Model organisms and developmental biology

仲寒冰

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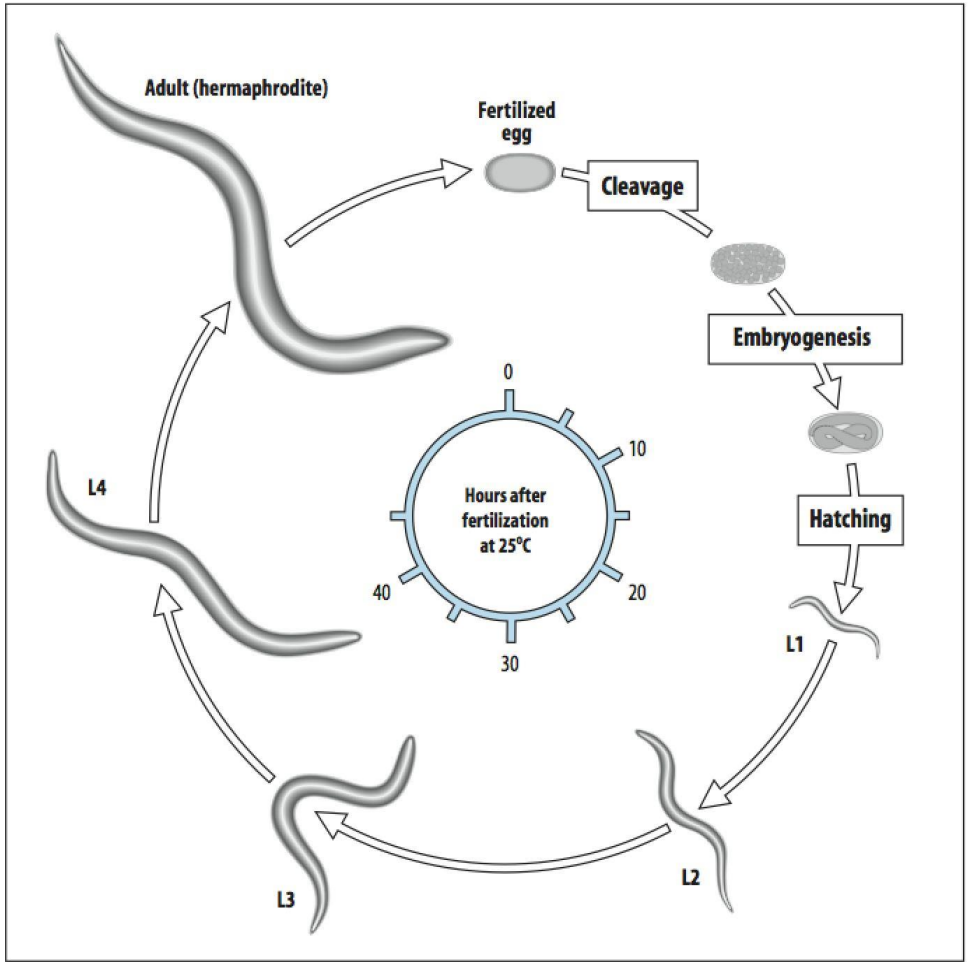
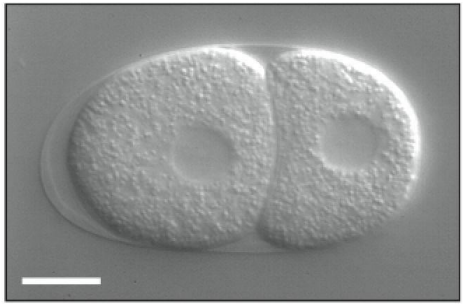
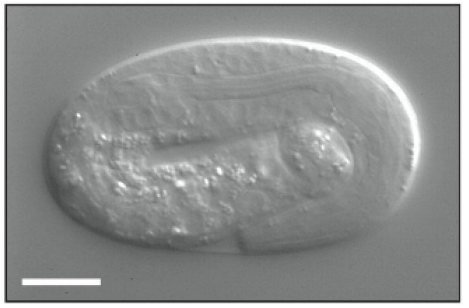
C. elegans

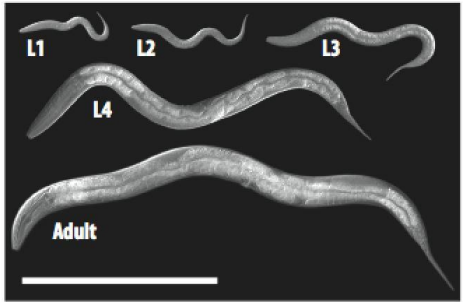
An adult hermaphrodite *C. elegans*, ~ 1 mm.

