

GFP: Lighting Up Life

You can observe a lot by watching.

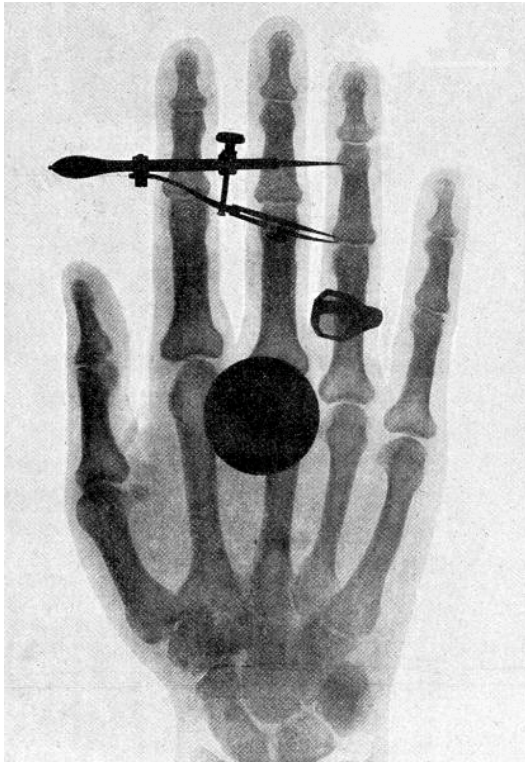
Yogi Berra

My companions and I then witnessed a curious spectacle. . . The Nautilus floated in the midst of. . . truly living light. . . an infinite agglomeration of colored. . . globules of diaphanous jelly. . .

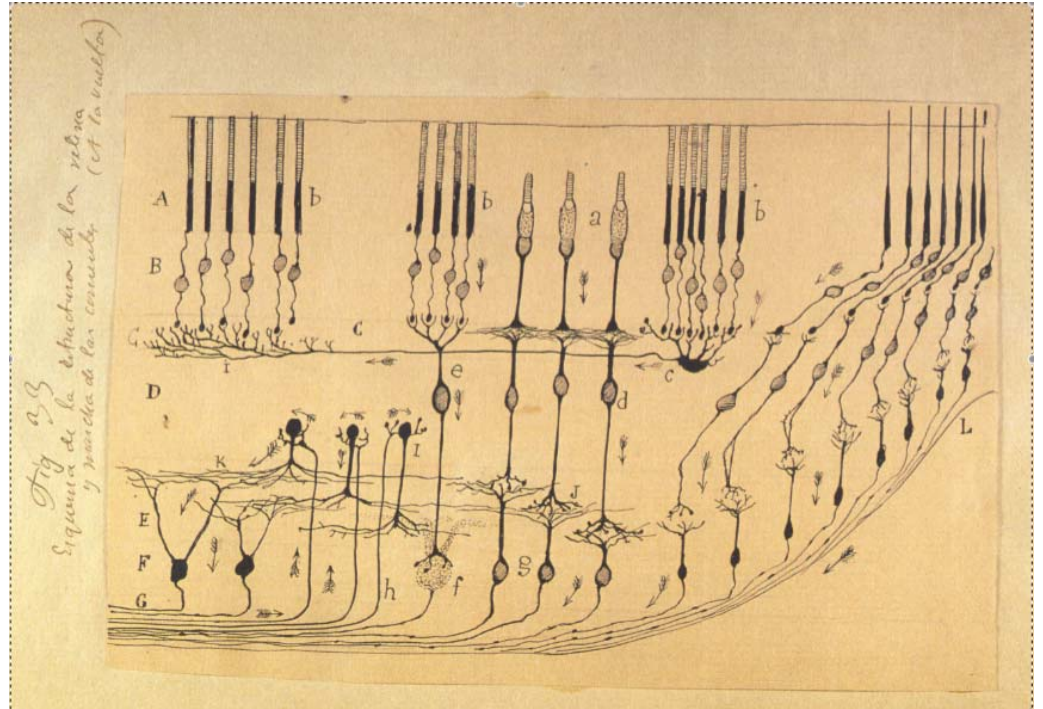
Twenty Thousand Leagues Under the Sea – Jules Verne

Now it is such a bizarrely improbable coincidence that anything so mind-bogglingly useful could have evolved purely by chance that some thinkers have chosen to see it as a final and clinching proof of the nonexistence of God.

The Hitchhiker's Guide to the Galaxy – Douglas Adams



Wilhelm Röntgen



Camillo Golgi



Santiago Ramón y Cajal



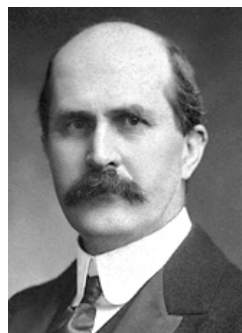
X-ray Crystallography

Ultramicroscope

Nuclear Magnetic Resonance

Phase Contrast
Microscope

Large-
Array
Radio
Telescopes



William
Bragg

Physics, 1915



Lawrence
Bragg



Richard
Zsigmondy

Chemistry, 1925



Felix
Bloch

Physics, 1952

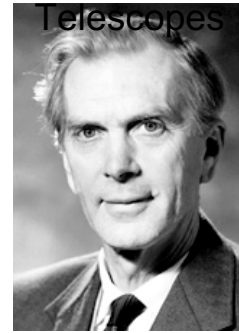


E. M.
Purcell



Frits
Zernike

Physics, 1953



Martin
Ryle

Physics, 1974

Electron
Microscope

Scanning Tunneling Microscope

Computer Assisted Tomography

Magnetic Resonance Imaging



Ernst
Ruska

Physics, 1986



Gerd
Binnig

Physics, 1986



Heinrich
Rohrer



Allan
Cormack

Physiology or Medicine, 1979



Godfrey
Hounsfield



Paul
Lauterbur

Physiology or Medicine, 2003



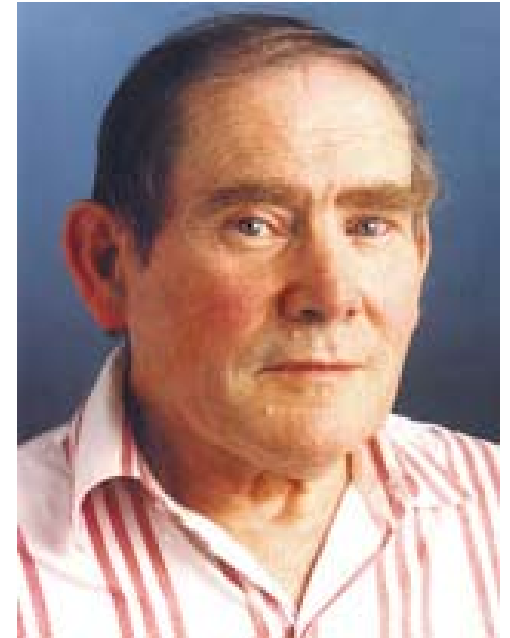
Peter
Mansfield



José Zadunaisky



Bob Perlman



Sydney Brenner



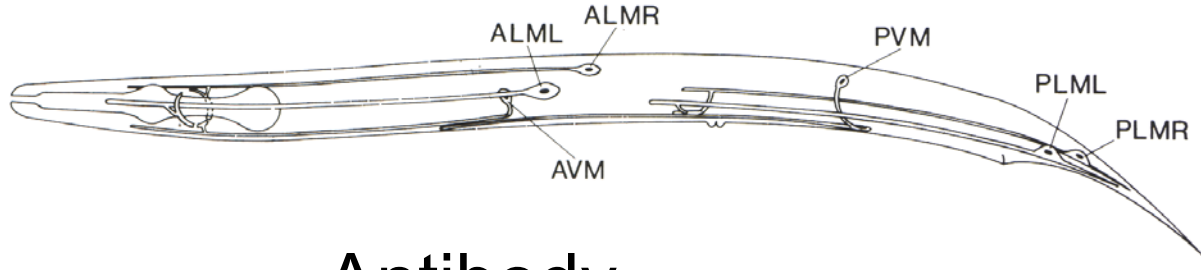
Sydney Brenner

Bob Horvitz

John Sulston



Caenorhabditis elegans



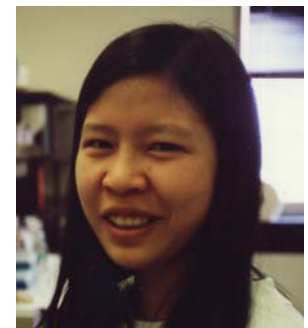
Antibody

MEC-7



β -galactosidase Activity

mec-9



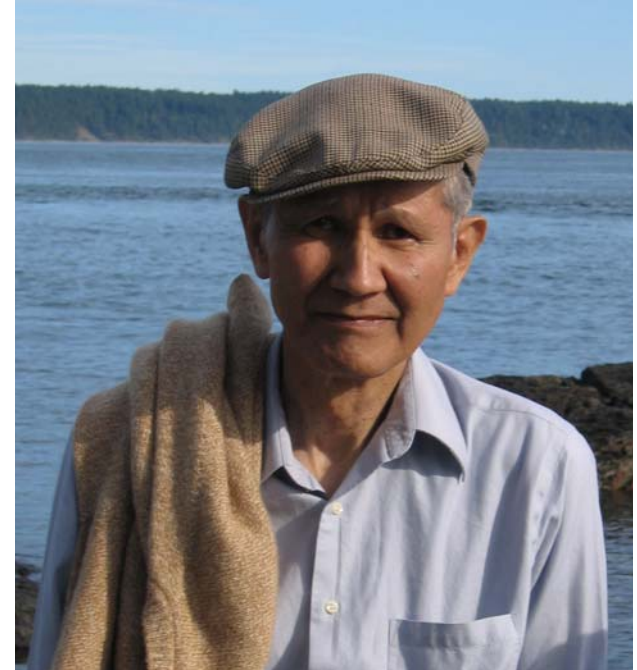
Hongping Du



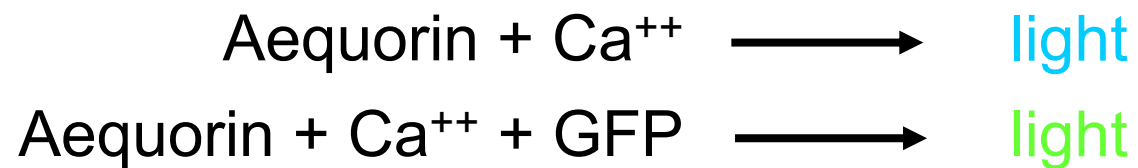
Paul Brehm



Aequorea victoria



Osamu Shimomura



Σ. Newton Harvey. - Bioluminescence.

