GFP: Lighting Up Life

You can observe a lot by watching.

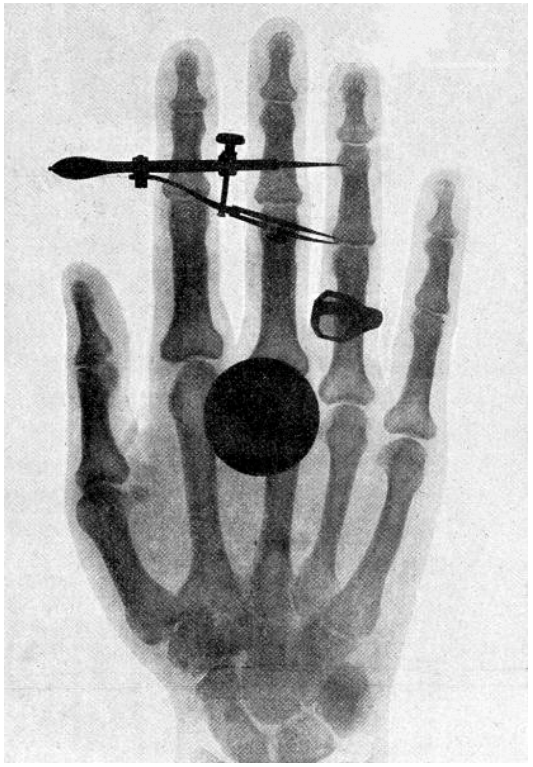
Yogi Berra

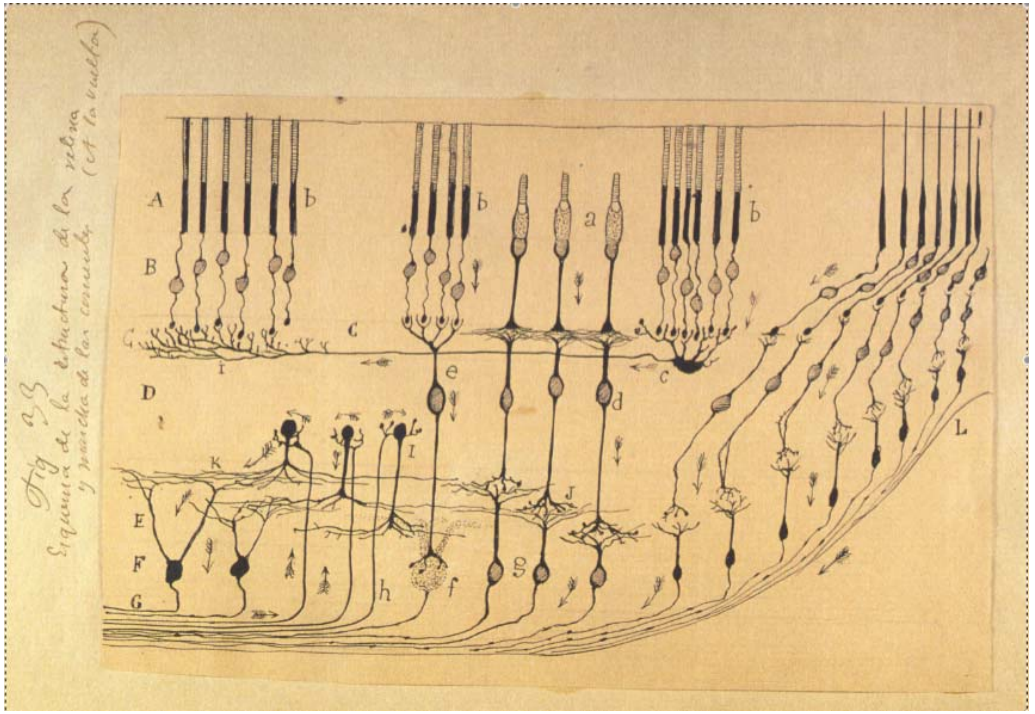
My companions and I then witnessed a curious spectacle. . .The Nautilus floated in the midst of. . . truly living light. . . an infinite agglomeration of colored. . . globules of diaphanous jelly. . .