Life cycle of *C. elegans*

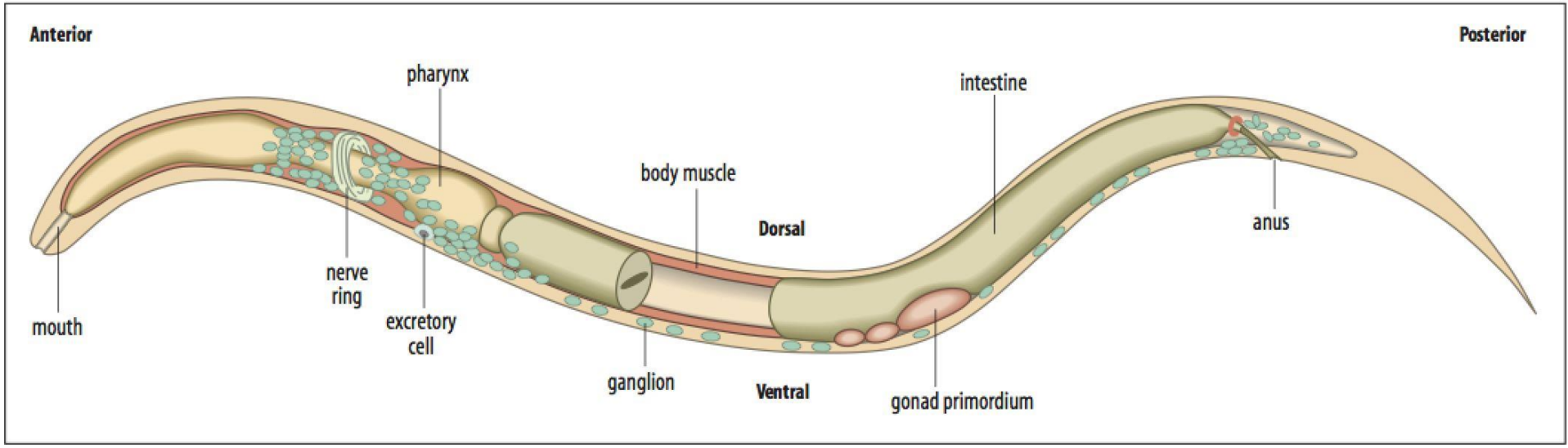




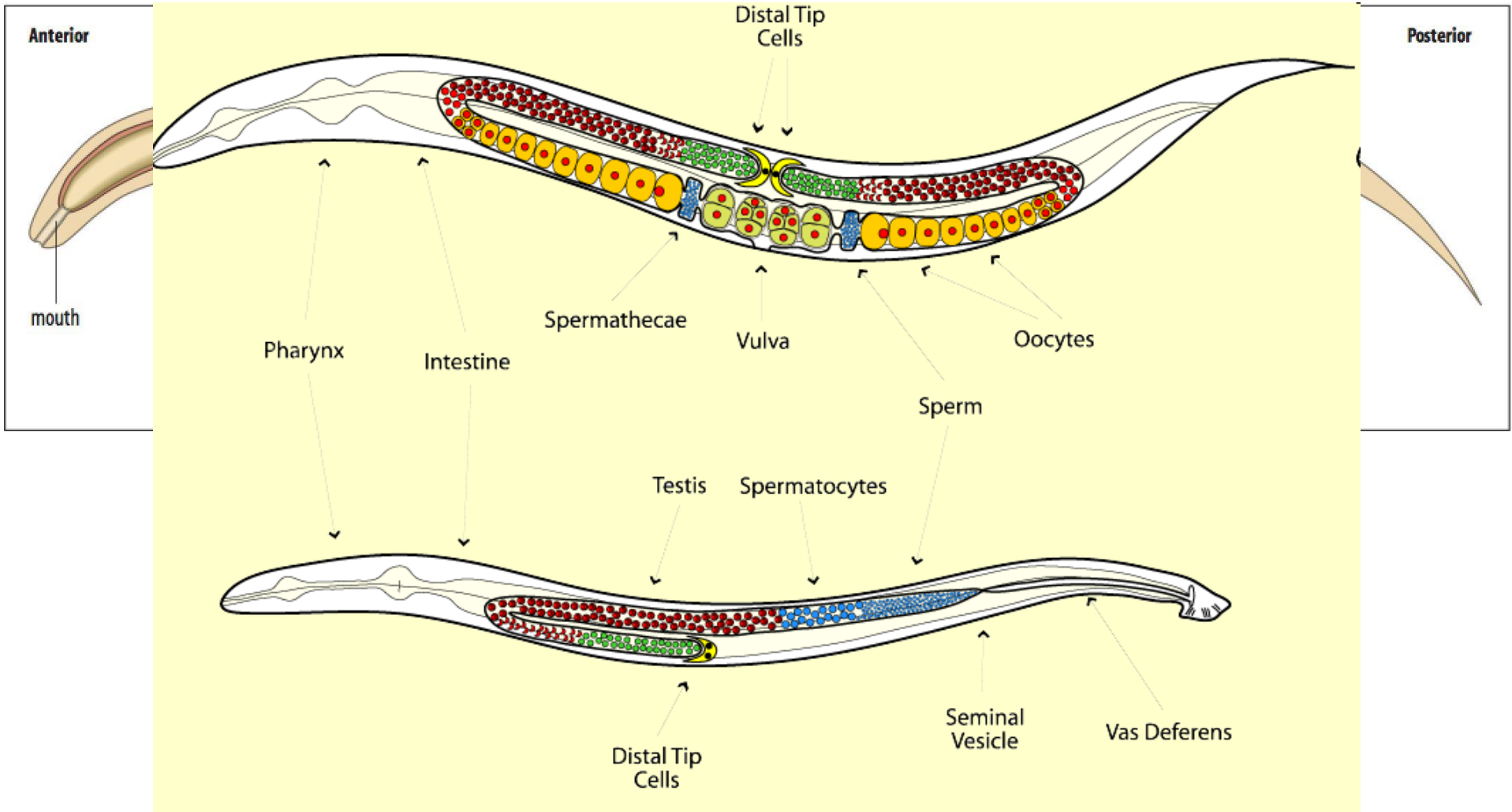




Schematic figures of adult hermaphrodite and male *C. elegans*



Schematic figures of adult hermaphrodite and male *C. elegans*



History

- 1. Sydney Brenner, with Francis Crick, Leslie Barnett and Richard J. Watts-Tobin, genetically demonstrated the triplet nature of the code of protein translation.

- 2. Central dogma is proposed by Crick.

- 3. Aim of Sydney Brenner, “How genes might specify the complex structures found in higher organisms is a major unsolved problem of biology.” -- The genetics of *Caenorhabditis elegans,* 1974

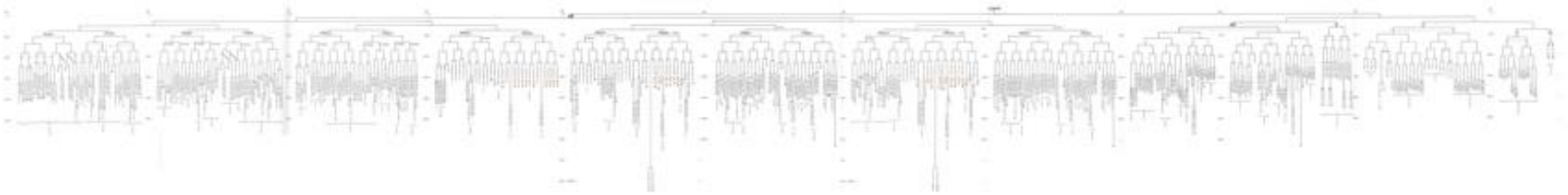
Advantage

- 1 mm long and 70 um in diameter.
- Definite cell numbers. 558 cells of the newly-hatched worm. 959 somatic cells with a variable number of germ cells, of the adult worm.
- Can be frozen and resuscitated.
- Hermaphrodite and male. It reproduces rapidly, ~ 80 hours @ 20C.
- Programmed cell death (PCD, apoptosis).
- Before it dies (after 2-3 weeks), it shows signs of aging and thus may provide general clues as to the aging process.

Definite cell numbers and lineage

- 1. Complete lineage is known by careful work of John Sulston.
- 2. When it hatches, 558 cells.
- 3. During development, 131 die in apoptosis.
- 4. Adult, 959 somatic cells with additional germ cells varying in number.

Cell lineage

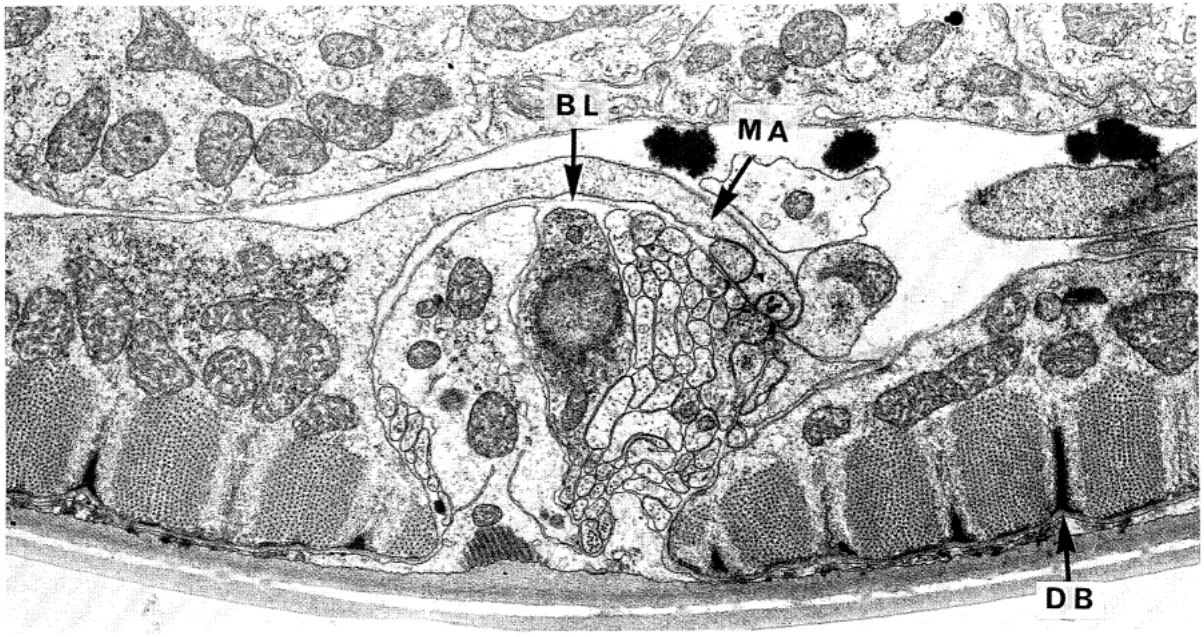


- http://wormweb.org/celllineage#c=EMS&z=1

The structure of the nervous system is known at EM level

directly from prints of micrographs of nervous tissue. In the region of the nerve ring, four-way montages were necessary; in other regions, single prints were sufficient. Every section was photographed in the region of the nerve ring and other areas of dense neuropile: photographs of every third section usually sufficed for following process bundles. Some use was made of a computer-aided reconstruction system described by White (1974) and Stevens & White (1979), but most of the reconstructions were done by hand from a total of about 8000 prints.