- 1747

Green fluorescent protein ^{Milner} ^{dimer} ⁴⁰⁴⁻⁵⁴²⁻¹³³⁴
Nalt Lorenz ^{gill library} ^{Milton Cormier (Georgia)}
Grad. stud.

Shimimura (Woods Hole)

Shimamura

617-956-6922
Paul Brehm
Tufts -

↓ yellowish
aquorea GFP - 32000 MW
monomer.

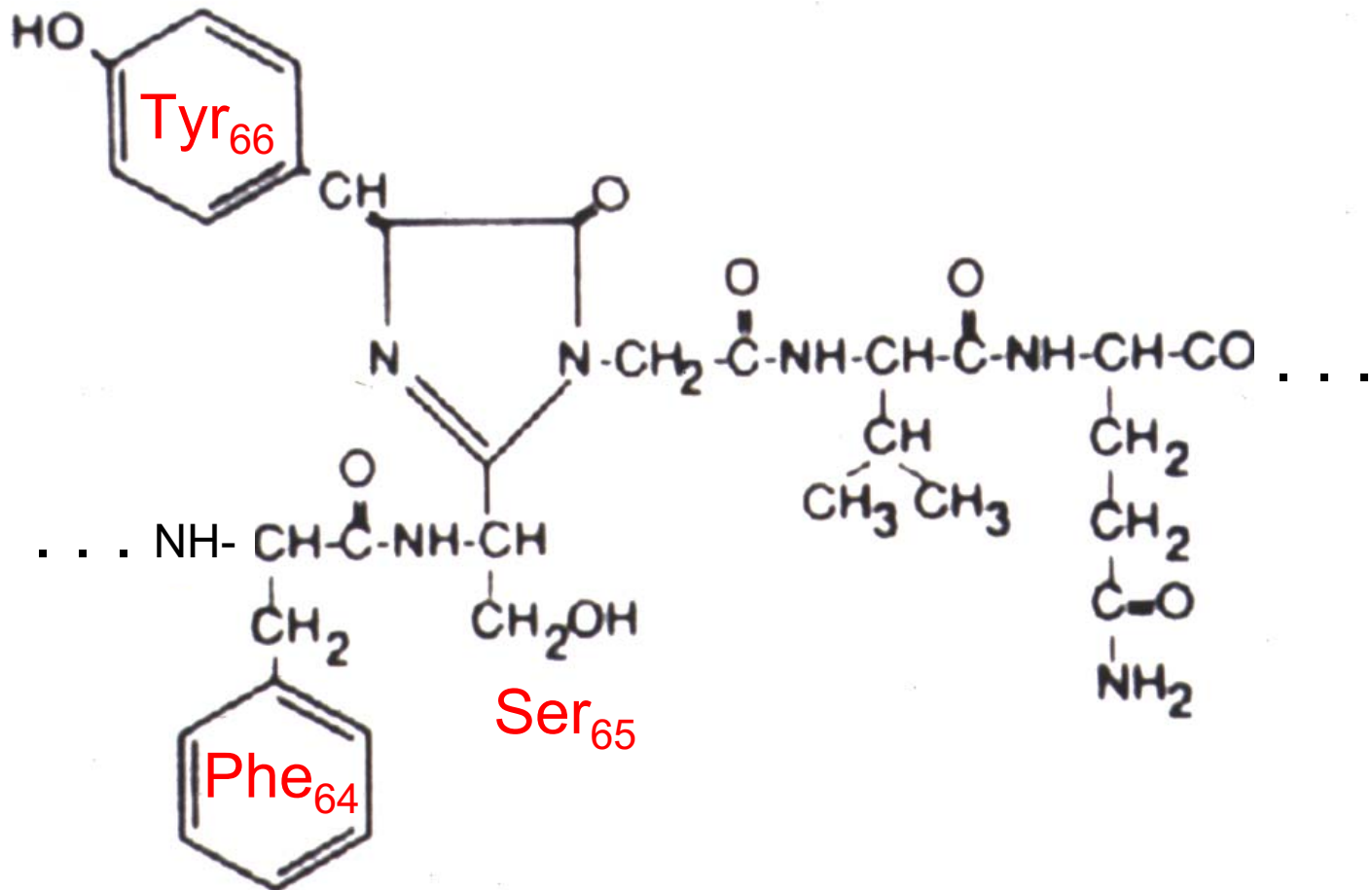
Chromophore - same in a + ren
cofactor - b a a - post translational
modification -
not know what
generates cofactor.

plasmid protein
monomer - 32000 MW
4/26/79
Douglas Prasher
50%
6/79 - 548-1400
x 2311
Univ of Georgia
John Wampler
404-542-1577



Douglas Prasher

The GFP Fluorophore



GENE 06296

Primary structure of the *Aequorea victoria* green-fluorescent protein

(Bioluminescence; Cnidaria; aequorin; energy transfer; chromophore; cloning)

Douglas C. Prasher^a, Virginia K. Eckenrode^b, William W. Ward^c, Frank G. Prendergast^d and Milton J. Cormier^b

Correspondence to: Dr. D.C. Prasher, Redfield Bldg., Woods Hole Oceanographic Institution, Woods Hole, MA 02543 (U.S.A.)

Tel. (508)457-2000, ext. 2311; Fax (508)457-2195.

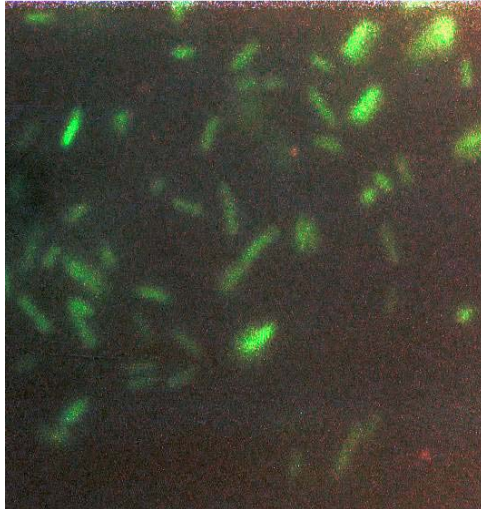
λGFP10

1 TACACACGAA TAAAAGATAA CAAAGATGAG TAAAGGAGAA GAACTTTTCA
CTGGAGTTGT
61 CCCAATTCTT GTTGAATTAG ATGGTGATGT TAATGGGCAC AAATTTTCTG
TCAGTGGAGA
121 GGGTGAAGGT GATGCAACAT ACGGAAAAC TACCCTTAAA TTTATTTGCA
CTACTGGAAA
181 ACTACCTGTT CCATGGCCAA CACTTGTCAC TACTTTCTCT TATGGTG TTC
AATGCTTTTC
241 AAGATACCCA GATCATATGA AACAGCATGA CTTTTTCAAG AGTGCCATGC
CCGAAGGTTA
361 CAAGTTTGAA GGTGATACCC TTGTTAATAG AATCGAGTTA AAAGGTATTG
ATTTTAAAGA
421 AGATGGAAAC ATTCTTGGAC ACAAATTGGA ATACAAC TAT AACTCACACA
ATGTATACAT
481 CATGGCAGAC AAACAAAAGA ATGGAATCAA AGTTAACTTC AAAATTAGAC
ACAACATTGA
541 AGATGGAAGC GTTCAACTAG CAGACCATTA TCAACAAAAT ACTCCAATTG
GCGATGGCCC
601 TGTCTTTT TA CCAGACAACC ATTACCTGTC CACACAATCT GCGCTTCGA



TTACACATGG
721 CATGGATGAA CTATACAAAT AAATGTCCAG ACTTCCAATT GACACTAAAG
TGTCCGAACA
781 ATTAATAAAA TCTCAGGGTT CCTGGTTAAA TTCAGGCTGA GATATTATTT
ATATATTTAT
841 AGATTCATTA AAATTGTATG AATAATTTAT TGATGTTATT GATAGAGGTT
ATTTTCTTAT

EcoRI



Ghia Euskirchen

Tuesday 13. October 1992

— continued —

Fluorescence Microscopy

— Used 'scope from 368' Eng. Terrace lab with
fluorescein block. — Also viewed by Ding & Chuck.
Viewed under oil immersion of 100x objective.

Check for fluorescence

E. coli from Ding
untreated

no autofluorescence
could be seen although the
field had a strong greenish cast

1 t = 2 hr (after
induction)

fluorescing E. coli (strongly)
fairly black field

2 t = 0 hr (before
induction)

Weakly fluorescing E. coli
fairly black field

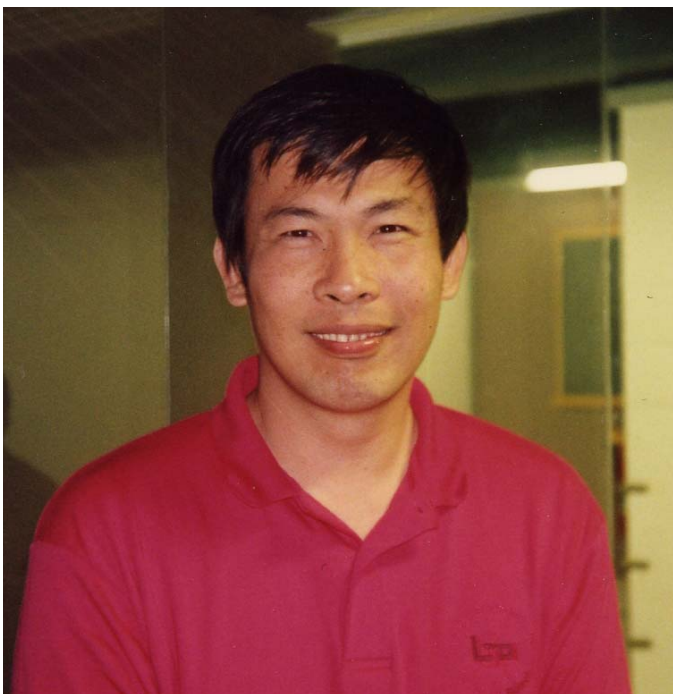
2 t = 2 hr (after
induction)

