- Used *C. elegans* to study a number of developmental systems
  - Programmed cell death
  - Genetics of cell lineage
- Director of the Sanger Center
- Was knighted for his services to science in 2001



Howard Robert Horvitz: 1947 - Present

- Born in Chicago, Illinois
- Received his PhD from Harvard University
- After his doctorate, went to the Medical Research Council in Cambridge to work with Sydney Brenner
- Used *C. elegans* to study a number of developmental systems
  - Neuronal development
  - Ras pathway
  - Genetics of cell lineage
- Co-founder of Idun Pharmaceuticals - develops therapeutics focusing on apoptosis
- Enjoys reading in his spare time, particularly British contemporary novels



Recipients of the Nobel Prize in Physiology or Medicine

- Awarded jointly in 2002 to Brenner, Horvitz, and Sulston
- Prize was awarded for development of the nematode worm into what is now one of the scientific community's favorite genetic models
- Genetic regulation of organ development and programmed cell death

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ABSTRACT

Methods are described for the isolation, complementation and mapping of mutants of *Caenorhabditis elegans*, a small free-living nematode worm. About 300 EMS-induced mutants affecting behavior and morphology have been characterized and about one hundred genes have been defined. Mutations in 77 of these alter the movement of the animal. Estimates of the induced mutation frequency of both the visible mutants and X chromosome lethals suggests that, just as in Drosophila, the genetic units in *C. elegans* are large.

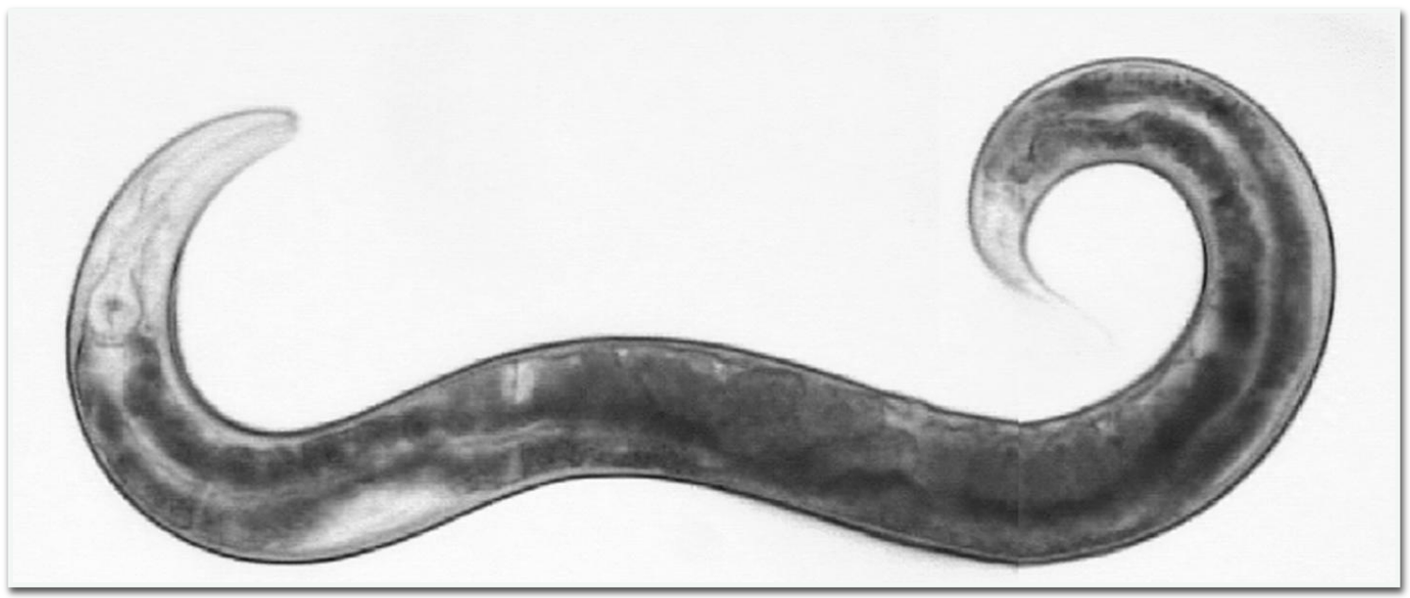
lo genes specify the complex structures found in higher organ

t are the molecular mechanisms used to switch genes on and

at controls the temporal sequences that we see in developme

C. elegans as a Model Organism

- Small, ~ 1 mm long
- Short generation time, approximately 3.5 days from egg laying until adulthood
- Small and possibly fixed number of cells (1/2 of which are neurons)
- Transparent - ease of observation



Main Points

1. Basic genetic features of C. elegans

2. Genetic specification of the nervous system

3. Effects of mutations on the nervous system

Hermaphrodite Genetics

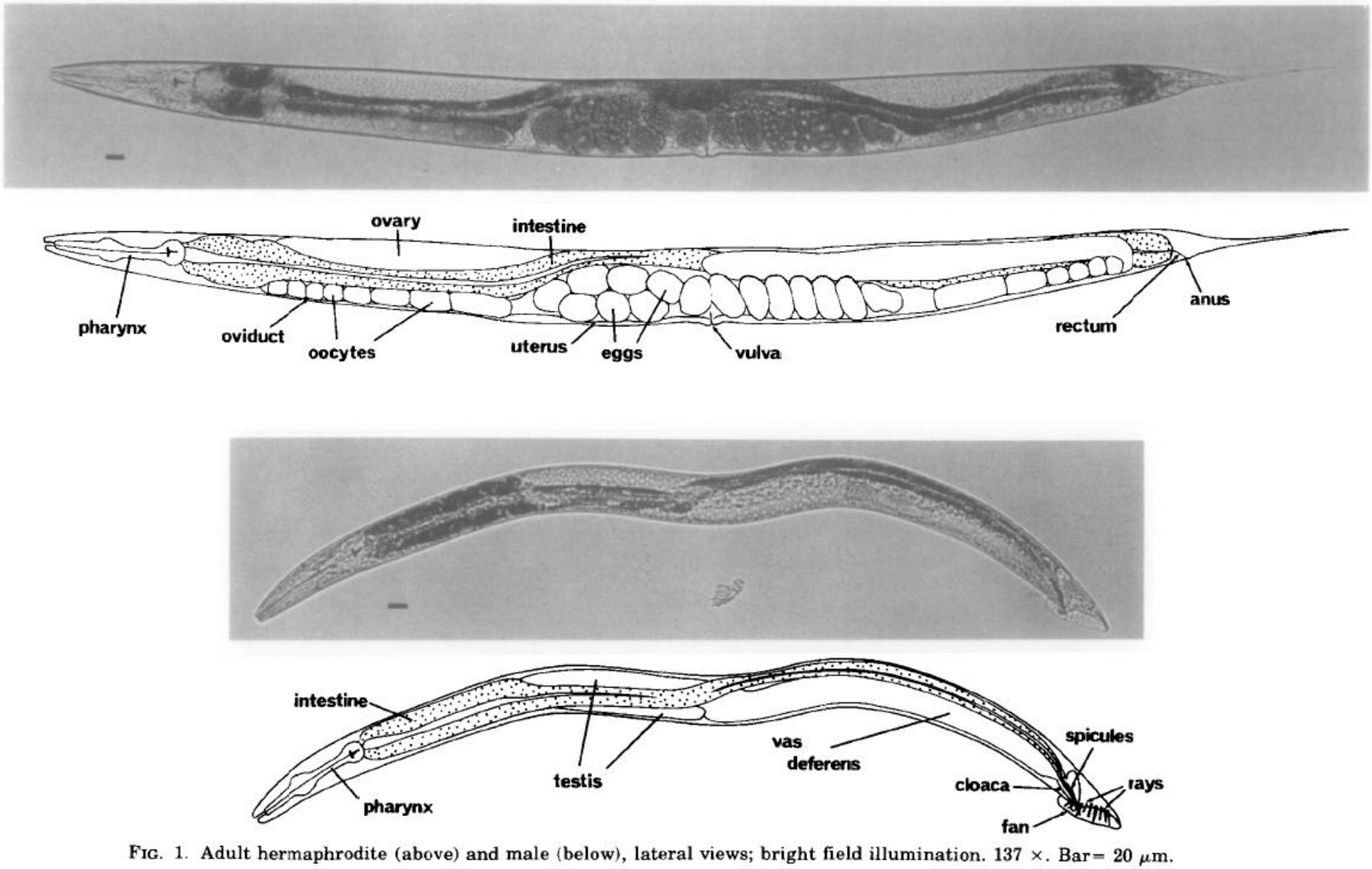
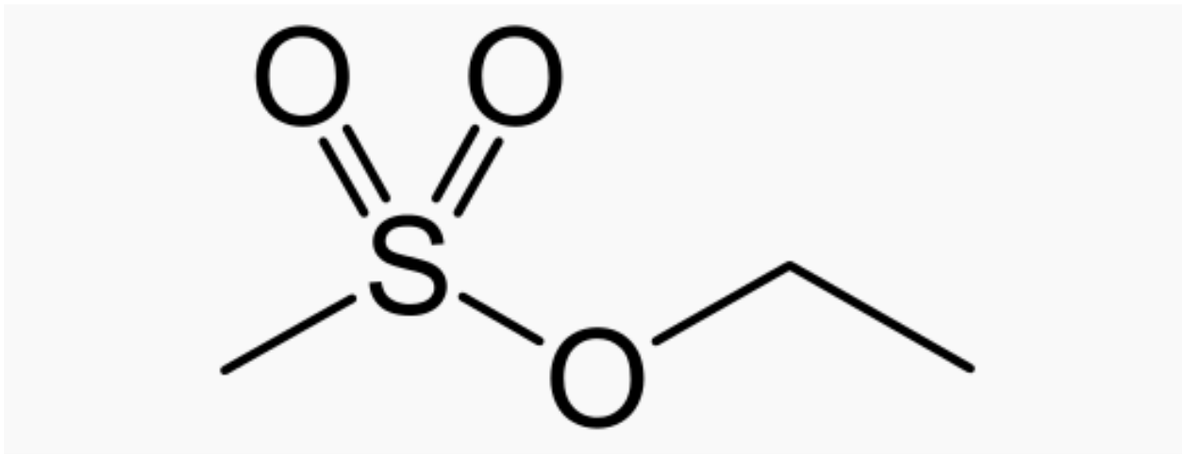


FIG. 1. Adult hermaphrodite (above) and male (below), lateral views; bright field illumination. 137 ×. Bar= 20 μm.