J. G. White, E. Southgate, J. N. Thomson and S. Brenner, 1986,
The Structure of the Nervous System of the Nematode *Caenorhabditis elegans*


BL
MA
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The Nobel Prize in Physiology or Medicine 2002

Sydney Brenner

H. Robert Horvitz

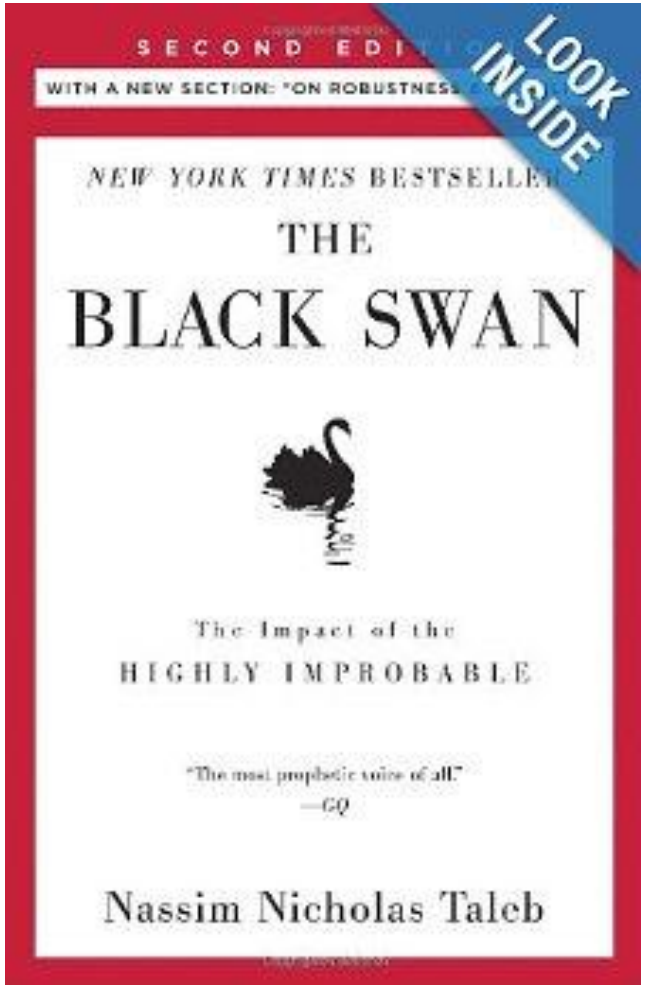
John E. Sulston

The Nobel Prize in Physiology or Medicine 2002 was awarded jointly to Sydney Brenner, H. Robert Horvitz and John E. Sulston *"for their discoveries concerning genetic regulation of organ development and programmed cell death"*.

Big achievements done with *C. elegans*

- 1. Molecular mechanisms of programmed cell death.
- 2. RNA interference (RNAi).
- 3. Aging and neural development.
- 4. MicroRNA (miRNA).

The Black Swan by Nassim Nicholas Taleb



The Impact of the Highly Improbable

A black swan is an event, positive or negative, that is deemed improbable yet causes massive consequences. In this groundbreaking and prophetic book, Taleb shows in a playful way that Black Swan events explain almost everything about our world, and yet we—especially the experts—are blind to them.

Discovery of RNA interference

The Plant Cell, Vol. 2, 279–289, April 1990 © 1990 American Society of Plant Physiologists

Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes *in trans*

Carolyn Napoli,[1] Christine Lemieux, and Richard Jorgensen[2]

DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, California 94608

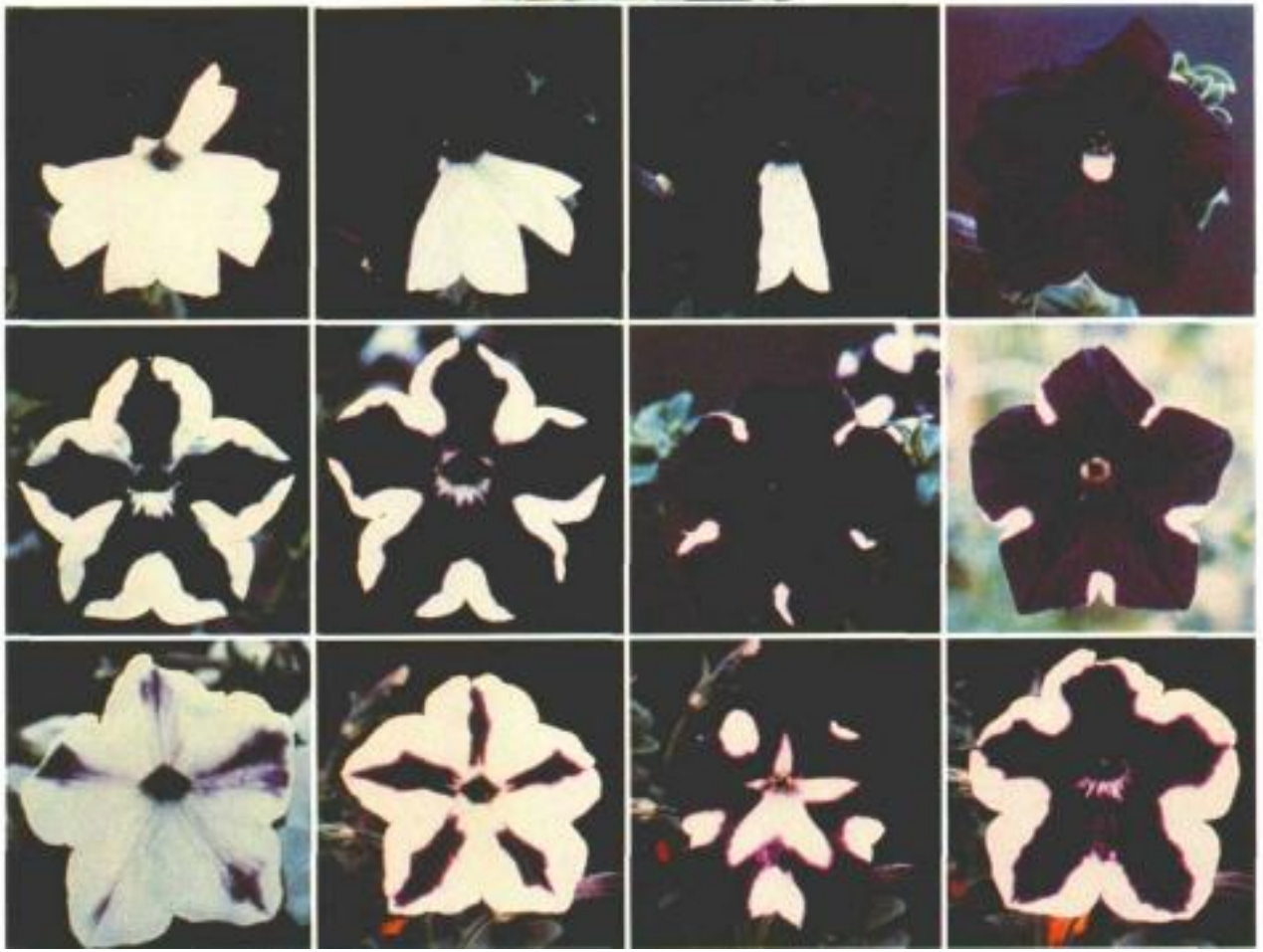
We attempted to overexpress chalcone synthase (CHS) in pigmented petunia petals by introducing a chimeric petunia CHS gene. Unexpectedly, the introduced gene created a block in anthocyanin biosynthesis. Forty-two percent of plants with the introduced CHS gene produced totally white flowers and/or patterned flowers with white or pale nonclonal sectors on a wild-type pigmented background; none of hundreds of transgenic control plants exhibited such phenotypes. Progeny testing of one plant demonstrated that the novel color phenotype co-segregated with the introduced CHS gene; progeny without this gene were phenotypically wild type. The somatic and germinal stability of the novel color patterns was variable. RNase protection analysis of petal RNAs isolated from white flowers showed that, although the developmental timing of mRNA expression of the endogenous CHS gene was not altered, the level of the mRNA produced by this gene was reduced 50-fold from wild-type levels. Somatic reversion of plants with white flowers to phenotypically parental violet flowers was associated with a coordinate rise in the steady-state levels of the mRNAs produced by both the endogenous and the introduced CHS genes. Thus, in the altered white flowers, the expression of both genes was coordinately suppressed, indicating that expression of the introduced CHS gene was not alone sufficient for suppression of endogenous CHS transcript levels. The mechanism responsible for the reversible co-suppression of homologous genes *in trans* is unclear, but the erratic and reversible nature of this phenomenon suggests the possible involvement of methylation.