same as # 1 t = 2 hr

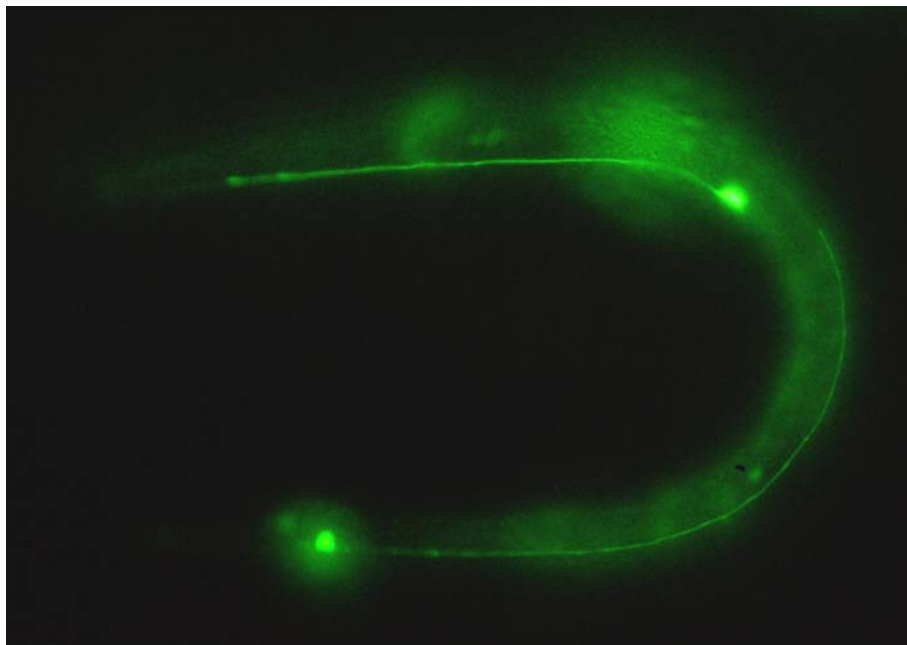
With Vroman's camera,
Kodak Ektar 100 ASA 35 mm
set on 100 ASA

1st group of exposures $\sim \# 16$ were the untreated E. coli from Ding.
2nd group $\sim \# 30$ were #2 t = 2 hr
3rd group (frames 31 +) were # 2 t = 0 hr

* For auto exposure time which was ~ 60 sec, cells had completely
BLEACHED.

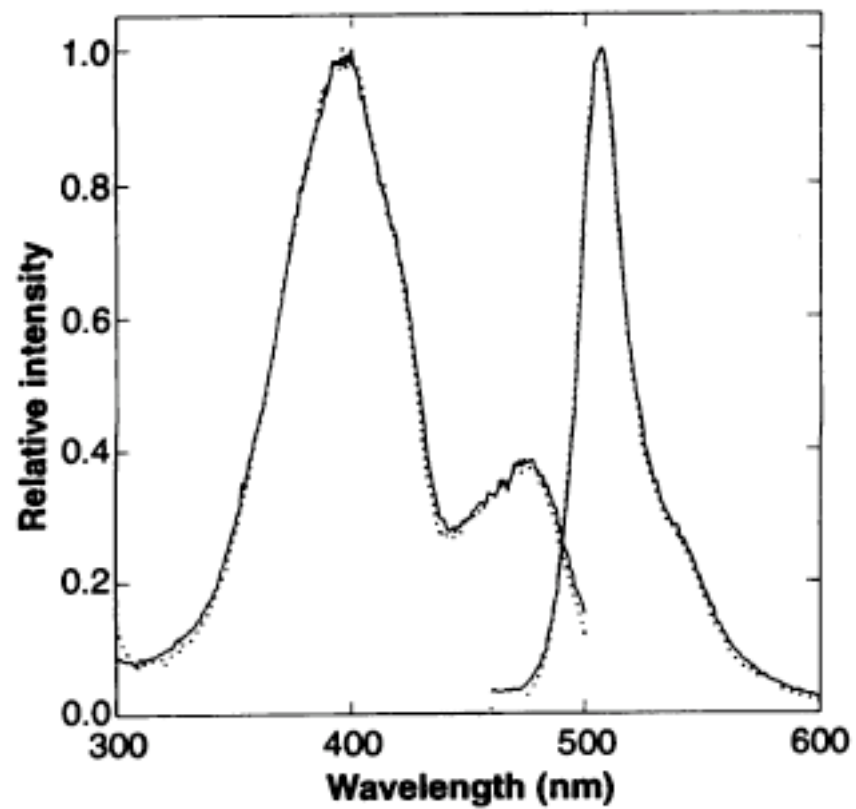


Yuan Tu





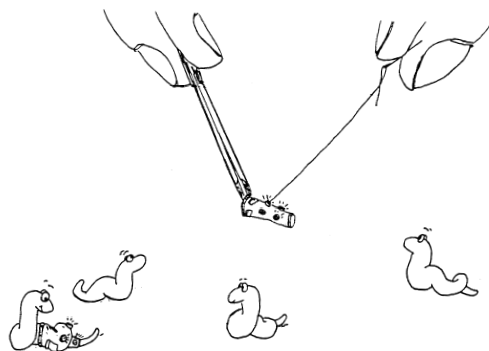
Bill Ward



Glow Worms - A New Method of Looking at *C. elegans* Gene Expression

Marty Chalfie and Yuan Tu, Dept. Biol. Sci., Columbia Univ., NY, NY 10027
Douglas Prasher, US Dept. Agriculture, Otis ANG Base, MA 02542.

The Worm Breeder's Gazette



The Worm Jean Sequins Project

Volume 13 No. 1

October 1, 1993

Glow Worms - A New Method of Looking at *C. elegans* Gene Expression

Marty Chalfie and Yuan Tu, Dept. Biol. Sci., Columbia Univ., NY, NY 10027
Douglas Prasher, US Dept. Agriculture, Otis ANG Base, MA 02542.

We have developed a new way to look at gene expression in *C. elegans* (and other organisms) that utilizes an inherently fluorescent protein (the green-fluorescent protein; GFP) from the jellyfish *Aequorea victoria*. GFP fluoresces bright green when illuminated with blue light. We have found that this fluorescence does not depend upon any other component specific to *A. victoria*, so *gfp* can be used instead of *lacZ*, for example, to make gene expression fusions.

We have made a *mec-7gfp* fusion using the *mec-7* promoter, transformed *C. elegans* with this construct, and generated two integrated lines to examine GFP expression. Both lines (and the parental non-integrated strain) were fluorescent, but one insertion gave very strong fluorescence (*uIs4*). Strong expression is seen in the four embryonic touch cells (the ALM and PLM cells) in *uIs4* animals. Even the terminal branches of these neurons can be followed. Other cells also fluoresce, but less strongly (BDU, FLP, a few cells in the tail, and the AVM and PVM touch cells). Two additional cells in the tail also show fairly strong fluorescence: by the projection of their processes, these appear to be the ALN cells. The staining of the ALM, AVM and PVM (but not to as great an extent in the PLM cells) was dependent on *mec-3*. These results are consistent with the previous expression pattern produced by this promoter [Hamelin et al., *EMBO J.* 11, 2885 (1992); Mitani et al. *Development*, in press] and seems to be equal to our most sensitive method (antibody staining). (The ALM and PLM cells are often displaced anteriorly in *uIs4* animals, but not in the other strains; this defect is probably due to a secondary mutation or a mutation at the site of insertion.)

We have not completely optimized the method of viewing the GFP fluorescence. The excitation spectrum for native and recombinant GFP has a major peak at 395 nm and a minor peak at 470 nm, and the emission spectrum has a major peak at 509 nm with a shoulder at 540 nm. Because we found that 395 nm light causes a very rapid photobleaching that is not seen at 470 nm (the fluorescence bleaches, but slowly; there is recovery from photobleaching at both wavelengths), we have tended to use the higher exciting wavelength. However, refinements can be made. For example, we find that it is better to use a long-pass emission filter (GFP looks green and the animals' autofluorescence is yellow) rather than a band-pass filter (both are green). (In preliminary observations with several of the *flu* strains we haven't seen any improvement. We haven't yet looked at *clr-1* animals, but these would presumably help eliminate the problem of the autofluorescence.) Another improvement comes from using a xenon rather than a mercury lamp for fluorescence (the output dips at 470 nm with the mercury lamp, but not with the xenon lamp). We have not yet tried low-intensity-light video cameras (the autofluorescence may pose a problem here).

We have lots of ideas of how *gfp* might be used and imagine that other people will have many more. We think it should be possible 1) to examine gene expression and protein localization at various stages (and to see changes in expression, e.g. through cell division); 2) to examine the outgrowth and migration of cells *in situ*; 3) to look for mutants that change the pattern of expression (e.g., looking for revertants of the degeneration-causing *mec-4(e1611)* mutation by mutating a *mec-4gfp*; *mec-4(e1611)* double and looking for the reappearance of fluorescing cells); 4) to mark cells for subsequent isolation and study (an experiment we hope to do soon with Shawn Lockery - who suggested the above title); and 5) to identify cells for laser ablations (the cells may also absorb more laser energy).

We have generated a set of plasmids that may be useful for *C. elegans* researchers. These are a Bluescript II KS (+) derivative (TU#65) containing a *Kpn I* - *EcoR I* fragment encoding GFP with an Age I site 5' to the translation start and a *Bsm I* site at the termination codon (suggested by Andy Fire) and *gfp* versions (TU#60 - TU#63) of the four *C. elegans lacZ* expression vectors (pPD16.43, pPD21.28, pPD22.04, and pPD22.11, respectively) described by Fire et al., *Gene* 93, 189 (1990). If you are interested in obtaining any of these clones, please write (or FAX or email) your request (include your FAX number; we'd like to know what you are interested in doing, but that's not essential) to Marty Chalfie and he will FAX you the necessary Columbia papers to sign (they can be returned by FAX) and we will try to send out the clones immediately.

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ASSOCIATION FOR THE
ADVANCEMENT OF
SCIENCE

SCIENCE

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VOL. 263 • PAGES 725-888

\$6.00

~~Green Fluorescent Protein: A New Marker
for Gene Expression~~

~~The *Aequorea victoria* Green Fluorescent
Protein Needs No Exogenously-Added
Component to Produce a Fluorescent
Product in Prokaryotic and Eukaryotic Cells~~

Green Fluorescent Protein as a Marker
for Gene Expression

Martin Chalfie, Yuan Tu, Ghia Euskirchen,
William W. Ward, Douglas C. Prasher

Science **263**: 802-805, 1994

Columbia University in the City of New York | New York, N.Y. 10027

DEPARTMENT OF BIOLOGICAL SCIENCES

SHERMAN FAIRCHILD CENTER
FOR THE LIFE SCIENCES

Martin Chalfie
Dept. of Biological Sciences
Columbia University
New York, N.Y. 10027


Dear Marty,

Nov. 11, 1993.