Mating with males permits the transfer of genetic markers

Isolation of Mutants

- Used EMS, a potent mutagen, to induce mutations
- Newly hatched larvae were treated with EMS to allow for the production of large clones of mutants in their progeny
- Treated males are mated and the mutants are isolated from the progeny of these crosses (based on phenotype)
- Mutants were divided into the M set (550) and the S set (69)



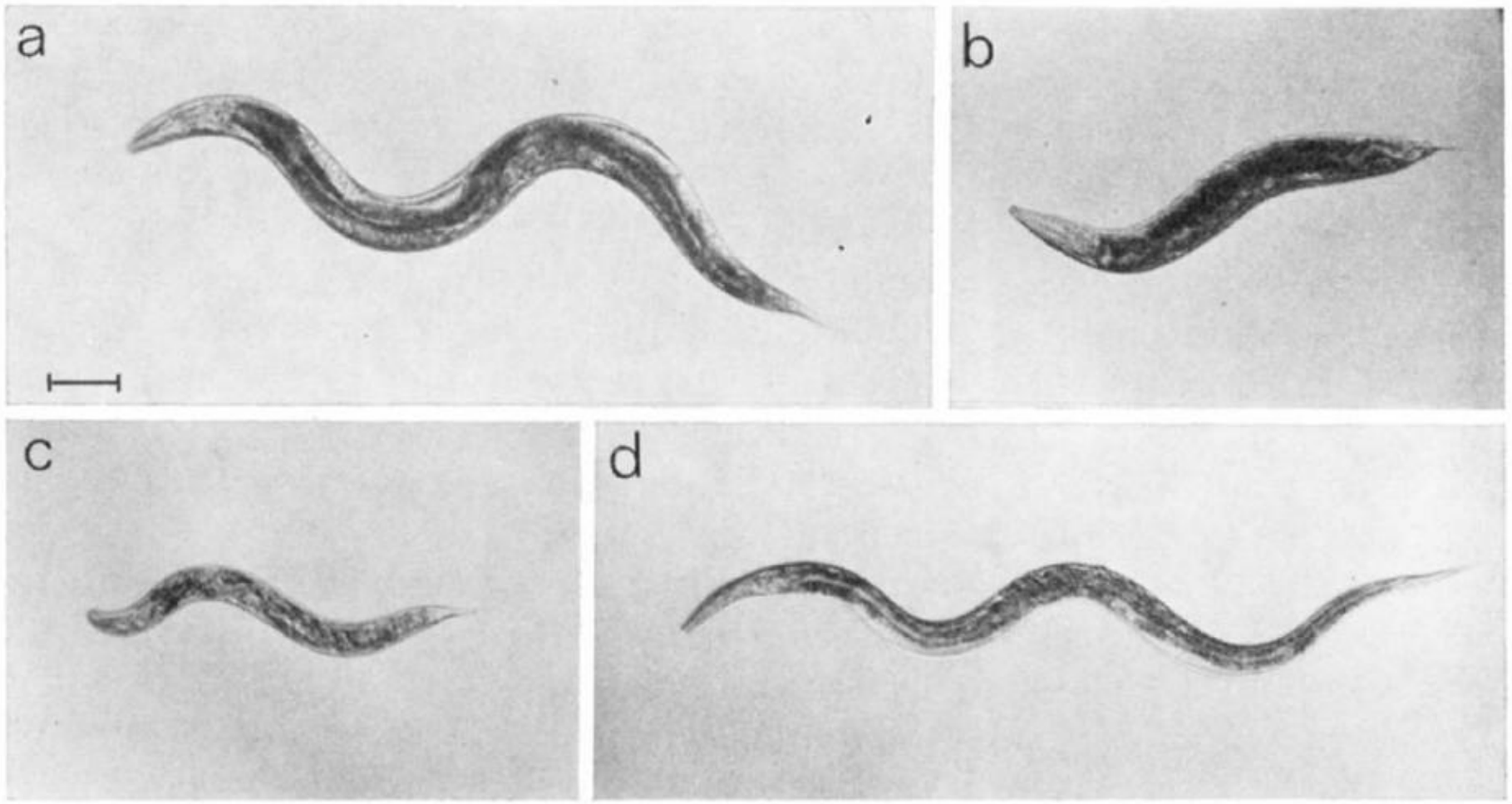


FIGURE 1.—Photomicrographs of *C. elegans* and some of its mutants. a: wild type, b: dumpy (*dyp-1*), c: small (*sma-2*), d: long (*lon-1*). The scale is 0.1 mm.

Mutants were selected based on…

- Movement defects
- Morphological abnormalities
- Size/Shape

Phenotypes of Mutants

1. **Uncoordinated**: any mutant that shows a detectable defect in movement; huge level of variation

2. **Roller**: body of the animal rotates around its long axis causing it to move in a circle

3. **Dumpy/Small**: shorter than wild type

4. **Long**: longer and thinner than wild type

5. **Blistered**: presence of fluid filled blisters

6. **Abnormal**: clear-cut morphological deformities; huge level of variation

TABLE 1

Phenotypes, linkage and summary of mapping for M and S mutants

Set	Phenotype	Autosomal		Sex-linked		Unassigned		Total
		Located	Not located	Located	Not located	Dominant	Other	
M	Uncoordinated	173	39	41	59	9	43	364
	Dumpy and small	71	8	5	24	2*	0	110
	Long	5	0	4	1	0	0	10
	Roller	2	2	0	1	2	0	7
	Blistered	8	0	0	0	1	0	9
	Abnormal	0	17	0	3	0	24	44
	Residual	..	..	..	..	..	..	6
		259	66	50	88	14	67	550
S	Uncoordinated	14	4	1	9	2	16	46
	Dumpy and small	11	0	0	1	0	0	12
	Long	0	0	0	0	1	0	1
	Roller	1	1	0	1	0	0	3
	Blistered	0	0	0	0	0	0	0
	Abnormal	0	1	0	1	0	5	7
		26	6	1	12	3	21	69

* Recessive lethals.

Classification of Mutants

- Mutants were crossed with WT males
- Classification was based on the phenotype of the progeny

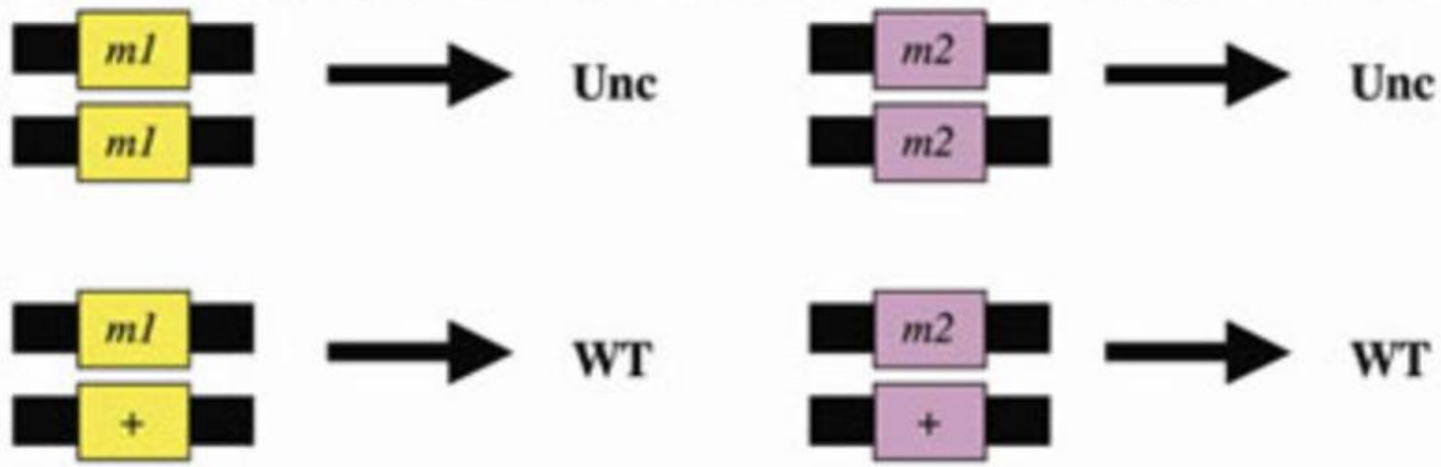
TABLE 2

Classification of mutants: progeny of cross with wild-type males

Phenotype of progeny		
♀	♂	Type
wild	wild	autosomal recessive
wild	mutant	sex-linked recessive
intermediate	intermediate	autosomal semidominant
intermediate	mutant	sex-linked semidominant
mutant	mutant	dominant

Genetic Complementation

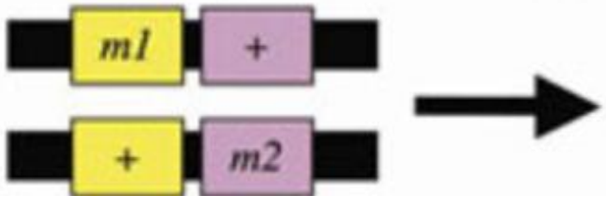
m1 and ***m2*** are two separate recessive mutations that both result in the same uncoordinated (Unc) behavior.