PARENTAL

Transgenote
218.11

Transgenote
218.43

Transgenote
218.56



Cell, Vol. 81, 611–620, May 19, 1995, Copyright © 1995 by Cell Press

par-1, a Gene Required for Establishing Polarity in C. elegans Embryos, Encodes a Putative Ser/Thr Kinase That Is Asymmetrically Distributed

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Summary

The first cleavage of C. elegans is asymmetric, generating daughter cells with different sizes, cytoplasmic components, and fates. Mutations in the *par-1* gene disrupt this asymmetry. We report here that *par-1* encodes a putative Ser/Thr kinase with similarity to kinases from yeasts and mammals. Two strong alleles have mutations in the kinase domain, suggesting that kinase activity is essential for *par-1* function. PAR-1 protein is localized to the posterior periphery of the zygote and is distributed in a polar fashion preceding the asymmetric divisions of the germline lineage. Be-

appear to play a role in at least the first division. Brief pulses of the microfilament-disrupting drug cytochalasin during a critical period of the first cell cycle prevent the posterior localization of the P granules (Strome and Wood, 1983; Hill and Strome, 1988). Cytochalasin pulses during this same period sometimes also lead to symmetric divisions producing daughter cells of equal sizes, similar cell cycle rates, and variable spindle orientations (Hill and Strome, 1988, 1990).

Maternal-effect lethal mutations in the *par* genes produce phenotypes similar to the effects of cytochalasin treatments (Kemphues et al., 1988). In *par* mutant embryos, normally unequal divisions are equal, cleavage spindles are misoriented, P granules are mislocalized, and the blastomere fates are altered. This implies that the *par* genes function in both spindle placement and cytoplasmic localization (Kemphues et al., 1988; Kirby et al., 1990; Morton et al., 1992; Cheng et al., 1995).

The *par-1* gene is required for several aspects of early

observed.

Surprisingly, injection of in vitro synthesized sense RNA from the cDNA *ZC22* also induced *par-1* phenotypes at a high frequency among the progeny of injected worms. It is not clear what accounts for this effect. Moreover, the sense effect appears to be restricted to the putative translated region of the RNA while the antisense effect is not. Injection of both sense and antisense RNA from the 5′ region (lacking the 3′ untranslated region) also gave *par-1* phenocopies, while only the antisense RNA from the 3′ untranslated region gave an effect (data not shown). Thus, the antisense and sense effects appear to be separable and probably involve different mechanisms. The basis for the sense effect is under investigation and will not be discussed further. Overall, the specificity of the antisense and sense phenocopies provides strong evidence that the *ZC22* cDNA represents the *par-1* transcript. Additional evidence is provided below.

with PBS, the precipitates were dissolved in SDS gel loading buffer and counted in a beta-scintillation counter using a window for ^3H. The bound ^3H radioactivity was typically in the range ~2–8% of the total added. For co-immunoprecipitation, 25% PIP_2 or PIP in 75% phosphatidylcholine (PC) background (30 μg PIP_2 or PIP (Boehringer Mannheim) and 90 μg phosphatidylcholine (Sigma)), both in chloroform, were dried down together and sonicated in 300 μl PBS to form mixed liposome. GST fusion proteins were first incubated with 25% PIP_2 or PIP liposome (100 μM) and PIP_2 antibodies (1:100 dilution) for 2 h and with protein A–Sepharose for a further 30 min. After one wash with PBS, the immunoprecipitates were separated by 10% SDS–PAGE, probed with specific antibodies[21,22], and visualized by ECL (Amersham). Each experiment was performed at least twice with similar results. The relative amount of immunoreactivity in each lane was quantified by serial dilutions of sample[21].

Received 6 June; accepted 13 October 1997.

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Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

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Experimental introduction of RNA into cells can be used in certain biological systems to interfere with the function of an endogenous gene[1,2]. Such effects have been proposed to result from a simple antisense mechanism that depends on hybridization between the injected RNA and endogenous messenger RNA transcripts. RNA interference has been used in the nematode *Caenorhabditis elegans* to manipulate gene expression[3,4]. Here we investigate the requirements for structure and delivery of the interfering RNA. To our surprise, we found that double-stranded RNA was substantially more effective at producing interference than was either strand individually. After injection into adult animals, purified single strands had at most a modest effect, whereas double-stranded mixtures caused potent and specific interference. The effects of this interference were evident in both the injected animals and their progeny. Only a few molecules of injected double-stranded RNA were required per affected cell, arguing against stochiometric interference with endogenous mRNA and suggesting that there could be a catalytic or amplification component in the interference process.

The Nobel Prize in Physiology or Medicine 2006

Photo: L. Cicero

Andrew Z. Fire

Photo: J. Mottern

Craig C. Mello

The Nobel Prize in Physiology or Medicine 2006 was awarded jointly to Andrew Z. Fire and Craig C. Mello ***"for their discovery of RNA interference - gene silencing by double-stranded RNA"***

RNA-dependent RNA polymerase (RdRP)

- An enzyme that catalyzes the replication of RNA from an RNA template.
- Present in petunia and *C. elegans*.
- Do the transgenic mRNA of chalcone synthase and *par-1* form some kind of unexpected structure?