It is perfectly fine with me if you cite S.Wang's and my unpublished results in your Science paper on GFP, provided you meet the following conditions:

1. You make coffee each Saturday morning for the next two months, ready by 8:30 a.m.
2. You prepare a special french dinner at a time of your choosing.
3. You empty the garbage nightly for the next month.

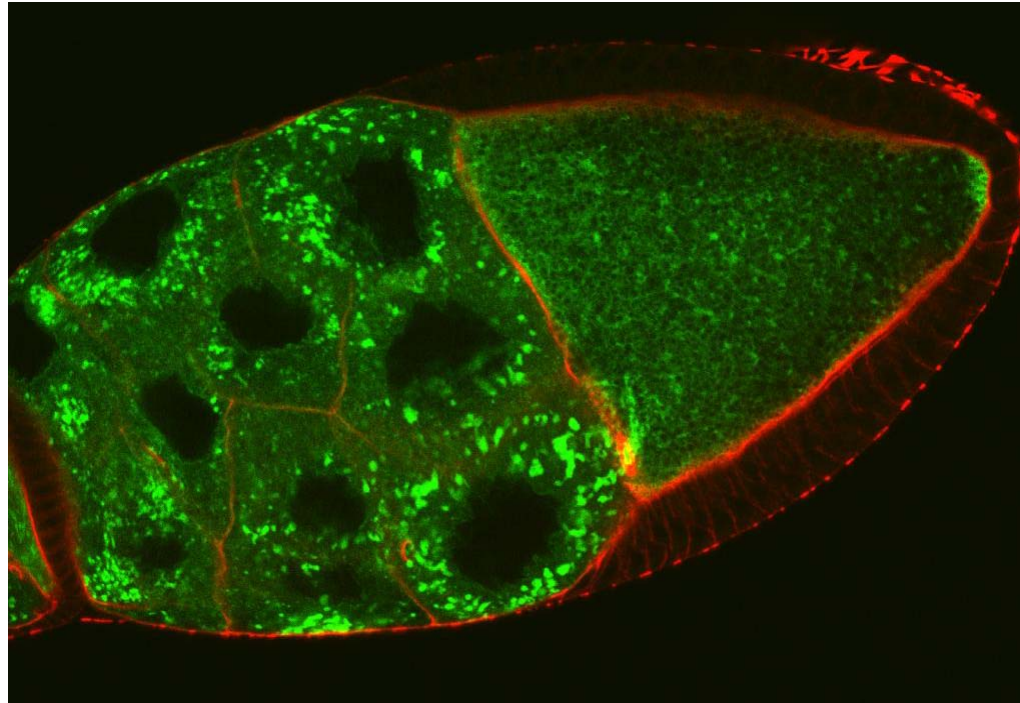
Your sincerely,


Tulle Hazelrigg



Tulle Hazelrigg

Sarah Chalfie



Implications for *bcd* mRNA localization from spatial distribution of *exu* protein in *Drosophila* oogenesis

Shengxian Wang and Tulle Hazelrigg

Nature **369**: 400-403, 1994



Tulle Hazelrigg



Shengxian Wang

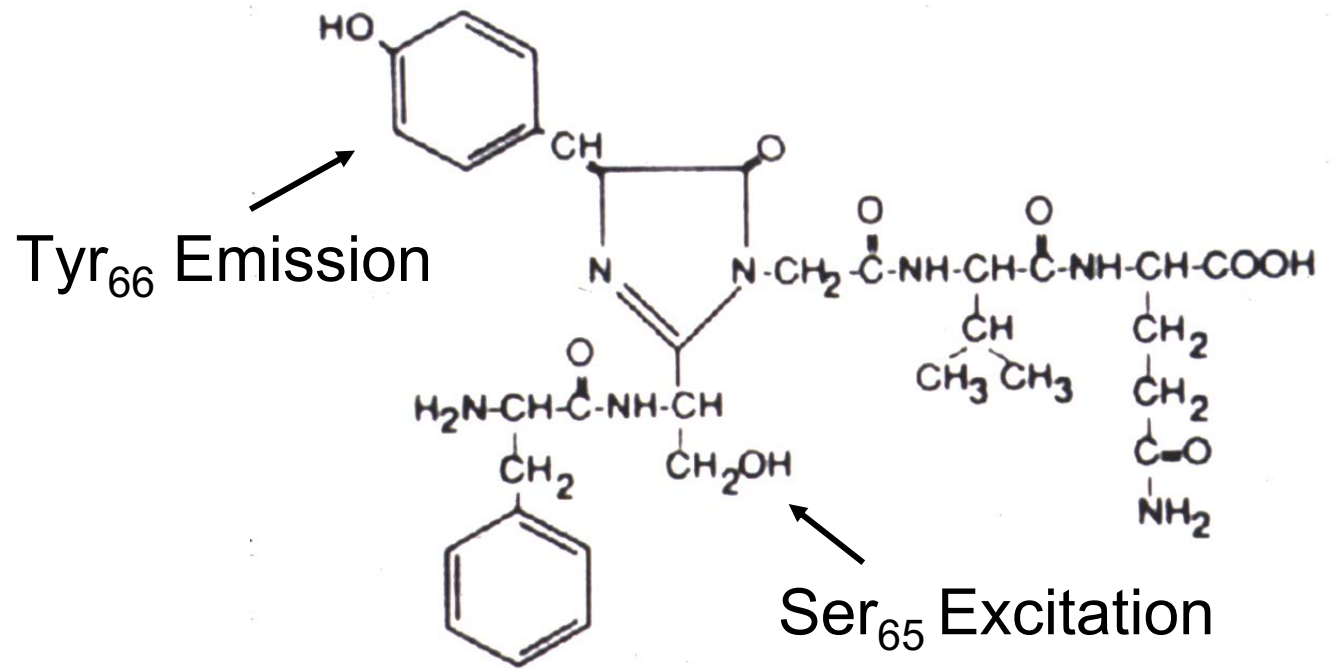
Advantages of GFP as a Biological Marker

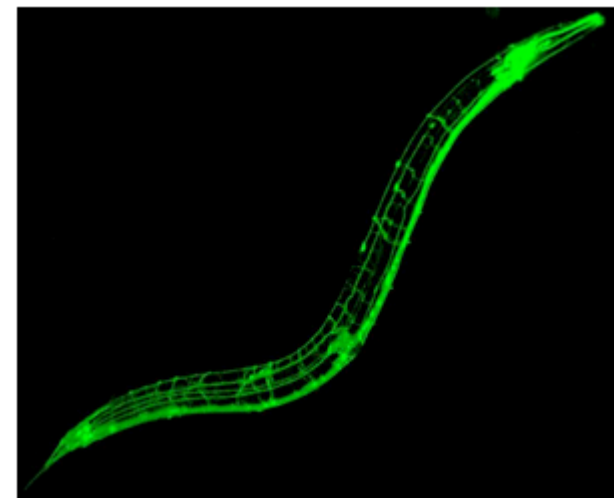
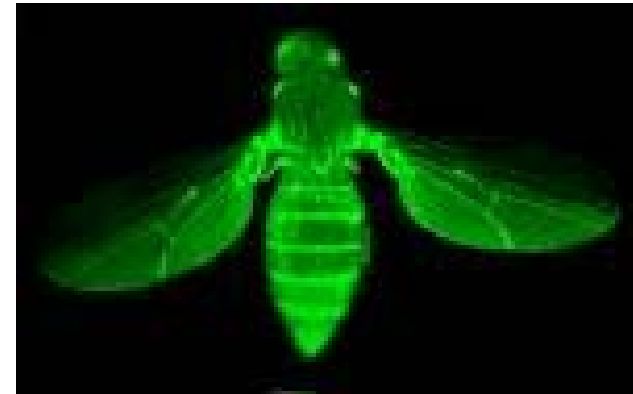
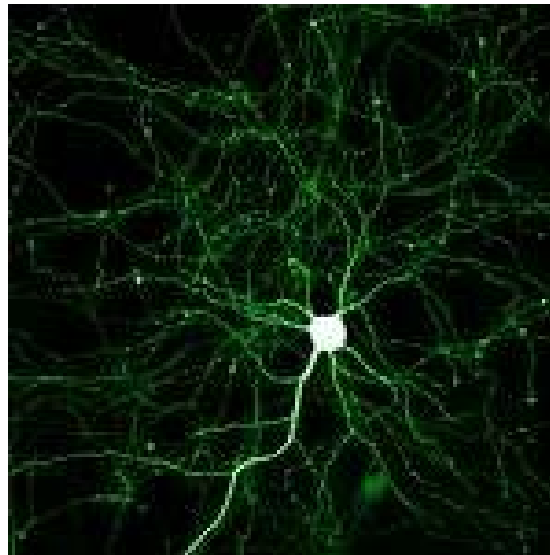
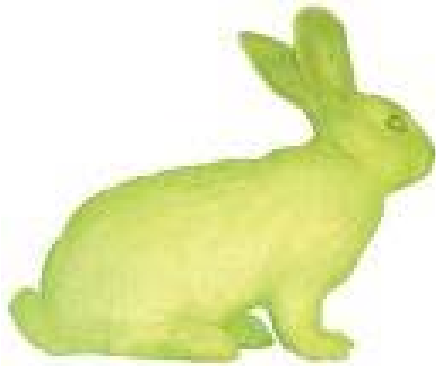
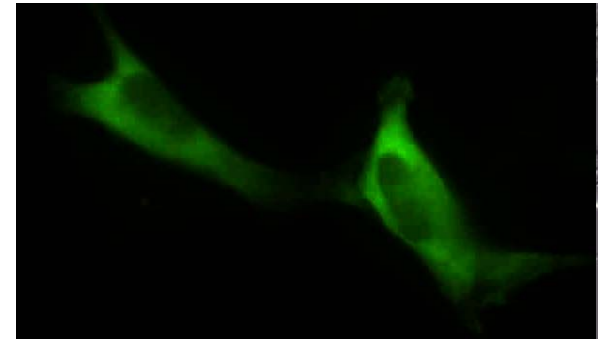
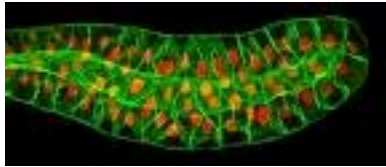
1. Heritable
2. Relatively Non-invasive
3. Small and Monomeric
4. Visible in Living Tissues