If both of these mutations are present in a *trans* configuration, and an uncoordinated behavior is observed

then these mutations DO NOT complement each other, and are alleles of the same gene.

But, if the *trans* configuration results in wild-type (WT) behavior



then these mutations DO complement each other, and are alleles of different genes.

Location of Mutants on Linkage Groups

- Linkage Groups: alleles are located closely together on the same chromosome; inherited together during meiosis
- Trans configuration is used to identify the linkage group of a new mutant
- Linkage is often signified by the absence of the AB class

TABLE 3

Patterns of segregation of phenotypes from doubly heterozygous hermaphrodites

		A: *trans* heterozygote $a+/+b$			
	Eggs	Parental		Recombinant	
Sperm		$a+$ ($1-p$)	$+b$	ab (p)	$++$
Parental $1-p$	$a+$	A	W	A	W
	$+b$	W	B	B	W
Recombinant p	ab	A	B	AB	W
	$++$	W	W	W	W

TABLE 4

Occurrences of EMS mutants in mapped genes

Linkage group	Gene	Reference mutant	Number of isolates M	Number of isolates S	Comments
I	*bli-3*	E767	1		
	unc-35	E259	1		
	unc-56	E403	2		
	unc-11	E47	2		
	unc-40	E271	1		
	unc-57	E406	2		
	unc-38	E264	4	1	Tetramisole-resistant
	unc-63	E384	2		Tetramisole-resistant
	dpy-5	E61	2		
	dpy-14	E188	1		Larvae abnormal
	unc-14	E57	5	1	Small, paralyzed body
	unc-37	E262	1		
	unc-15	E73	1		Paralyzed; defect in body muscle cells
	unc-55	E402	2		
	unc-13	E51	17		Paralyzed; pharyngeal movement irregula
	unc-21	E330	1		
	unc-29	E193	2		Tetramisole-resistant
	unc-54	E190	5		Paralyzed; defect in body muscle cells
	unc-59	E261	1		
II	*rol-2*	E489	1		
	bli-2	E768	4		
	dpy-2	E8	8		All alleles have roller phenotype
	dpy-10	E128	6	1	
	unc-4	E120	5		
	bli-1	E769	2		
	unc-53	E404	1		
	rol-1	E91	1		
	unc-52	E444	2		Progressive dystrophy of body musculatur
III	*unc-45*	E286	1		Slow moving; defect in body muscle cells
	dpy-1	E1	20	3	
	dpy-17	E164	3	1	Larvae abnormal
	sma-4	E729		1	Semidominant
	sma-3	E491	2		
	unc-16	E109	1		
	lon-1	E185	5		
	sma-2	E502	3		
	unc-32	E189	1		
	unc-36	E251	3		
	unc-47	E307	3		
	unc-69	E587	2		
	unc-50	E306	2		
	unc-49	E382	3	1	
	dpy-18	E364		2	
	unc-71	E541	1		
	bli-5	E518	1		
	unc-25	E156	3		
	unc-64	E246	1		
	unc-67	E713		1	
IV	*dpy-9*	E12	2		
	unc-33	E204	2	1	Paralyzed
	unc-17	E113	2		One allele lannate-resistant
	dpy-13	E184	5		Semidominant
	unc-77	E625	1		
	dpy-16	E225	1		
	unc-28	E15	1		Semidominant
	unc-5	E53	9		
	unc-8	E49	1		
	unc-44	E362	2	3	Small paralyzed
	unc-24	E138	2		
	unc-43	E408	2	1	
	unc-31	E169	7	1	Slow movement
	unc-22	E66	21		Twitching superimposed on normal movement
	unc-26	E205	9		
	unc-30	E191	6		
V	*unc-66*	E677	1		Paralyzed; defect in body muscle cells
	unc-60	E723		1	Paralyzed; defect in body muscle cells
	unc-34	E315	2		
	unc-62	E644	1		
	unc-46	E177	2	1	
	dpy-15	E24	1		Larvae abnormal
	unc-70	E524	1		
	unc-68	E540	3	1	
	dpy-11	E224	9	3	Same alleles are extreme dumpies
	unc-23	E25	5		Progressive dystrophy of head musculature
	unc-41	E268	5		
	rol-3	E754		1	
	sma-1	E30	8		Larvae have shortened round heads
	unc-42	E270	3		
	unc-65	E351	2		
	unc-61	E228	1		
	unc-39	E257	1		
	unc-51	E369	3	1	Paralyzed
X	*unc-1*	E94	12	1	
	dpy-3	E27	1		
	unc-2	E55	3		
	unc-20	E112	1		Temperature-sensitive
	dpy-8	E130	2		One allele temperature-sensitive
	lon-2	E678	4		
	dpy-6	E14	1		
	unc-6	E78	3		

Mapping of Mutants

B: *cis* heterozygote $++/ab$

Sperm	Eggs	Parental $1-p$ $++$	Parental $1-p$ ab	Recombinant p $a+$	Recombinant p $+b$
Parental $1-p$	$++$	W	W	W	W
	ab	W	AB	A	B
Recombinant p	$a+$	W	A	A	W
	$+b$	W	B	W	B

a and b are mutant recessive alleles and A and B their corresponding phenotypes.
W is wild type phenotype.

- **Two point mapping**: used to assign mutations to a specific chromosome; rough indication of distance between mutations
- Double mutant is crossed with the WT male to allow for production of the cis double heterozygote
- Production of the recombinant phenotypes A and B
- Restricted to mutants with different phenotypes

TABLE 7

Recombination between dpy *and* unc *genes on different linkage groups*

Linkage group	*dpy-*	Mutant	*unc-*	Mutant	W	D	U	UD	Percent recombination
X	6	E14	6	E78	1308	24	25	394	2.8
	6	E14	3	E95	826	97	110	213	18.3
I	5	E61	13	E51	1771	28	—	—	2.4
	5	E61	13	E312	865	12	—	—	2.1
	5	E61	54	E190	582	106	—	—	26.7
	5	E61	54	E843	632	133	—	—	30.8
III	1	E1	32	E189	1090	149	163	261	21.0
	1	E745	32	E189	808	114	123	177	21.8
IV	13	E184	17	E113	757	14	—	—	2.7
	13	E184	17	E245	1270	26	—	—	3.0
	13	E184	17	E464	990	20	—	—	3.0
	13	E184	30	E191	623	32	—	—	7.7
	13	E184	30	E318	773	45	—	—	8.6

The phenotypes—W = wild, D = dumpy, U = uncoordinated and UD = uncoordinated dumpy—were scored in the progeny of *cis* heterozygotes (+-+/*dpy unc*). In some of the crosses only the wild and dumpy animals were counted.

3 Factor Crosses

TABLE 8

Three-factor crosses: segregants of recombinant heterozygotes

Heterozygote	Recombinant phenotype		Predominant genetic structures	Phenotypes of segregants		
$\frac{+\ a\ b}{c\ +\ +}$	A		$\frac{+\ a\ +}{+\ a\ b}$	A	AB	
	B		$\frac{c\ +\ b}{+\ a\ b}$	B	AB	CB
$\frac{a\ +\ b}{+\ c\ +}$	A		$\frac{a\ +\ +}{a\ +\ b}$	A	AB	
		and	$\frac{a\ c\ +}{a\ +\ b}$	A	AB	AC
	B		$\frac{+\ +\ b}{a\ +\ b}$	B	AB	
		and	$\frac{+\ c\ b}{a\ +\ b}$	B	AB	BC

Three factor crosses are used to to order genes unambiguously, give a better indication of recombination distances.