The AP axis in *C. elegans* is determined by asymmetric cell division

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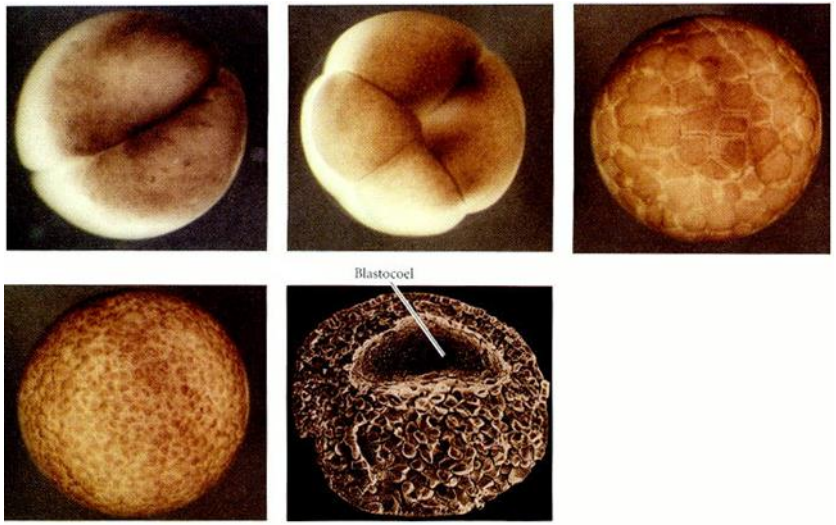
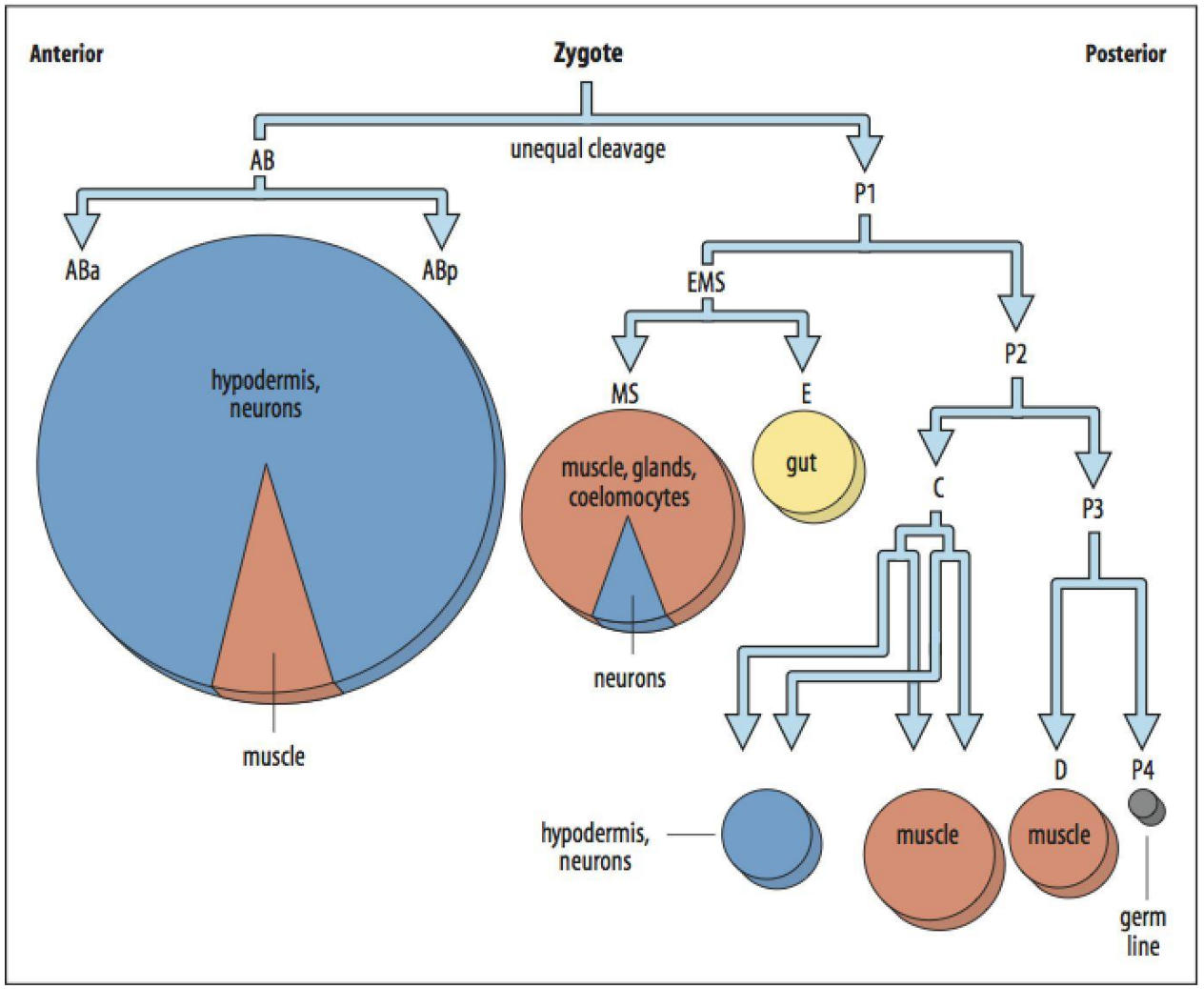
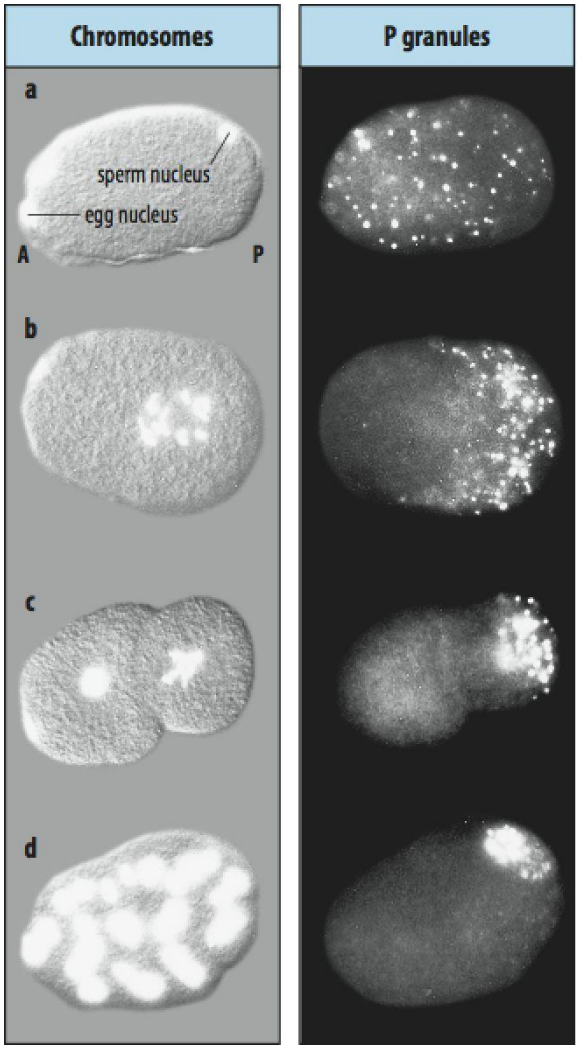