Improving GFP

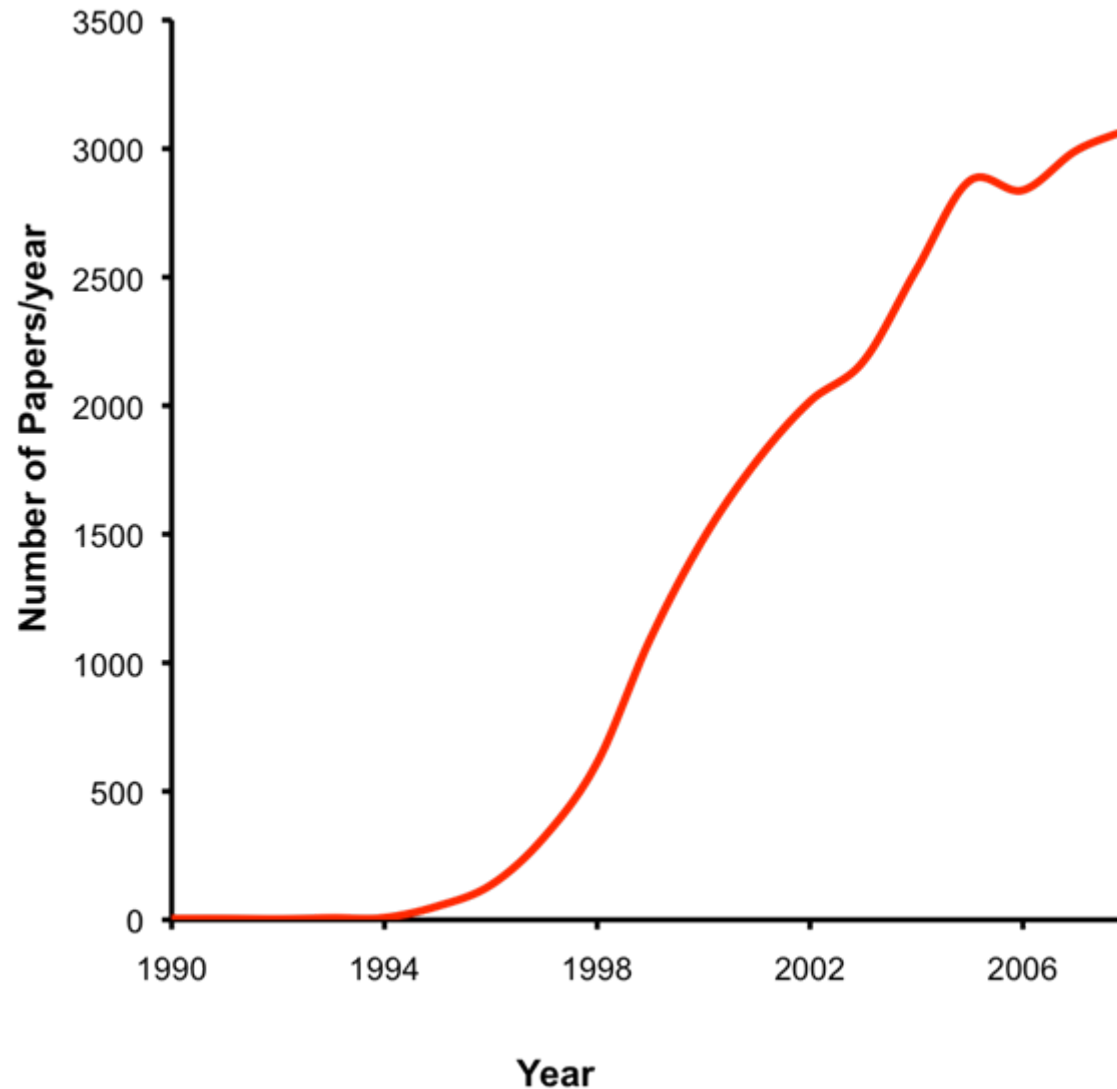


Roger Tsien





Papers Using Green Fluorescent Protein

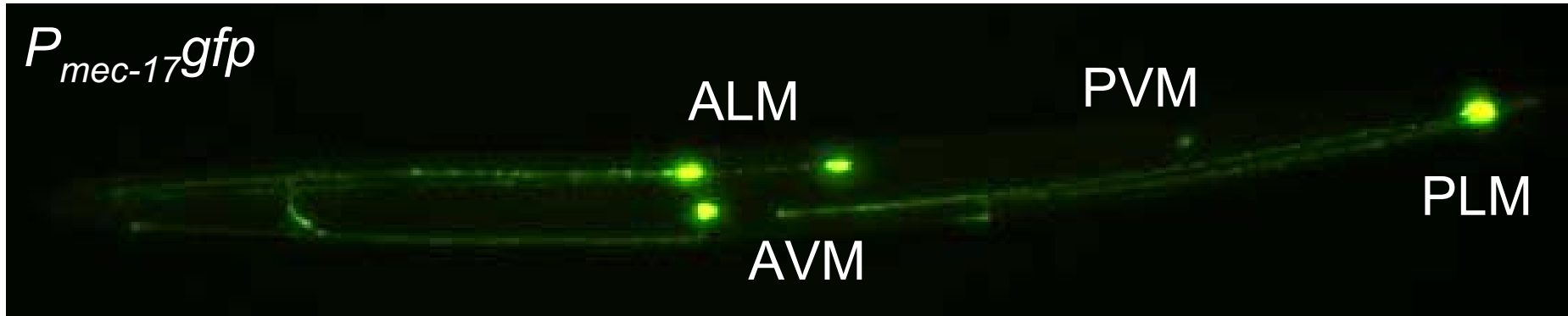
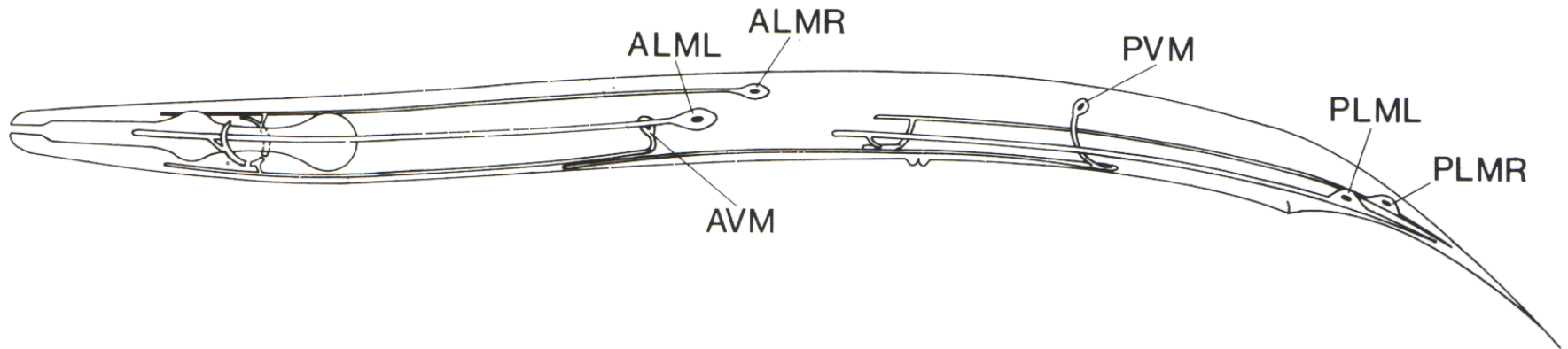


The First Human GFP Transgenic?



Ang Lee

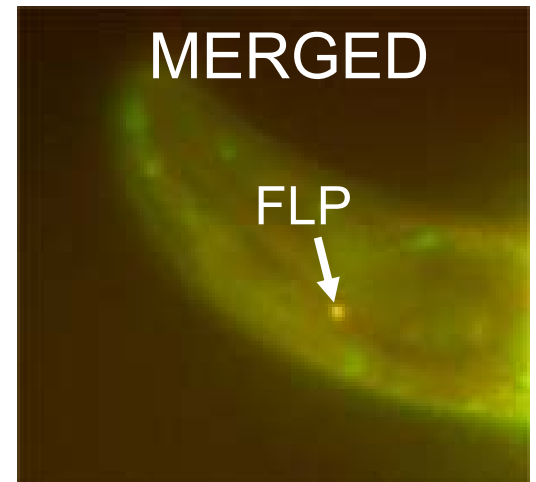
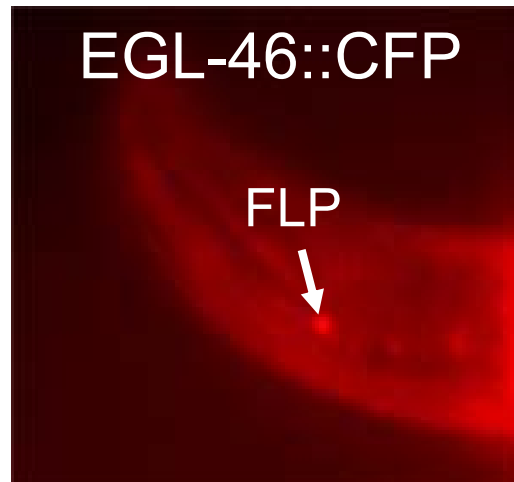
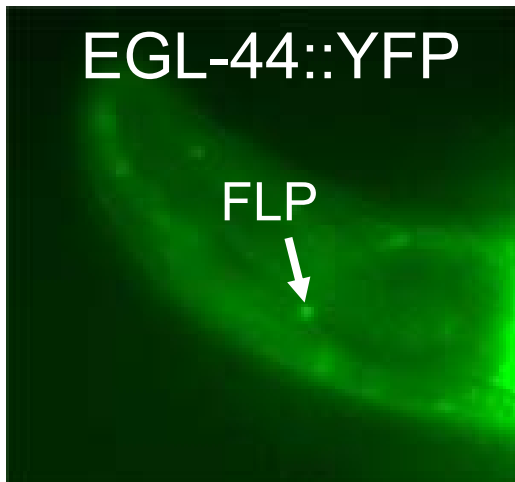
Gene Expression