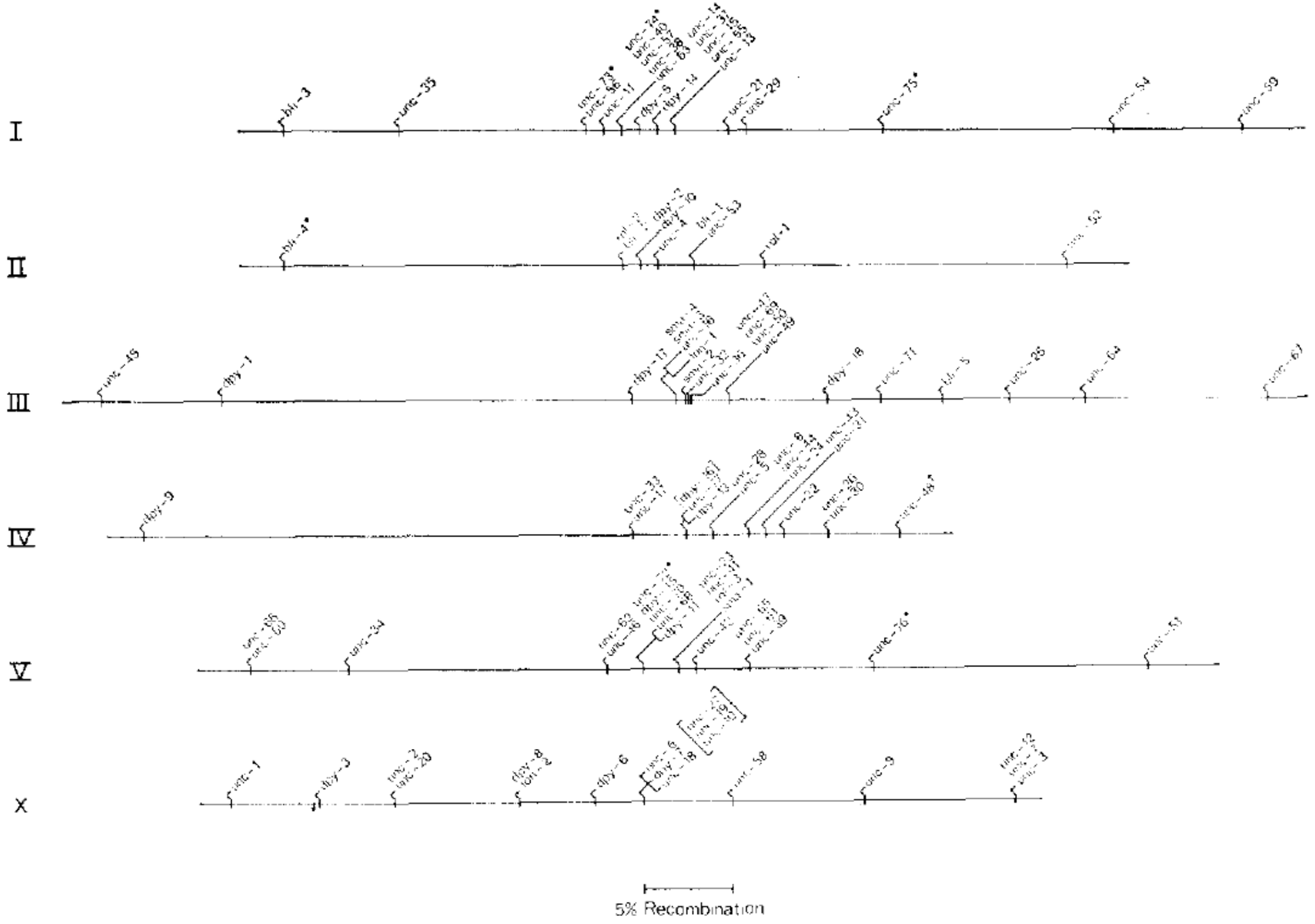


FIGURE 2.—Genetic map of *C. elegans*. The locations are shown only for mutants that have been unambiguously ordered. Two genes joined to the same location means that no recombination has been detected between them ($< 0.5\%$). In other cases, the genes are known to be closely linked but the internal order has not been determined. The map position of bracketed markers is only approximately known. All the genes are represented by EMS-induced mutants, except for those marked * which were isolated after ^{32}P decay (BABU and BRENNER, unpublished results), and for the site marked †, which is a spontaneous mutant. Four of the genes with a blistered phenotype (*bli-1* to *bli-4*) were mapped by MR. JONATHAN HODGKIN.

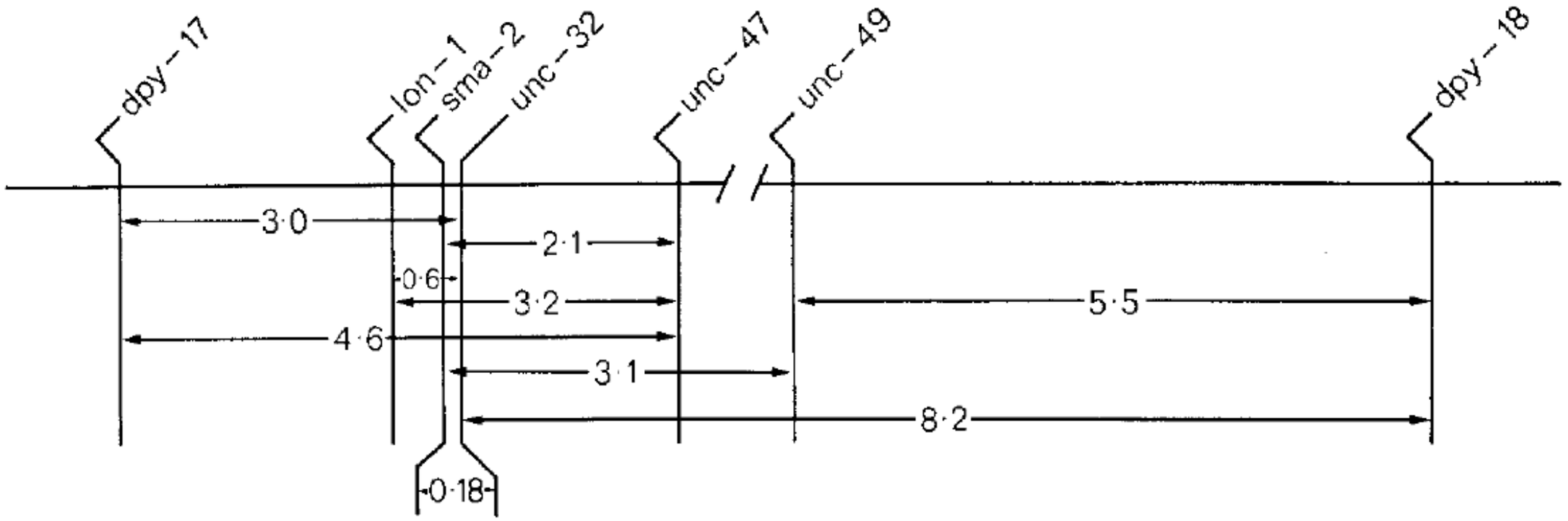
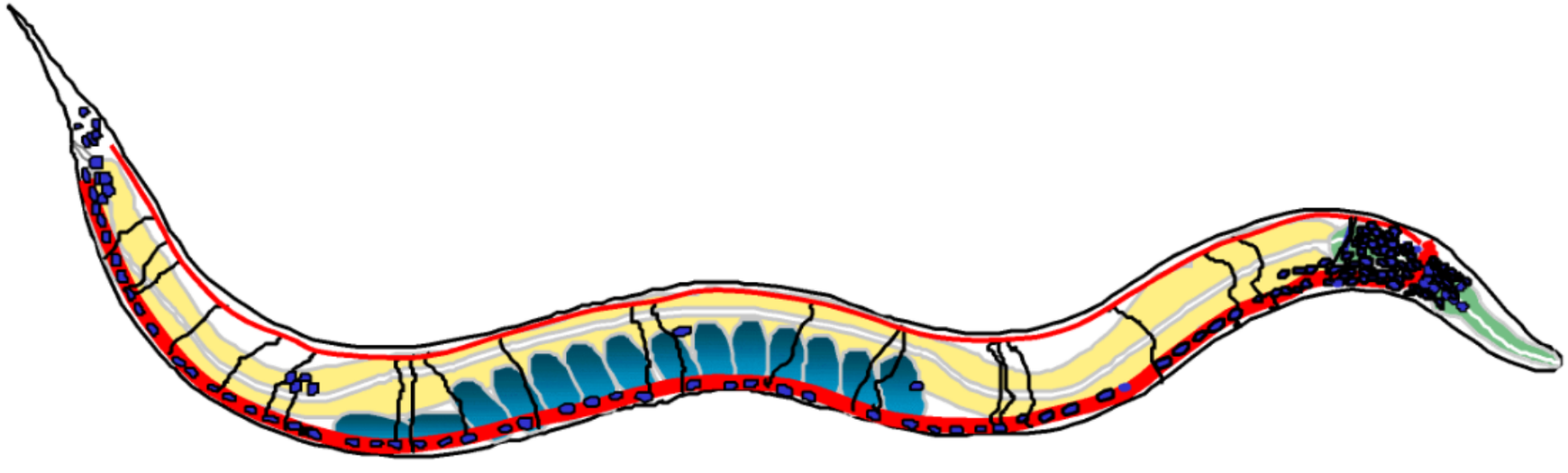


FIGURE 3.—Two-factor recombination values for a region of linkage group III. With the exception of *unc*-47 and *unc*-49, all of the sites have been ordered independently by three-factor crosses.

Conclusions/Observations

- *C. elegans* are a favorable organism for genetic analysis
- Development of methods for complementing and mapping mutations
- 6 linkage groups, which correspond with the number of haploid chromosomes
- Observable mutations seem to occur in only a small number of genes



Post-embryonic Cell Lineages of the Nematode, *Caenorhabditis elegans*

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Received August 23, 1976; accepted November 4, 1976