Cell lineage and cell fate in the early *C. elegans* embryo

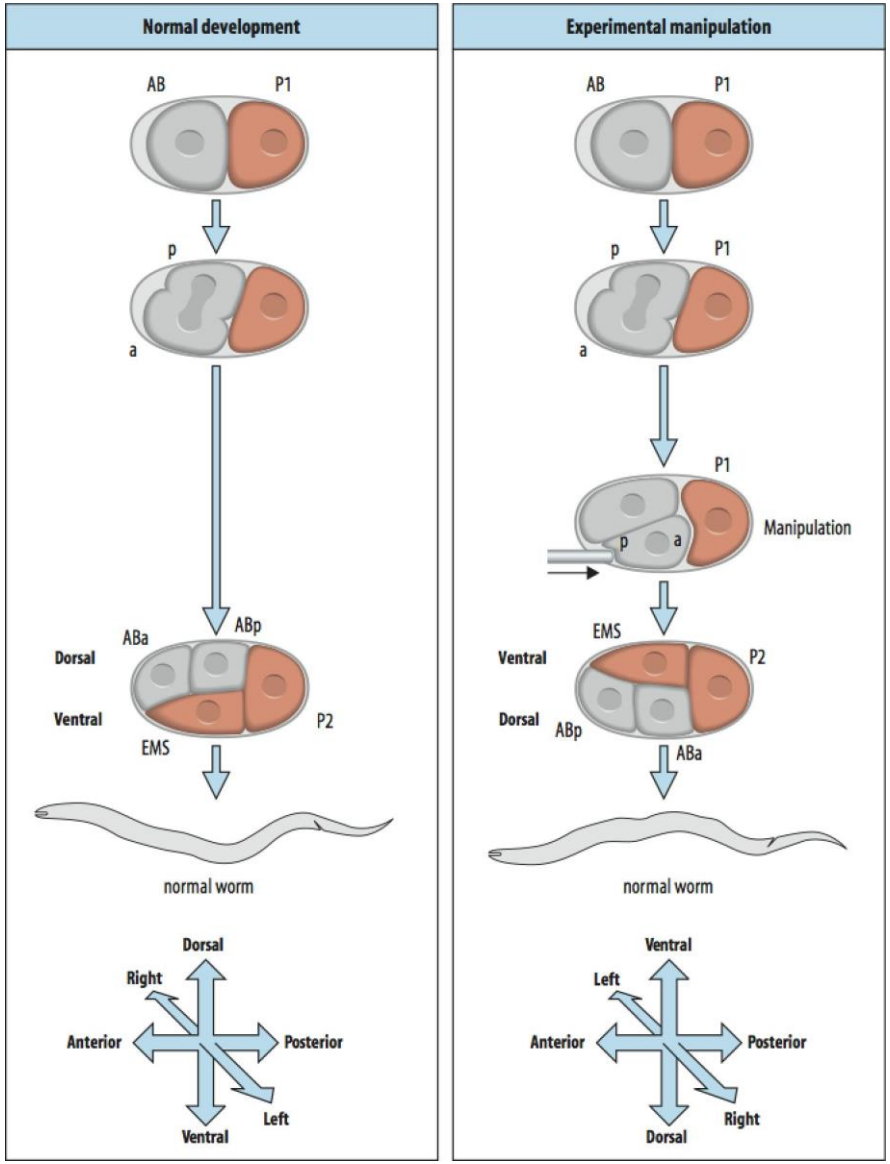


Localization of P granules

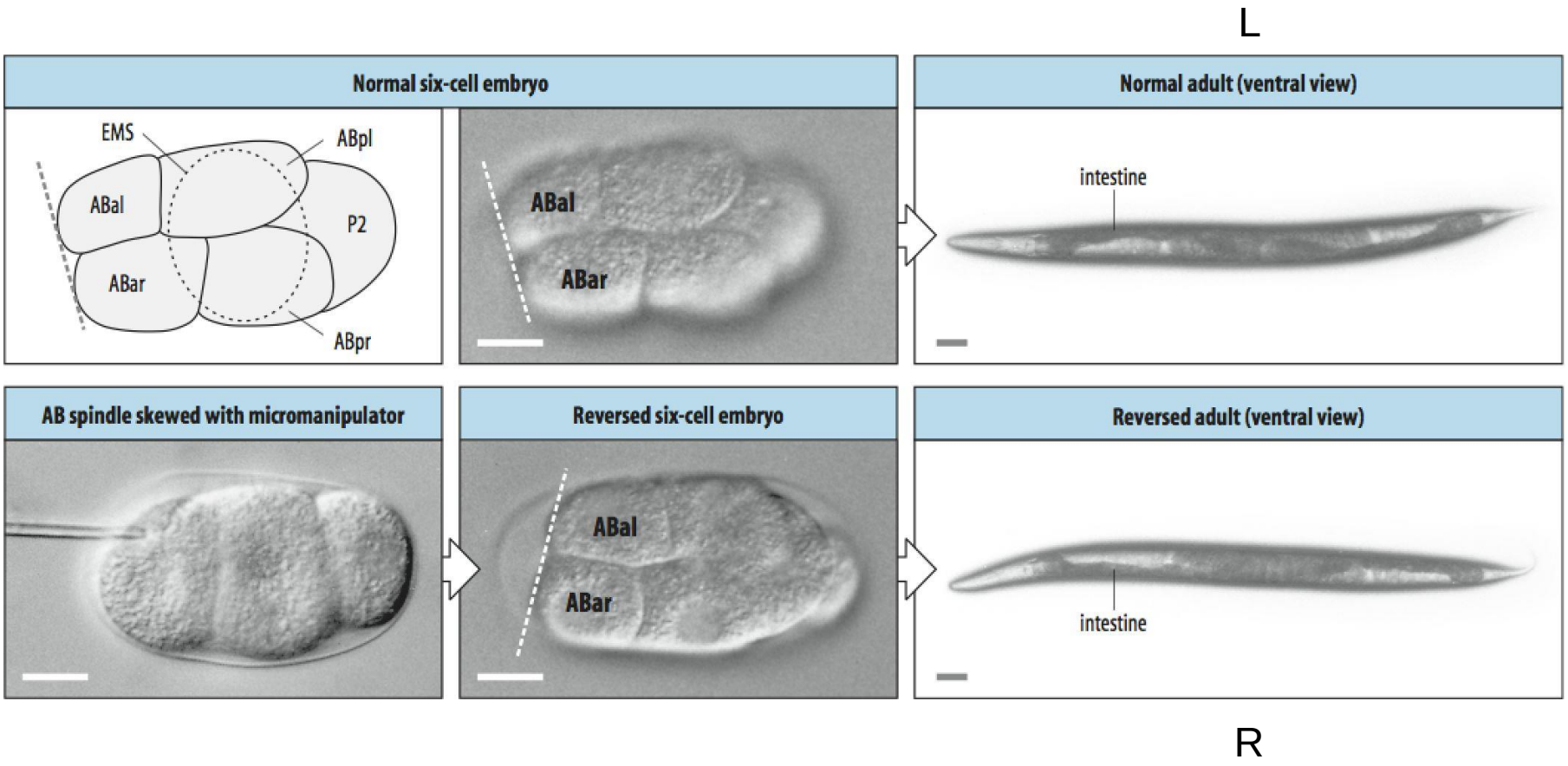


- Before fertilization, there is no evidence of any asymmetry.
- P granules, which contain maternal mRNAs and proteins, move to posterior end after fertilization.