Yun Zhang



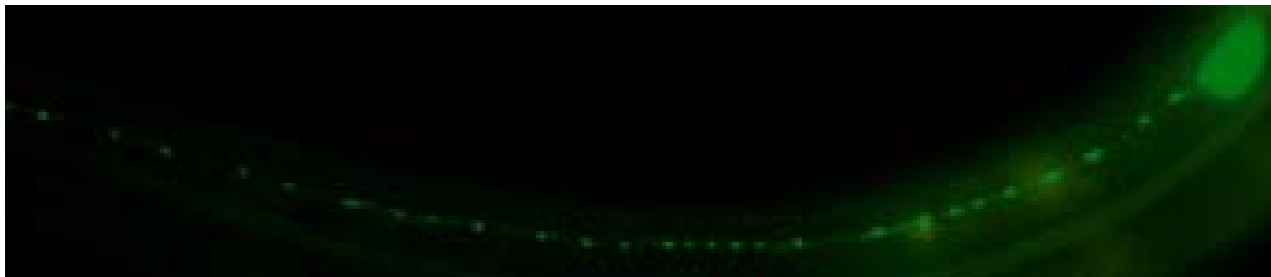
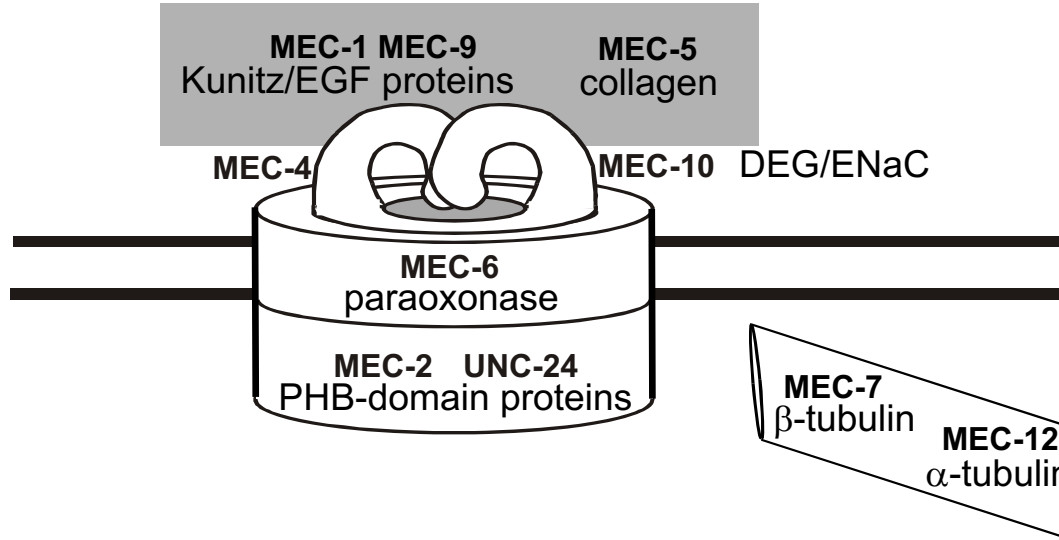
Co-expression



Ji Wu



Protein Localization

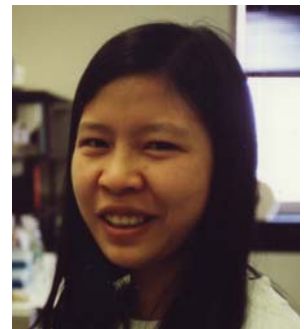
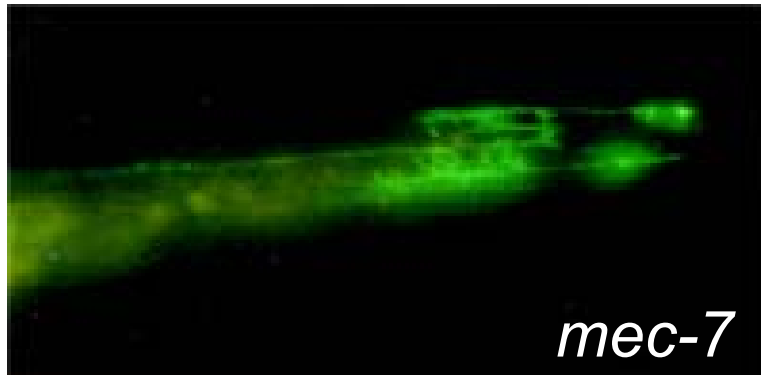
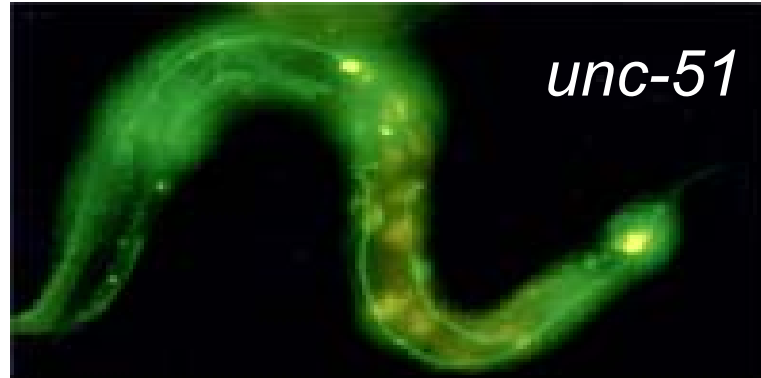
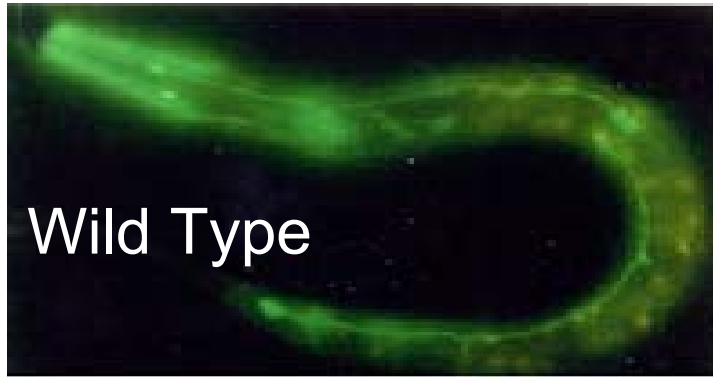


mec-4::yfp

Dattananda Chelur



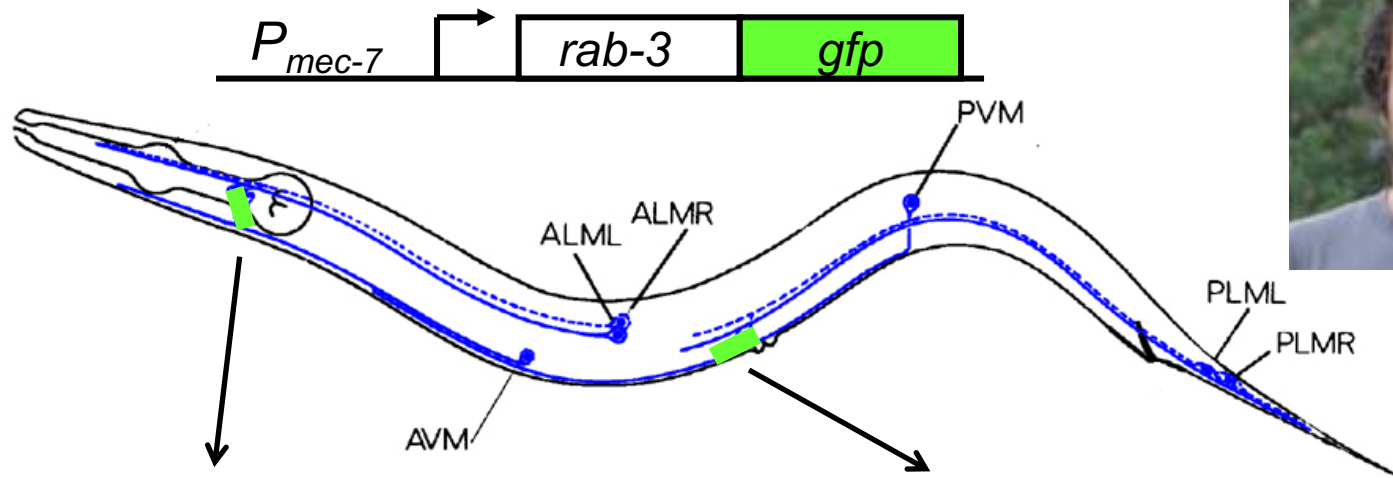
Mutant Screens and Characterization



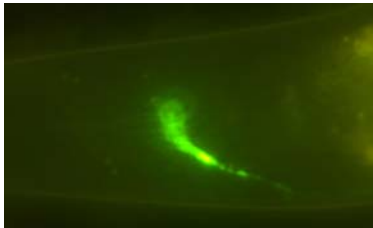
Hongping Du

Visualizing Synapses

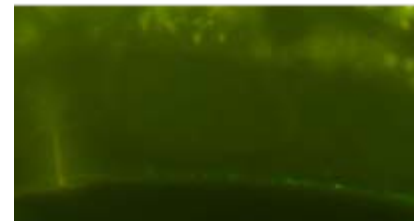
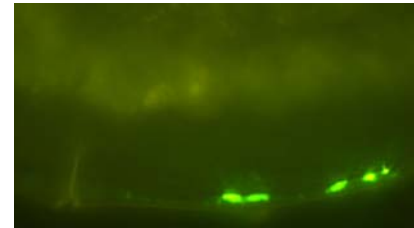
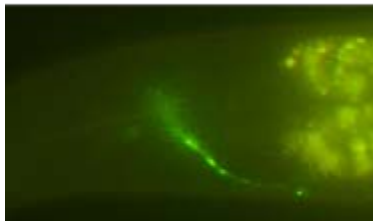
Mike Nonet



wild type

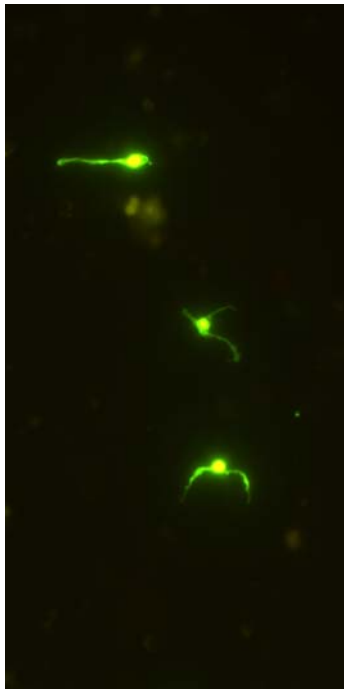
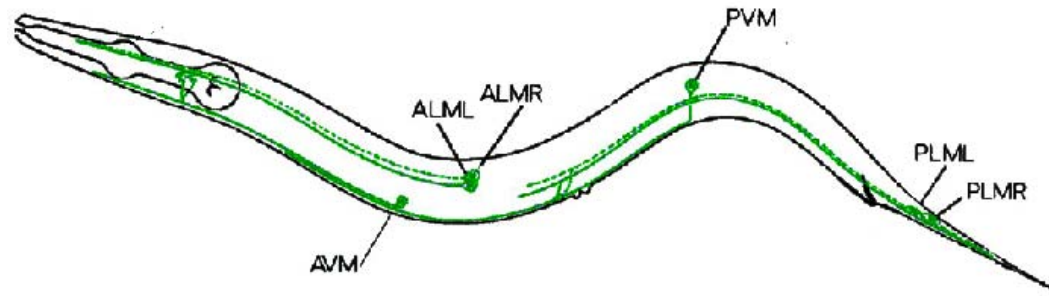