Main Points

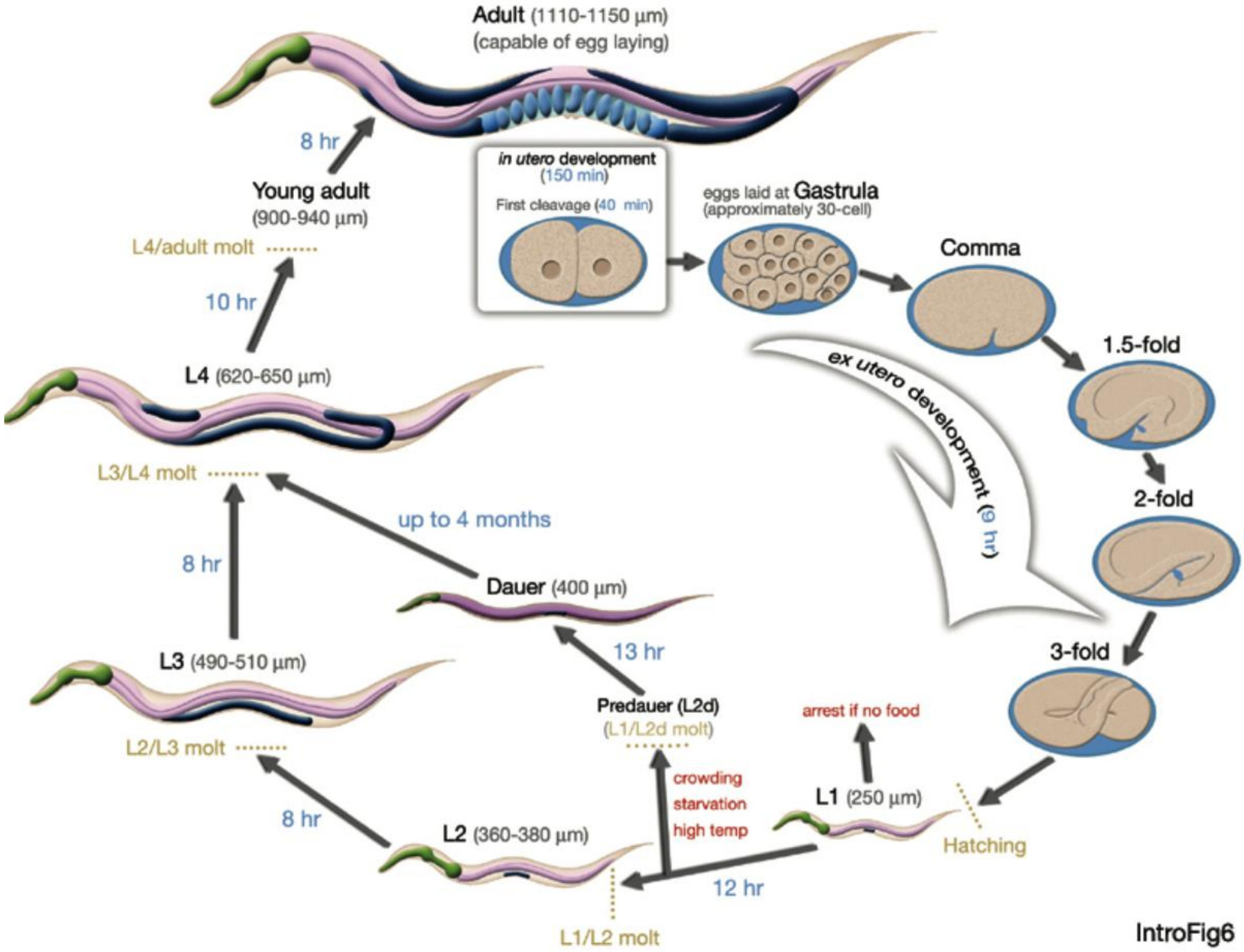
1. **Cell lineage**: all cells in our body are descendants from a fertilized, egg cell

2. Cells have to differentiate in a correct manner and at the right time during development

3. *C. elegans* have a predetermined number of cell progeny, which increases from 550 to about 810 in mature hermaphrodites or 970 in the mature male

Introduction

- C. elegans are an excellent organism for the study of cell lineages
  - Anatomically simple: tubular body consisting of a hypodermal wall and underlying musculature
  - Relatively few cells
  - Obvious developmental changes
- Development of a technique with which it is possible to determine cell lineage by observing living nematodes
- Extend previous studies to look at the development of the ventral nervous system


Adult (1110-1150 µm)
(capable of egg laying)
8 hr
Young adult
(900-940 µm)
L4/adult molt
10 hr
L4 (620-650 µm)
L3/L4 molt
up to 4 months
8 hr
Dauer (400 µm)
13 hr
L3 (490-510 µm)
Predauer (L2d)
(L1/L2d molt)
L2/L3 molt
crowding
starvation
high temp
8 hr
L2 (360-380 µm)
12 hr
L1/L2 molt
arrest if no food
L1 (250 µm)
Hatching
in utero development
(150 min)
First cleavage (40 min)
eggs laid at Gastrula
(approximately 30-cell)
Comma
1.5-fold
2-fold
3-fold
ex utero development (9 hr)

IntroFig6

Anatomy/Gross Morphology

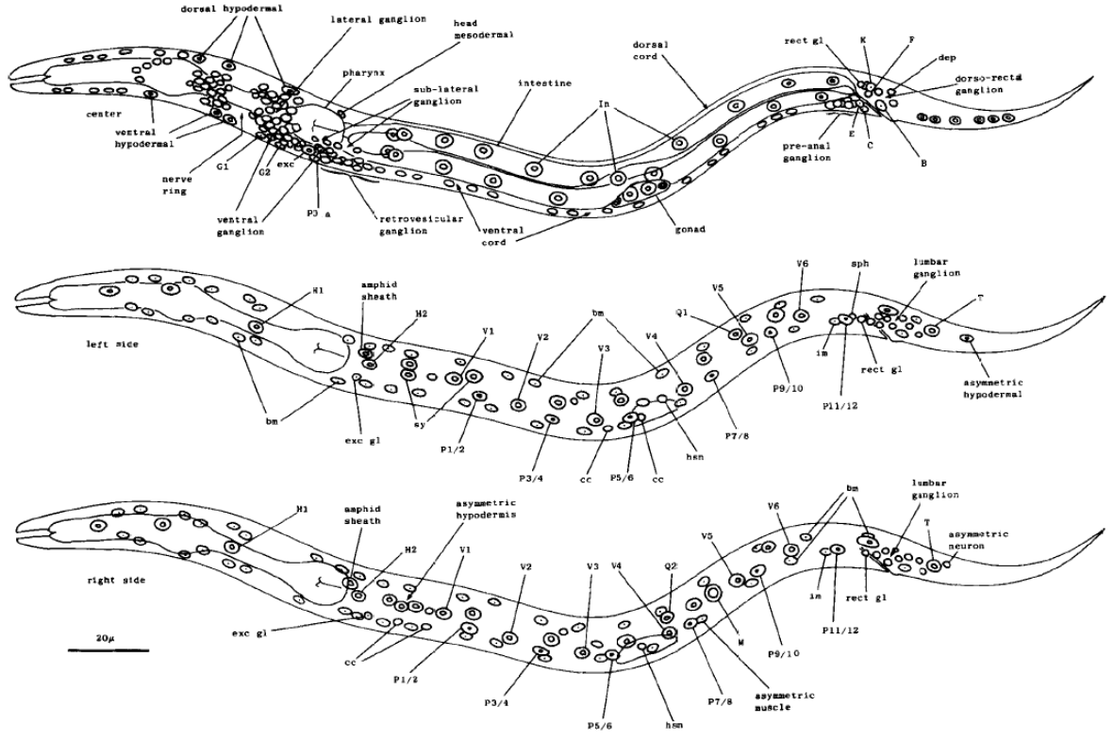


FIG. 4. Young L1 hermaphrodite, lateral views, showing nuclei as seen in central, left, and right optical sections. Not all nuclei in the head ganglia are shown. These figures are somewhat schematic, as nuclei are not arranged precisely into three planes. Left–right pairs of P cells (e.g., P1/2) are not finally named until after their subsequent migration into the ventral cord (see Cell Lineages, Development). bm, body muscle; cc, coelomocyte; dep, anal depressor muscle; exc, excretory cell; exc gl, excretory gland; hsn, hermaphrodite-specific neuron; im, intestinal muscle; rect gl, rectal gland; sph, anal sphincter muscle; sy, syncytial hypodermal nuclei.

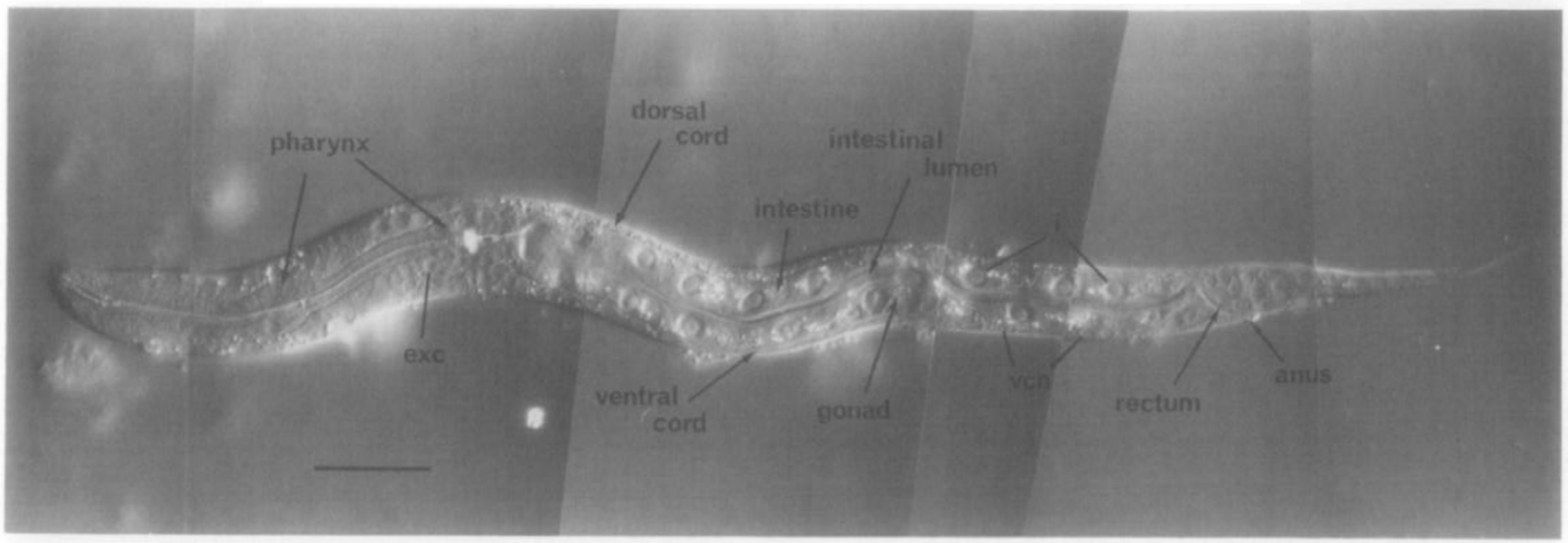


FIG. 2. Young L1 hermaphrodite, lateral view; Nomarski optics. Montage of photographs of a single living animal. exc, excretory cell; i, intestinal nuclei; vcn, ventral cord neurons. Bar = 20 μm.