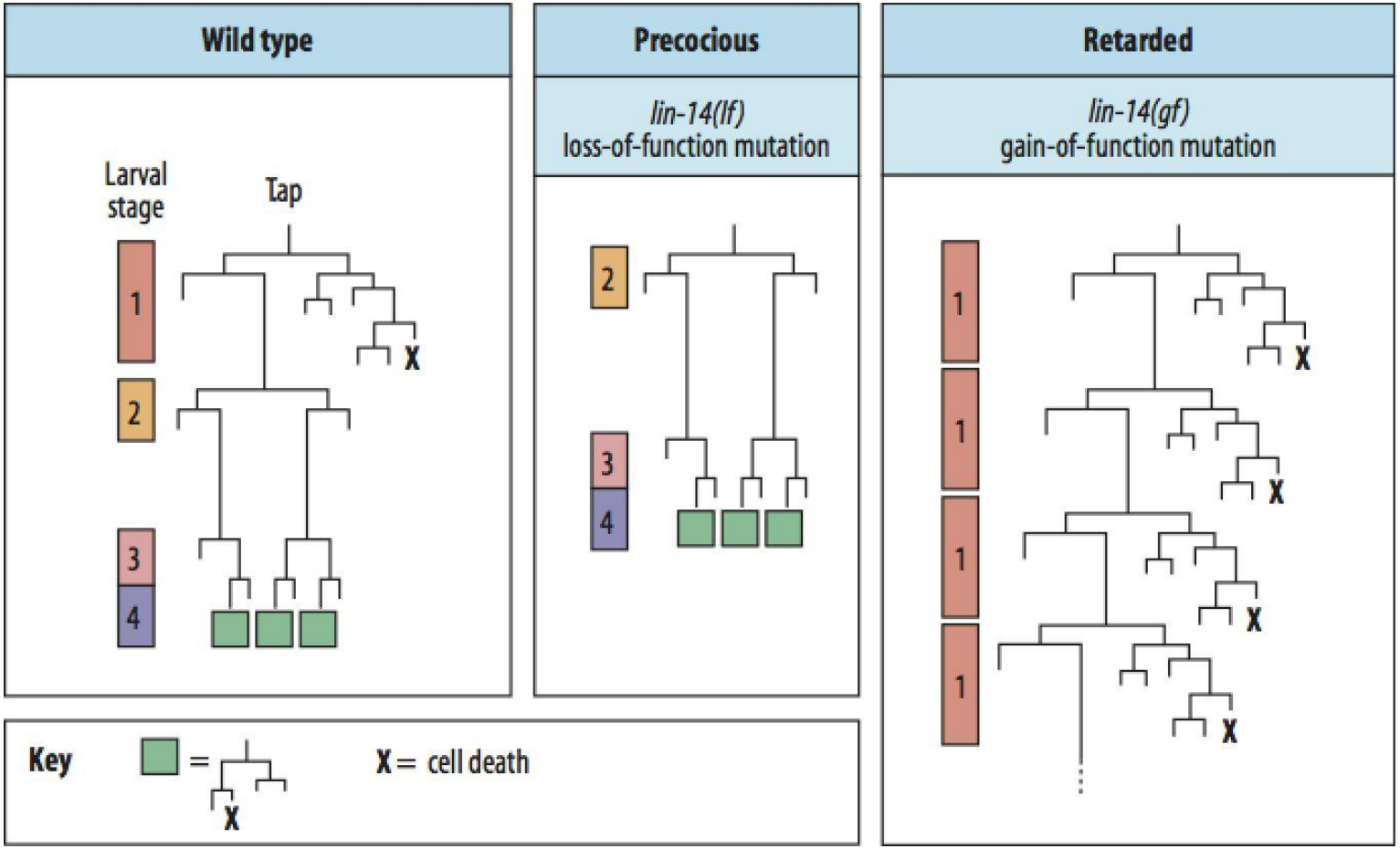
The DV axis in *C. elegans* is determined by cell-cell interaction



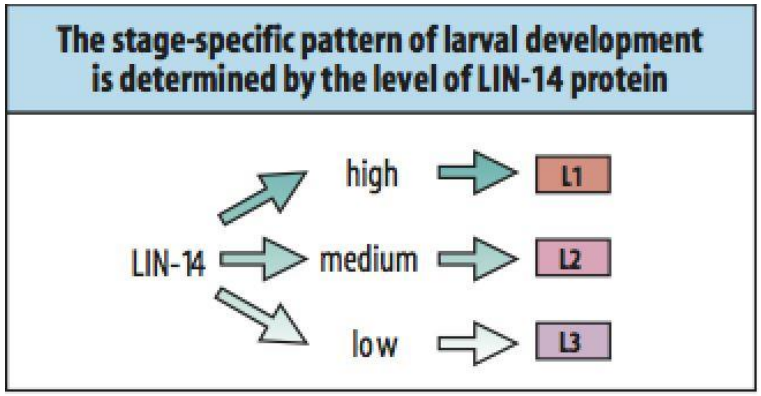
The LR axis is determined at the third cleavage

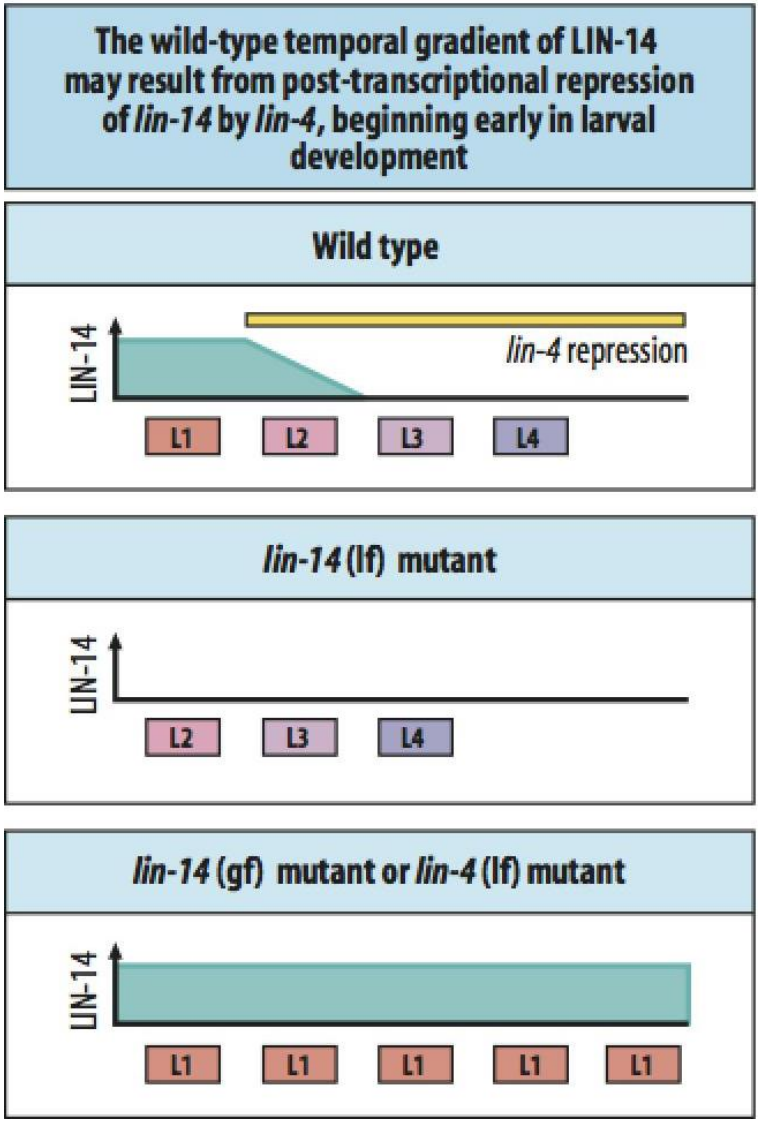


Precocious and Retarded



A model for the control of the temporal pattern





Discovery of MicroRNA

Cell, Vol. 75, 843–854, December 3, 1993, Copyright © 1993 by Cell Press

The C. elegans Heterochronic Gene *lin-4* Encodes Small RNAs with Antisense Complementarity to *lin-14*

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Department of Cellular and Developmental Biology
Cambridge, Massachusetts 02138

Summary

***lin-4* is essential for the normal temporal control of diverse postembryonic developmental events in C. elegans. *lin-4* acts by negatively regulating the level of LIN-14 protein, creating a temporal decrease in LIN-14 protein starting in the first larval stage (L1). We have cloned the C. elegans *lin-4* locus by chromosomal walking and transformation rescue. We used the C. elegans clone to isolate the gene from three other Caenorhabditis species; all four Caenorhabditis clones functionally rescue the *lin-4* null allele of C. elegans. Comparison of the *lin-4* genomic sequence from these four species and site-directed mutagenesis of potential open reading frames indicated that *lin-4* does not encode a protein. Two small *lin-4* transcripts of approximately 22 and 61 nt were identified in C. elegans and found to contain sequences complementary to a repeated sequence element in the 3′ untranslated region (UTR) of *lin-14* mRNA, suggesting that *lin-4* regulates *lin-14* translation via an antisense RNA–RNA interaction.**