mec-15

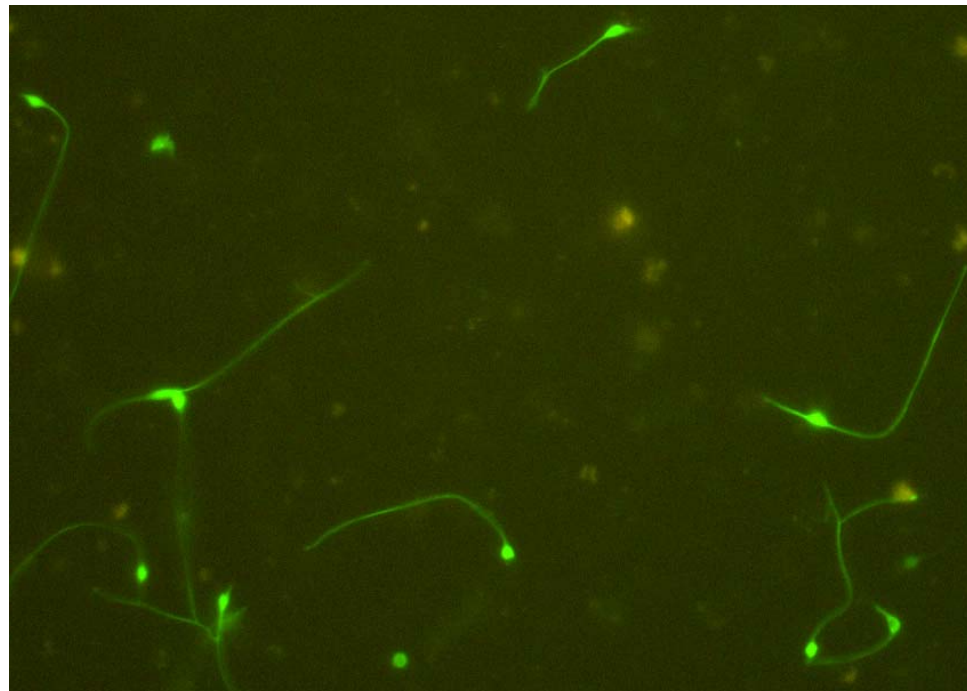


Alex Bounoutas

Cell Isolation



FLP neurons



Touch neurons

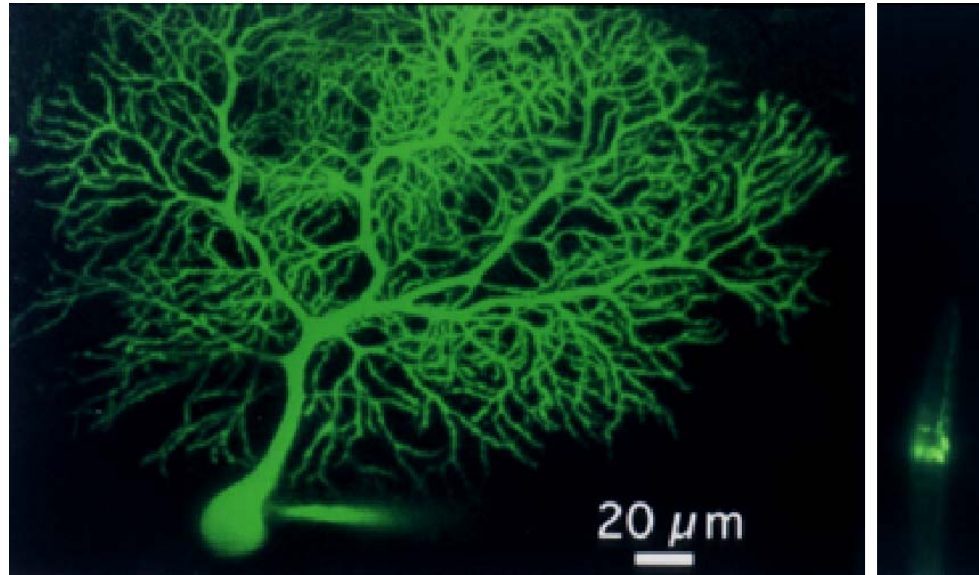


Irini Topalidou

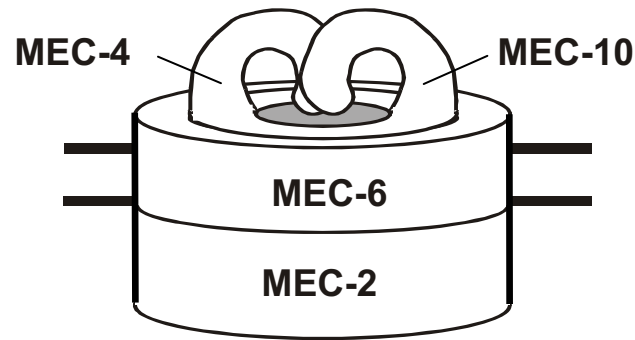
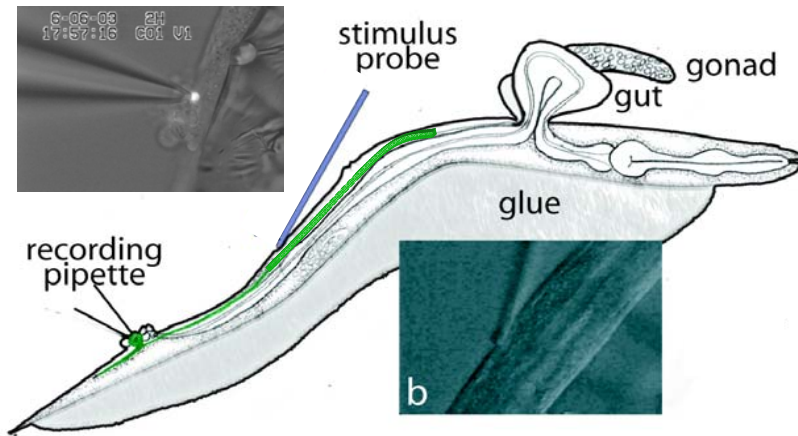


Yun Zhang

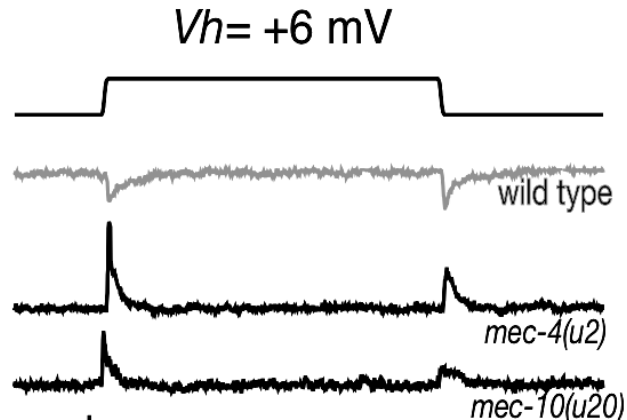
The Problem with *C. elegans* Electrophysiology



Cell-specific Electrophysiology

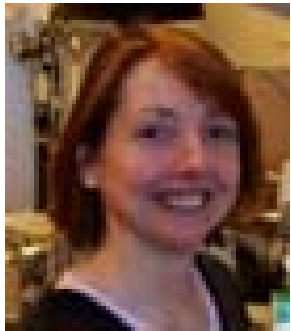
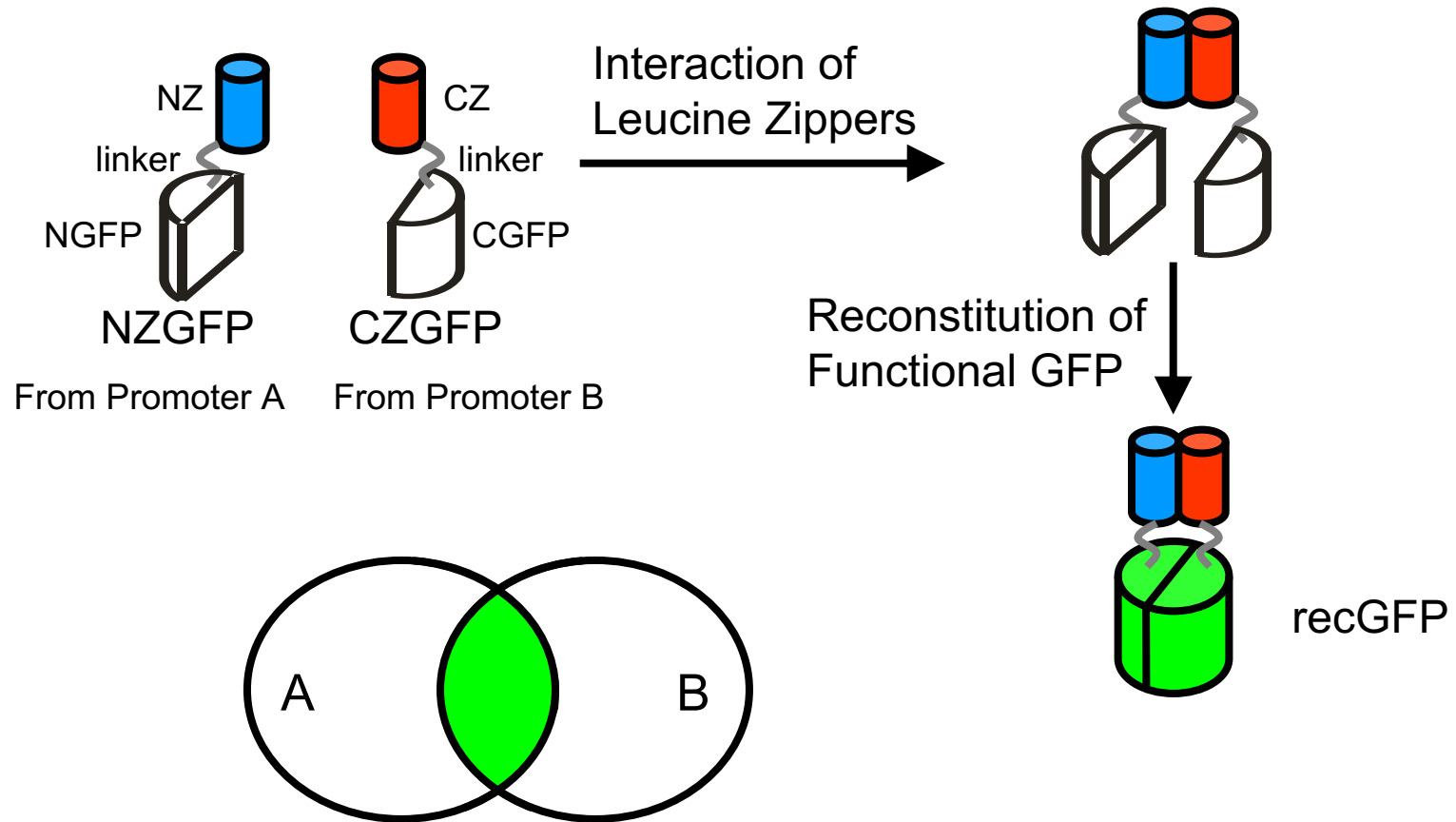