Cell Lineages: Cell Division

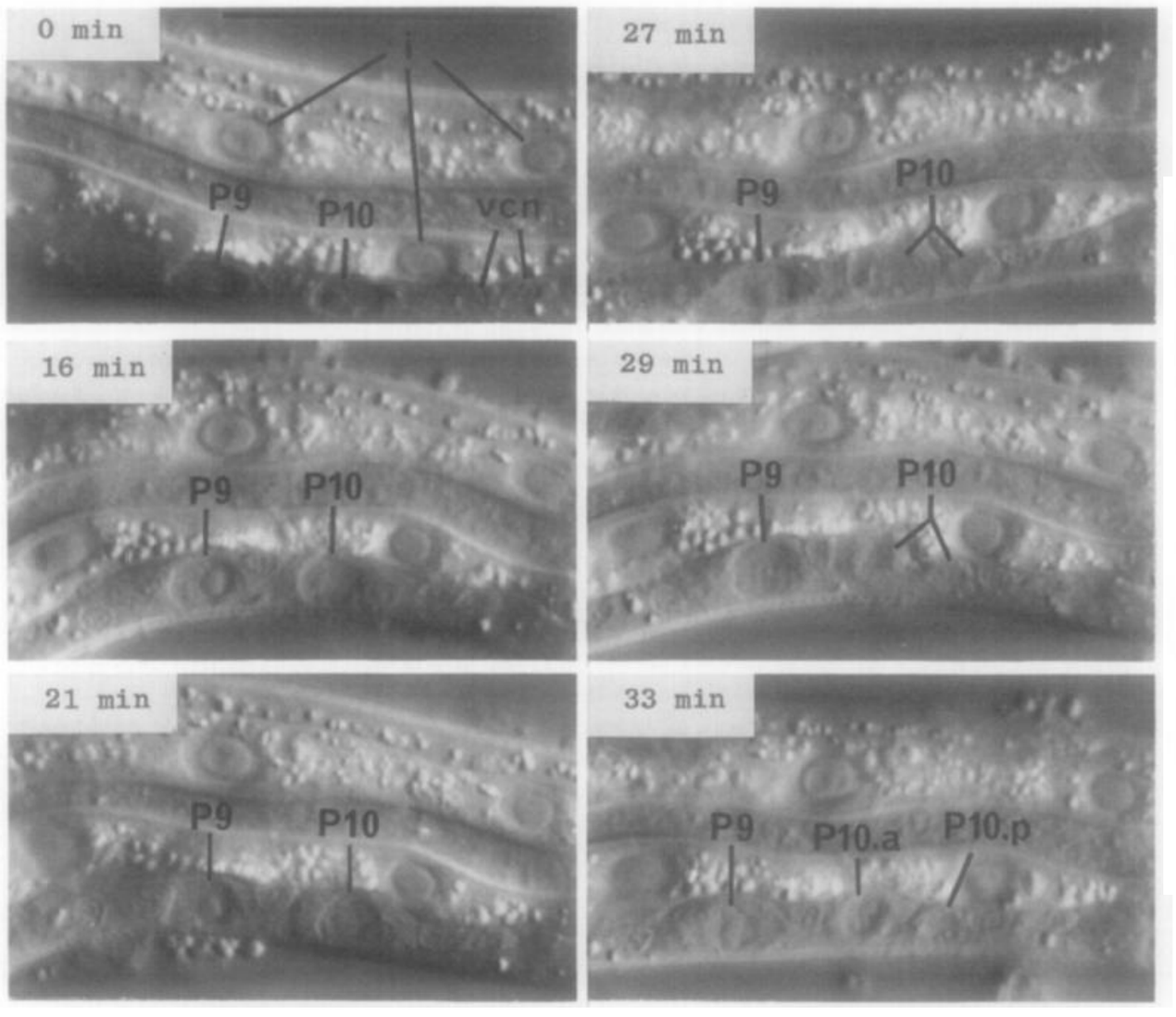


FIG. 7. Cell division. Sequential photographs of an L1 hermaphrodite, lateral view; Nomarski optics. vcn, ventral cord neurons. 0 min, interphase; 16 and 21 min, P10 prophase; 24 min, P10 metaphase; 26 min, P10 anaphase; 27 min, P10 telophase; 29 min, P9 prophase; 33 and 34 min, P9 metaphase. Bar = 20 μm.

Overall uniform process of cell division.

Cell Lineages: Programmed Cell Death

- Certain cells undergo a series of morphological changes interpreted to be programmed cell death
- Posterior daughters from anterio-posterior divions
- Pattern is different among males and hermaphrodites

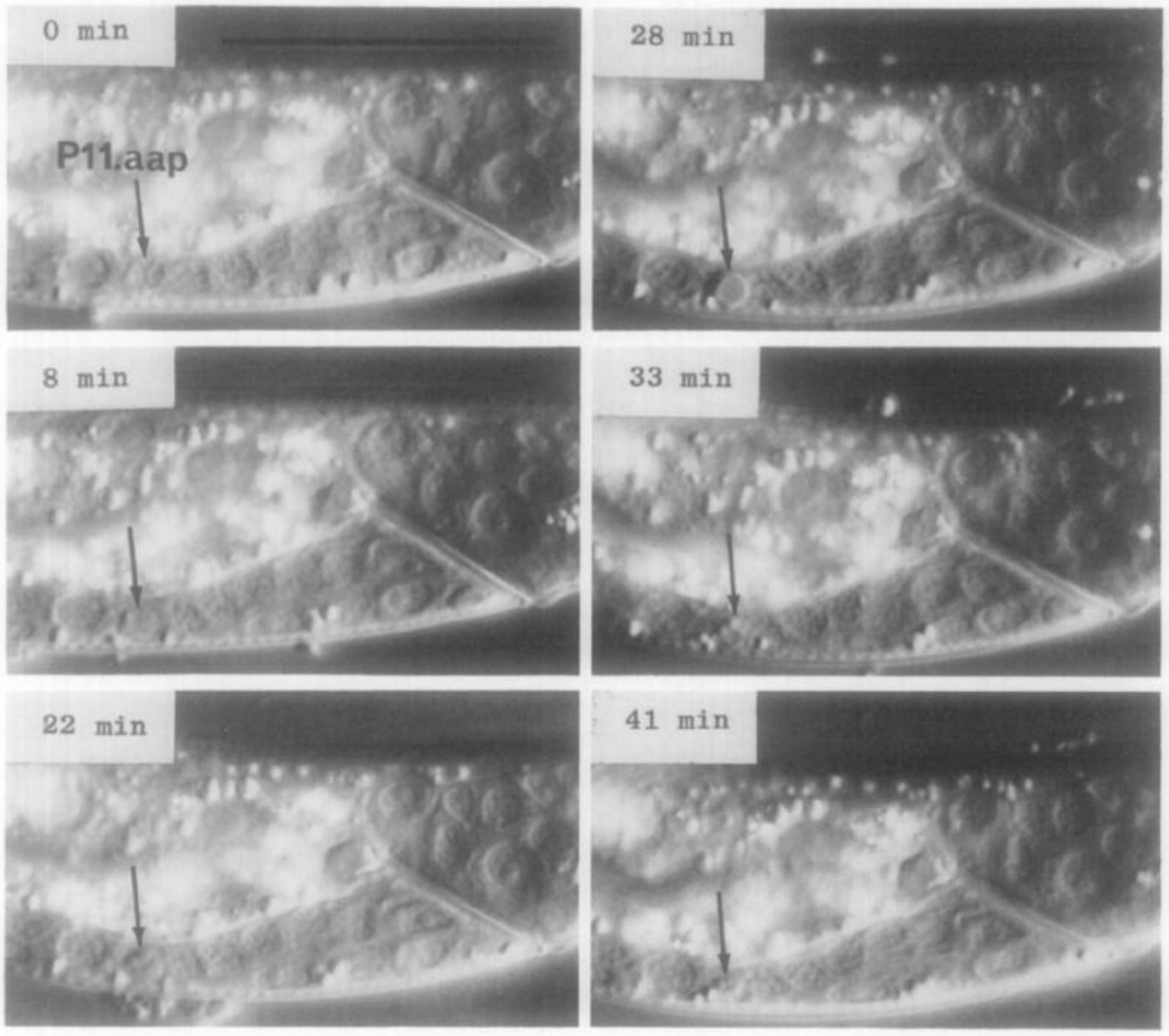
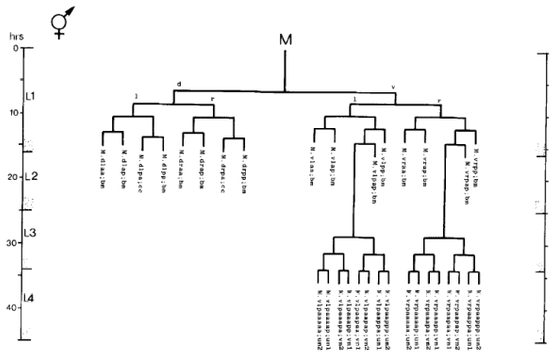
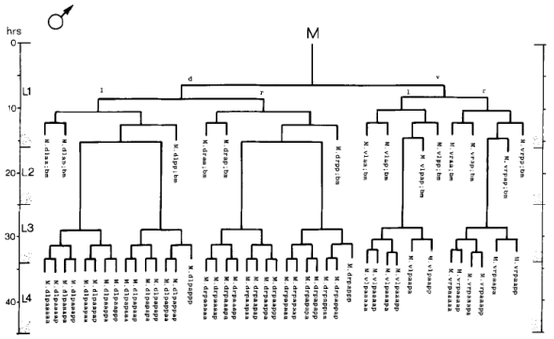
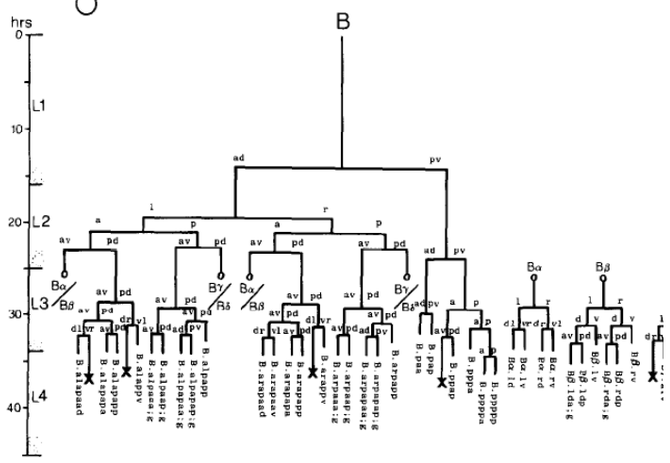