Ambros and Horvitz, 1987). Animals carrying a *lin-4* loss-of-function (*lf*) mutation, *lin-4(e912)*, display reiterations of early fates at inappropriately late developmental stages; cell lineage patterns normally specific for the L1 are reiterated at later stages, and the animals execute extra larval molts (Chalfie et al., 1981). The consequences of these heterochronic developmental patterns include the absence of adult structures (such as adult cuticle and the vulva) and the prevention of egg laying.

lin-14 null *(0)* mutations cause a phenotype opposite to that of *lin-4(lf)* and are completely epistatic to *lin-4(lf)*, which is consistent with *lin-4* acting as a negative regulator of *lin-14* (Ambros and Horvitz, 1987; Ambros, 1989). *lin-14(0)* mutants skip the expression of L1-specific events and precociously execute programs normally specific for the L2, L3, L4, and adult stages. *lin-14* gain-of-function (*gf*) mutations, which cause inappropriately high *lin-14* activity at late stages of development, result in a retarded phenotype virtually identical to that of *lin-4(lf)* (Ambros and Horvitz, 1987). These observations indicate that in wild-type development a high level of *lin-14* activity in the early L1 stage specifies L1-specific programs, and lower levels of *lin-14* activity in the late L1 specify later stage-specific programs. Thus, the normal developmental progression from the execution of L1 programs to later programs depends critically on the *lin-4*-dependent decrease in *lin-14* activity.

The temporal decrease in *lin-14* activity reflects a decrease in the level of LIN-14 protein. LIN-14 protein is normally abundant in the nuclei of late-stage embryos and

Discovery of MicroRNA

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The C. elegans Heterochronic Gene *lin-4* Encodes Small RNAs with Antisense Complementarity to *lin-14*

Rosalind C. Lee,*† Rhonda L. Feinbaum,*‡

Ambros and Horvitz, 1987). Animals carrying a *lin-4* loss-

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The temporal decrease in *lin-14* activity reflects a decrease in the level of LIN-14 protein. LIN-14 protein is normally abundant in the nuclei of late-stage embryos and

slower charge relaxations; this in turn implies that both rate constants leading away from that state (k_{-1} and k_2 in Fig. 4a) are relatively large.

The strictly sequential nature of the three charge components shown here indicates that the three Na^+ may be released from the Na^+/K^+ pump in a fixed order. Ordered occlusion/de-occlusion of two K^+ by kidney microsomal Na^+/K^+-ATPase[14] and sequential occlusion, translocation and release of the two Ca^{2+} ions transported by the sarcoplasmic reticulum Ca^{2+}-ATPase[15] have been detected using isotopes and rapid filtration techniques (time resolution ~10 ms), but the far higher time resolution and sensitivity of the electrical recording methods used here permit extraction of finer molecular kinetic detail[8,12,16]. Closer examination, using these methods, of the interactions of extracellular Na^+ ions with their binding sites within the Na^+/K^+ pump will now be required to discern the precise molecular rearrangements that surround these principal charge movements in the Na^+/K^+ transport cycle. □

Methods

Giant axons from the squid *Loligo pealei* were voltage clamped[17], internally dialysed and externally superfused at 20–22 °C with Cl^--free solutions[7,18] designed to restrict the pump to Na^+ de-occlusion/release steps (Fig. 1). Intracellular (in mM; pH adjusted with HEPES): 80 Na-HEPES, 57 *N*-methyl-D-glucamine (NMG)-HEPES, 50 glycine, 50 phenylpropyltriethylammonium-sulphate, 5 dithiothreitol, 2.5 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA), 15 Mg-HEPES, 5 Tris-ATP, 5 phospho(enol)pyruvate tri-Na^+-salt and 5 phospho-L-arginine mono-Na^+-salt. Extracellular (in mM): 400 Na-isethionate, 75 Ca-sulphamate, 1 3,4-diaminopyridine, 2×10^{-4} tetrodotoxin, 5 Tris-HEPES and 0.05 EDTA (pH 7.7). Osmolality of all solutions was ~930 mOsmol kg^{-1}. To lower $[Na]_o$, Na-isethionate was replaced by tetramethylammonium-sulphamate or NMG-sulphamate. Voltage pulses were generated and currents recorded using a 16-bit PC44 A-D/D-A converter board (Innovative Technologies) with software developed in-house. Currents were filtered at 12.5–200 kHz, then sampled at 20 kHz–2 MHz. Current records were sometimes acquired after subtraction of appropriately amplified small current signals, obtained in a voltage range where pump-mediated

(e-mail: gadsby@rockvax.rockefeller.edu), or to M.H.
(e-mail: miguel_holmgren@hms.harvard.edu).

The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*

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The *C. elegans* heterochronic gene pathway consists of a cascade of regulatory genes that are temporally controlled to specify the timing of developmental events[1]. Mutations in heterochronic genes cause temporal transformations in cell fates in which stage-specific events are omitted or reiterated[2]. Here we show

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slower charge relaxations; this in turn implies that both rate constants leading away from that state (k_{-1} and k_2 in Fig. 4a) are relatively large.

The strictly sequential nature of the three charge components shown here indicates that the three Na^+ may be released from the Na^+/K^+ pump in a fixed order. Ordered occlusion/de-occlusion of two K^+ by kidney microsomal Na^+/K^+-ATPase[14] and sequential occlusion, translocation and release of the two Ca^{2+} ions transported by the sarcoplasmic reticulum Ca^{2+}-ATPase[15] have been

50 phenylpropyltriethylammonium-sulphate, 5 dithiothreitol, 2.5 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA), 15 Mg-HEPES, 5 Tris-ATP, 5 phospho(enol)pyruvate tri-Na^+-salt and 5 phospho-L-arginine mono-Na^+-salt. Extracellular (in mM): 400 Na-isethionate, 75 Ca-sulphamate, 1 3,4-diaminopyridine, 2×10^{-4} tetrodotoxin, 5 Tris-HEPES and 0.05 EDTA (pH 7.7). Osmolality of all solutions was ~930 mOsmol kg^{-1}. To lower $[Na]_o$, Na-isethionate was replaced by tetramethylammonium-sulphamate or NMG-sulphamate. Voltage pulses were generated and currents recorded using a 16-bit PC44 A-D/D-A converter board (Innovative Technologies) with software developed in-house. Currents were filtered at 12.5–200 kHz, then sampled at 20 kHz–2 MHz. Current records were sometimes acquired after subtraction of appropriately amplified small current signals, obtained in a voltage range where pump-mediated

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The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*

whereas increased *let-7* gene dosage causes precocious expression of adult fates during larval stages. *let-7* encodes a temporally regulated 21-nucleotide RNA that is complementary to elements in the 3′ untranslated regions of the heterochronic genes *lin-14*, *lin-28*, *lin-41*, *lin-42* and *daf-12*, indicating that expression of these genes may be directly controlled by *let-7*. A reporter gene

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Thanks!

C. Elegans dumpy (*dpy*) mutant

wild type

dumpy

- The concept of "programmed cell-death" was used by Lockshin & Williams[9] in 1964 in relation to insect tissue development, around eight years before "apoptosis" was coined.
- http://www.sciencedirect.com/science/article/pii/0022191064900344