Bob O'Hagan



Miriam Goodman

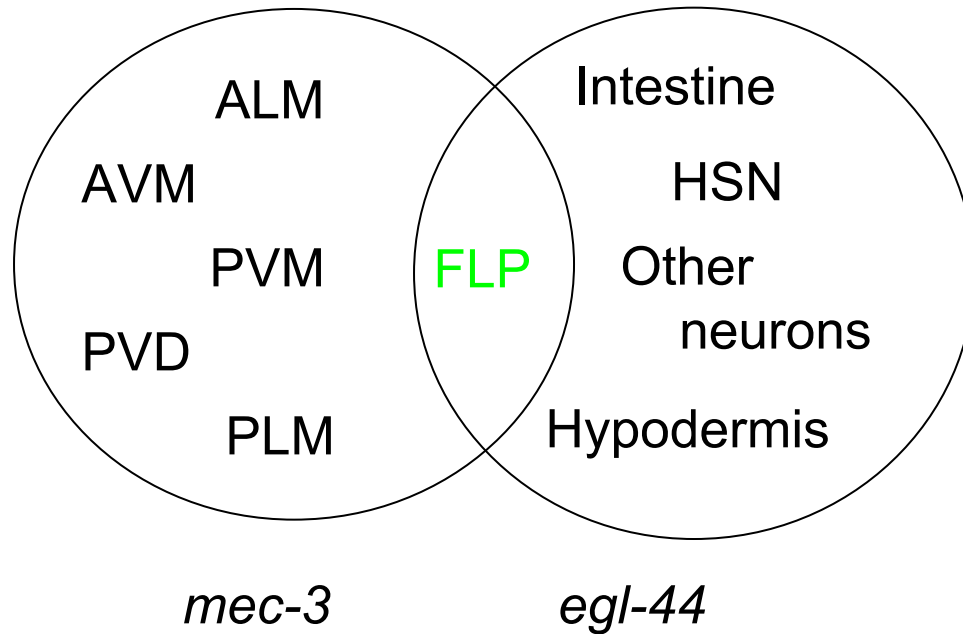
Non-covalent Reconstitution of GFP



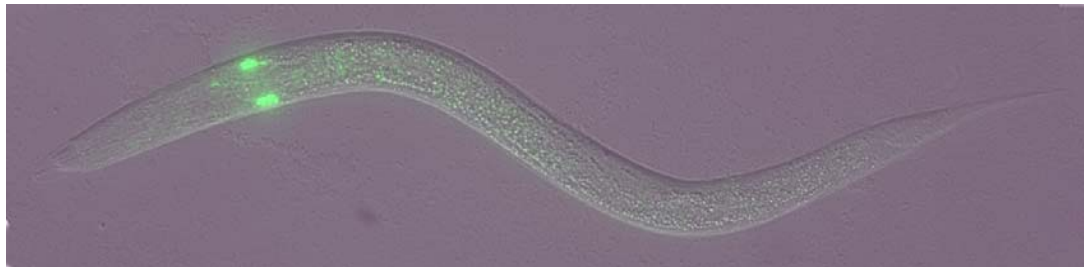
Lynne Regan

Indraneel Ghosh, Andrew D. Hamilton, and Lynne Regan (2000) Antiparallel Leucine Zipper-Directed Protein Reassembly: Application to the Green Fluorescent Protein. *J. Am. Chem. Soc.* **122**: 5658–5659.

Refining Cell Labeling



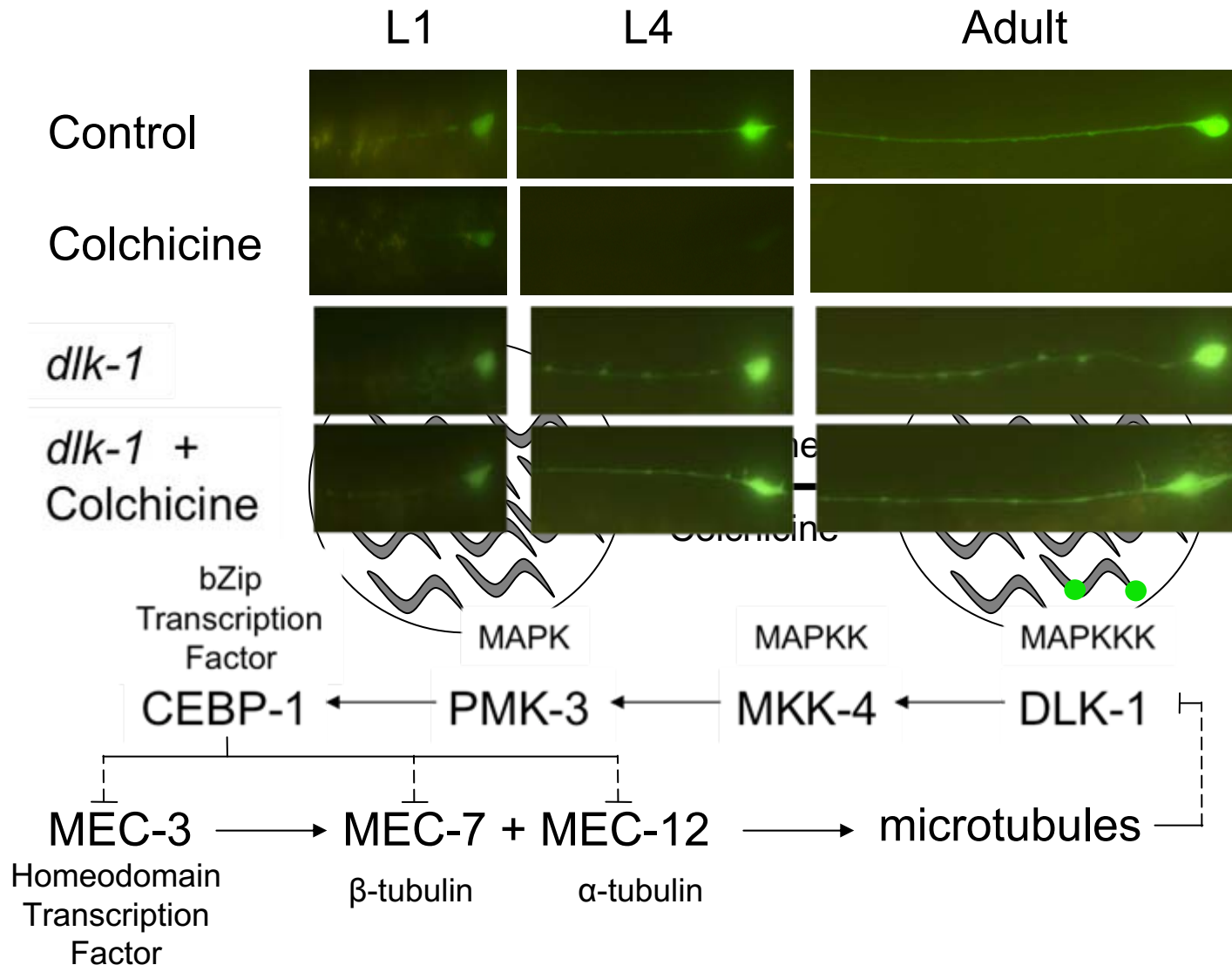
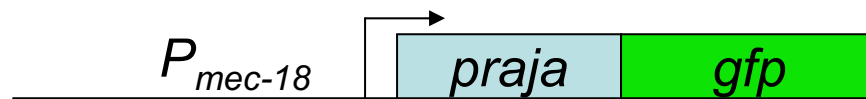
Shifang Zhang



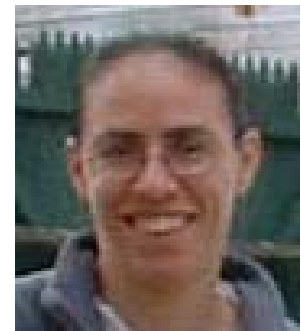
$P_{mec-3}nzgfp$ & $P_{egl-44}czgfp$



Chuck Ma



Chuck Ma



Leslie Emtage



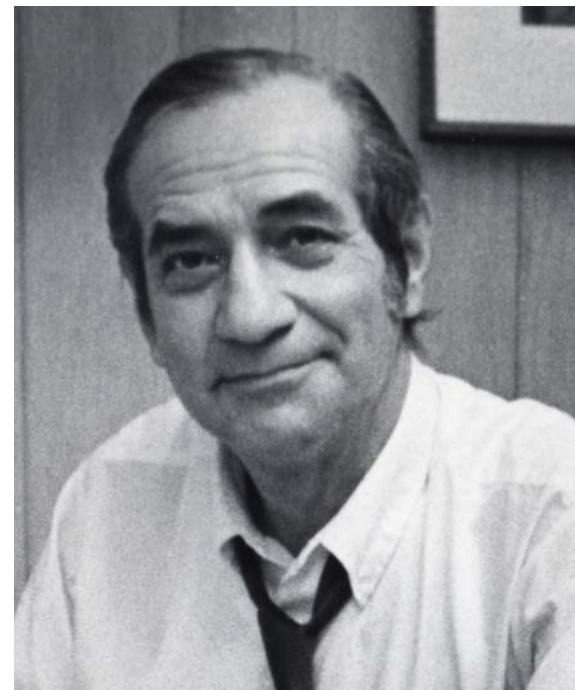
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