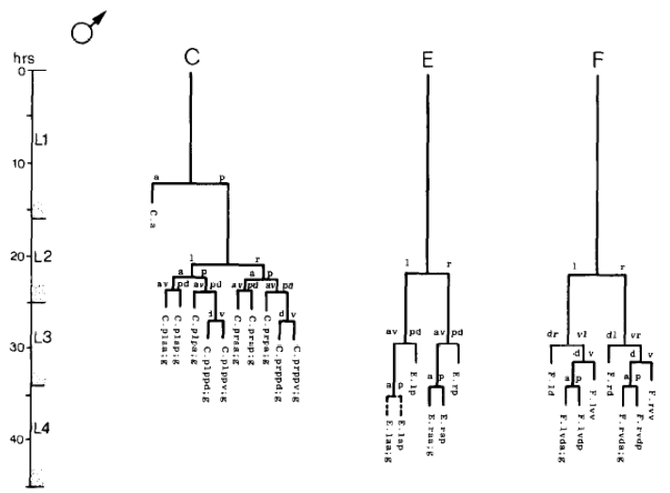
FIG. 8. Cell death. Sequential photographs of an L1 hermaphrodite, lateral view; Nomarski optics. The arrow points to the dying cell, P11.aap. Bar = 20 μm.

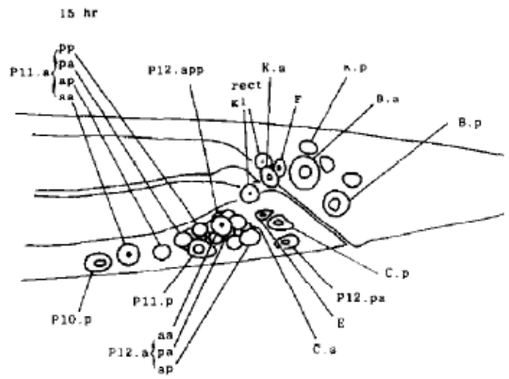
What goes on in a 60 page paper?

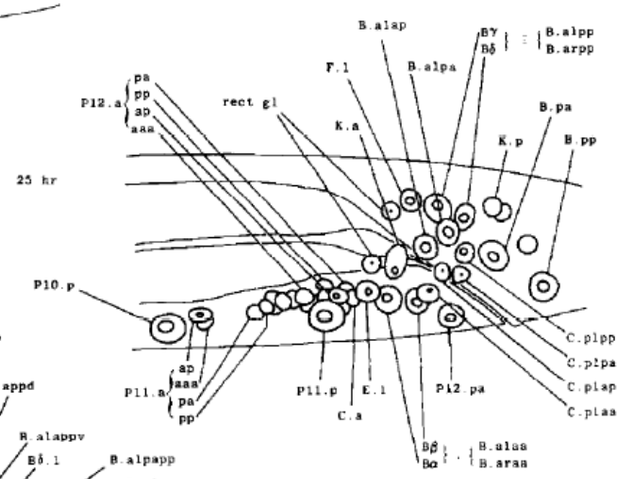


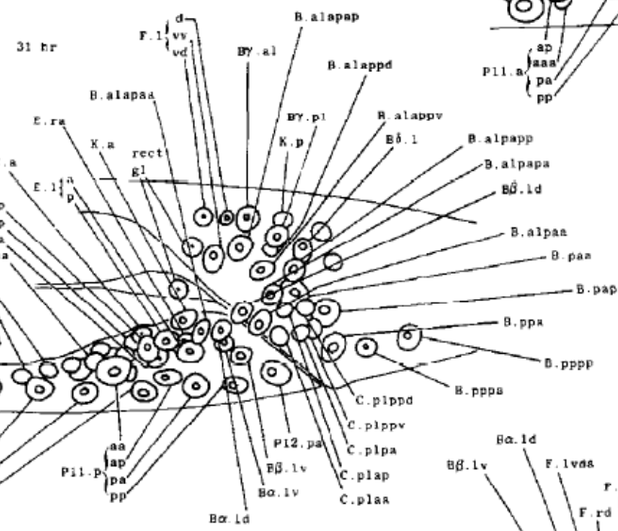


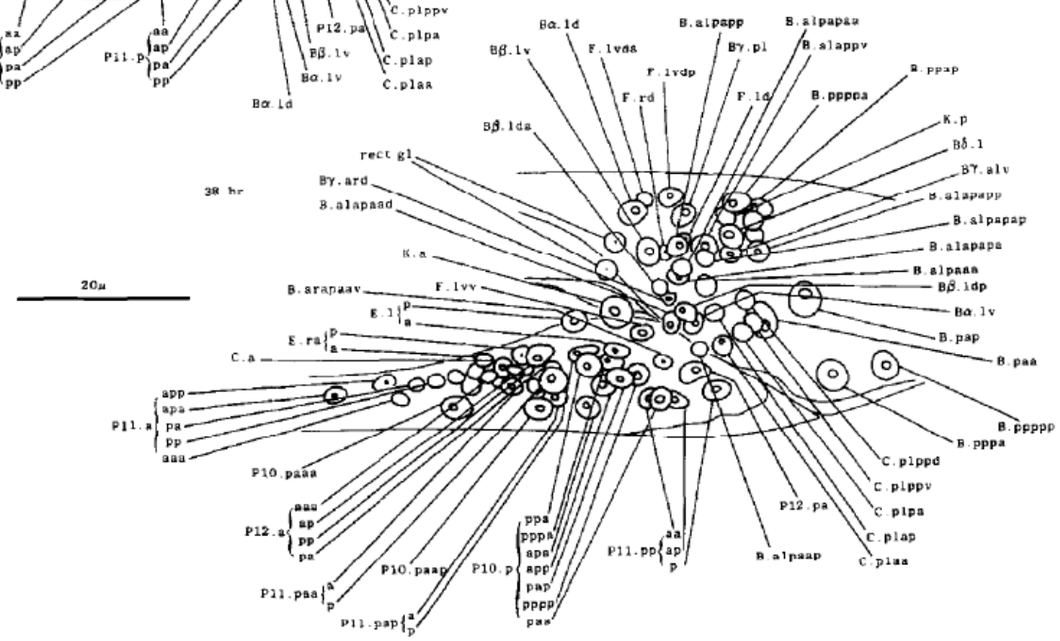












Summary of Their Findings

- Number of non-gonadal nuclei in the hermaphrodite increases from about 550 at hatching to about 810 in the mature adult
- Invariant cell lineages generate a fixed number of progeny cells of rigidly determined fates
- Post-embryonic cell lineages range in length from one to eight sequential divisions
- Discussion…
  - Functions of post-embryonic cell divisions
  - Invariance
  - Patterns of Cell Divisions
  - Mechanisms of Determination
  - Symmetry & Migrations

TABLE 2

NUCLEAR COUNTS, HERMAPHRODITE

	Present in young L1		Derived from postembryonic lineages		
	Nondividing nuclei	Blast cells	Surviving nuclei	Cell deaths	Present in adult
Lateral hypodermis					
Seam	2	–	30	–	32
Syncytial	20	–	98	–	118
Neuronal or glial	8	–	28	–	36
Total	30	20 (H,V,T,Q)	156	8	186
Ventral cord and associated ganglia					
Neuronal or glial	33	–	56	9	89
Hypodermal	0	–	12	1	12
Vulva	0	–	22	0	22
Total	33	13 (P)	90	10	123
Mesoderm					
Body muscles	81	–	14	0	95
Sex muscles	0	–	16	0	16
Coelomocytes	4	–	2	0	6
Digestive tract muscles	4	–	0	0	4
Head mesodermal cell	1	–	0	0	1
Total	90	1 (M)	32	0	122
Intestine	6[a]	14[a] (I)	28	0	34
Head					
Neuronal, glial, small structural	201[b]	–	4	0	205[b]
Hypodermal (dorsal, ventral)	15	–	1	0	16
Pharynx	80	–	0	0	80
Pharyngeal-intestinal valve	6	–	0	0	6
Excretory system	4	–	0	0	4
Total	306	2 (G)	5	0	311
Tail					
Neuronal or glial	19	–	1	0	20
Hypodermal	7	–	1	0	8
Rectal glands	3	–	0	0	3
Total	29	1 (K)	2	0	31
Other tail ectoderm (B,C,E,F)	4	0	0	0	4
Total, excluding gonad	498	51	313	18	811

[a] The L1 intestine contains 20 nuclei. Of these, 6 never divide; in a given individual, 10–14 of the others divide.

[b] Estimate.

TABLE 3

NUCLEAR COUNTS, MALE

	Present in young L1		Derived from postembryonic lineages		
	Nondividing nuclei	Blast cells	Surviving nuclei	Cell deaths	Present in adult
Lateral hypodermis					
Seam	2	–	36	–	38
Syncytial	20	–	104	–	124
Neuronal or glial	6	–	28	–	34
Rays	0	–	54	–	54
Total	28	20 (H,V,T,Q)	222	26	250
Ventral cord and associated ganglia					
Neuronal or glial	33	–	70	4	103
Hypodermal	0	–	10	1	10
Unknown	0	–	16	0	16
Total	33	13 (P)	96	5	129
Mesoderm					
Body muscles	81	–	14	0	95
Sex muscles	0	–	} 42	} 0	} 46
Coelomocytes	4	–			
Unknown	0	–			
Digestive tract muscles	4	–	0	0	4
Head mesodermal cell	1	–	0	0	1
Total	90	1 (M)	56	0	146
Intestine	6[a]	14[a] (I)	28	0	34
Head					
Neuronal, glial, small structural	205[b]	–	4	0	209[b]
Hypodermal (dorsal, ventral)	15	–	1	0	16
Pharynx	80	–	0	0	80
Pharyngeal-intestinal valve	6	–	0	0	6
Excretory system	4	–	0	0	4
Total	310	2 (G)	5	0	315
Tail					
Neuronal or glial	19	–	1	0	20
Hypodermal	7	–	1	0	8
Rectal glands	3	–	0	0	3
Total	29	1 (K)	2	0	31
Other tail ectoderm	0	4 (B,C,E,F)	66	5	66
Total, excluding gonad	496	55	475	36	971

[a] The L1 intestine contains 20 nuclei. Of these, 6 never divide; in a given individual, 10–14 of the others divide.

[b] Estimate.

Functions of Post-Embryonic Cell Divisions

- At hatching, the hermaphrodite and male are almost identical with respect to cell number, position, and (presumably) function
- By the adult stage, gross anatomic differences are obvious with the development of sex-specific structures
- Post-embryonic differences may relate to growth

Invariance

Post-embryonic cell lineages are generally invariant; however 5 types of variations have been observed…

1. Variation in the pattern of cell divisions

2. Variation in the pattern of cell deaths

3. Variation in which of two alternative lineage programs a given cell will follow

4. Variation in the precise order of specific events

5. Variation in the precise positions of cell nuclei

Patterns of Cell Divisions

Two distinguishable types of cell divisions occur during the post-embryonic development of C. elegans

1. Symmetric: daughters are equivalent in morpholoy and in subsequent development

2. Asymmetric: two distinct daughter cells are produces; posterior-anterior divisions

Most lineages involve both symmetrical and asymmetrical cell divisions, but at least one daughter cell retains the morphology of the mother.

Mechanisms of Determination

Correlation between lineage and function could arise in essentially two different ways…

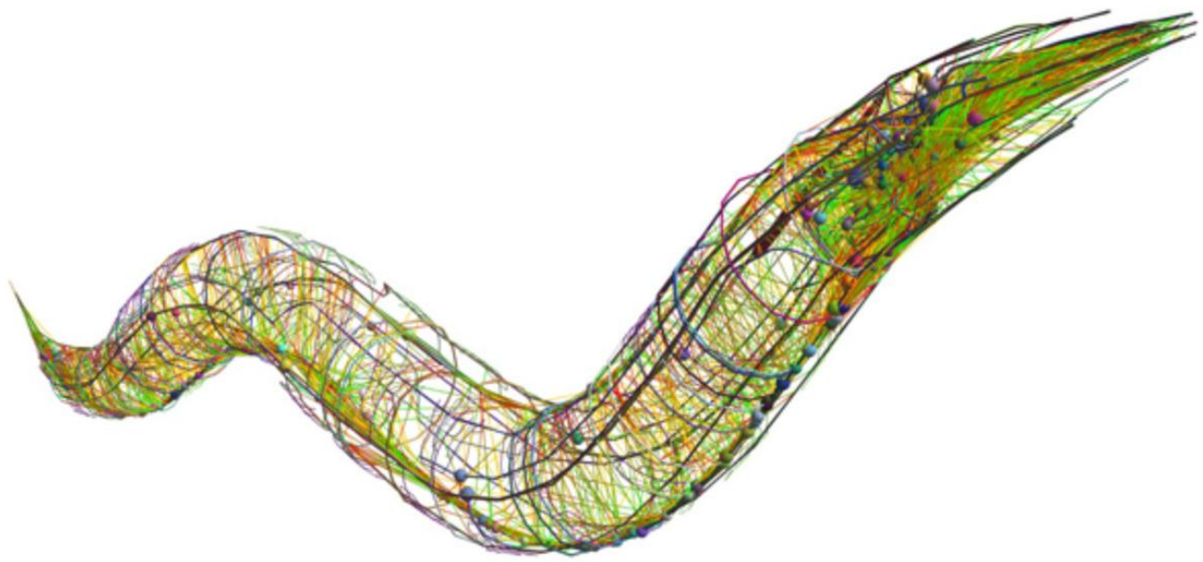
1. Ultimate differentiation of a cell could be determined extrinsically according to its position

2. Fate could be determined intrinsically according to its lineage history

Observations correlate with the hypothesis that much of the development of C. elegans is based upon a lineal determination of cell function.

Symmetry & Migrations

- *C. elegans* are essentially bilaterally symmetrical with minor differences between the left and right sides
- Cellular migrations occur frequently during post-embryonic development
- Migrations occur as both passive events and those that are rigidly determined, with cells traveling long distances



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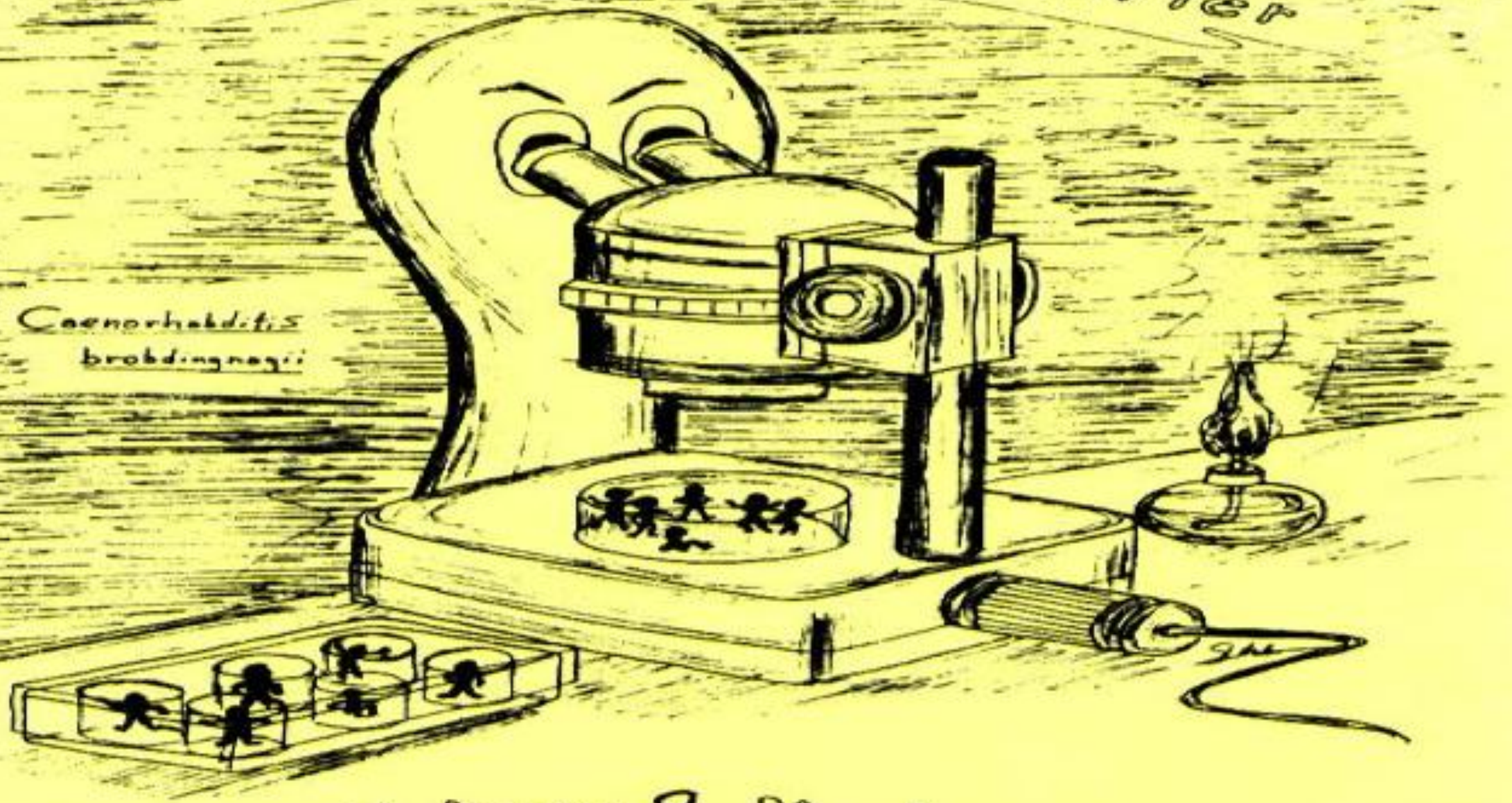

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