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



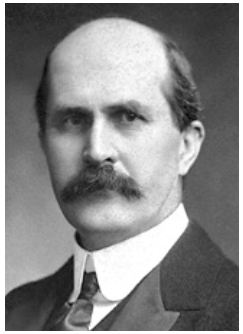


Wilhelm Röntgen

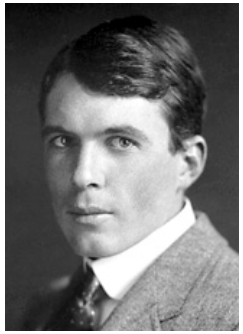
Camillo Golgi

Santiago Ramón y Cajal

X-ray Crystallography



William Bragg



Lawrence Bragg

Physics, 1915

Ultramicroscope

Richard Zsigmondy

Chemistry, 1925

Nuclear Magnetic Resonance

Felix Bloch

E. M. Purcell

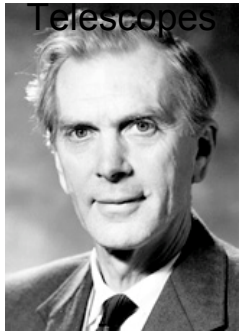
Physics, 1952

Phase Contrast Microscope

Frits Zernike

Physics, 1953

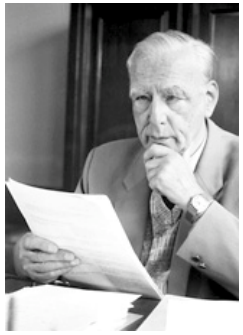
Large-Array Radio Telescopes



Martin Ryle

Physics, 1974

Electron Microscope



Ernst Ruska

Physics, 1986

Scanning Tunneling Microscope

Gerd Binnig

Heinrich Rohrer

Physics, 1986

Computer Assisted Tomography

Allan Cormack

Godfrey Hounsfield

Physiology or Medicine, 1979

Magnetic Resonance Imaging

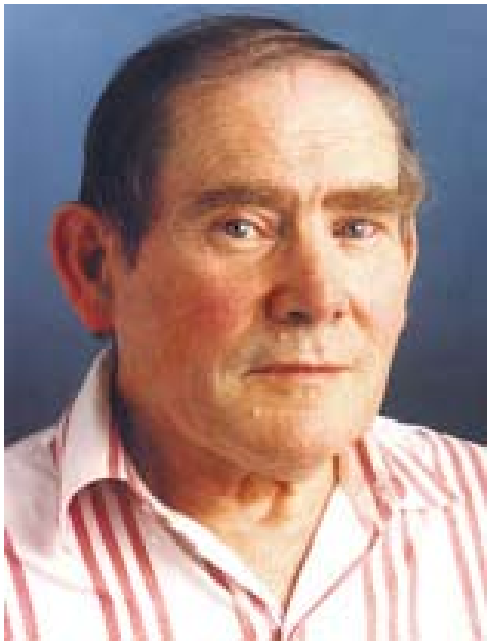
Paul Lauterbur

Peter Mansfield

Physiology or Medicine, 2003

José Zadunaisky

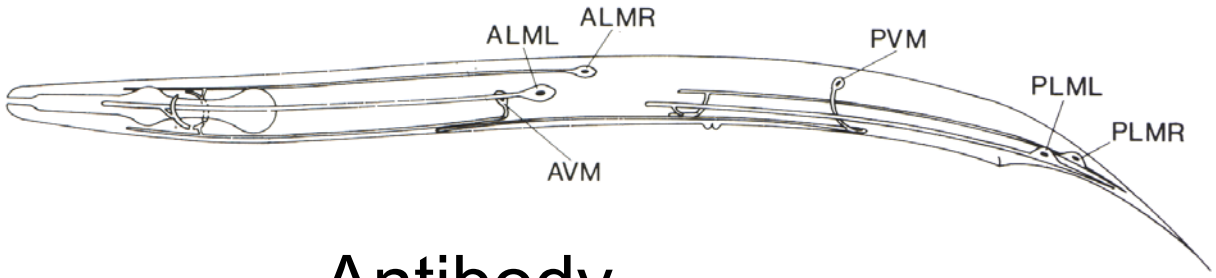
Bob Perlman



Sydney Brenner

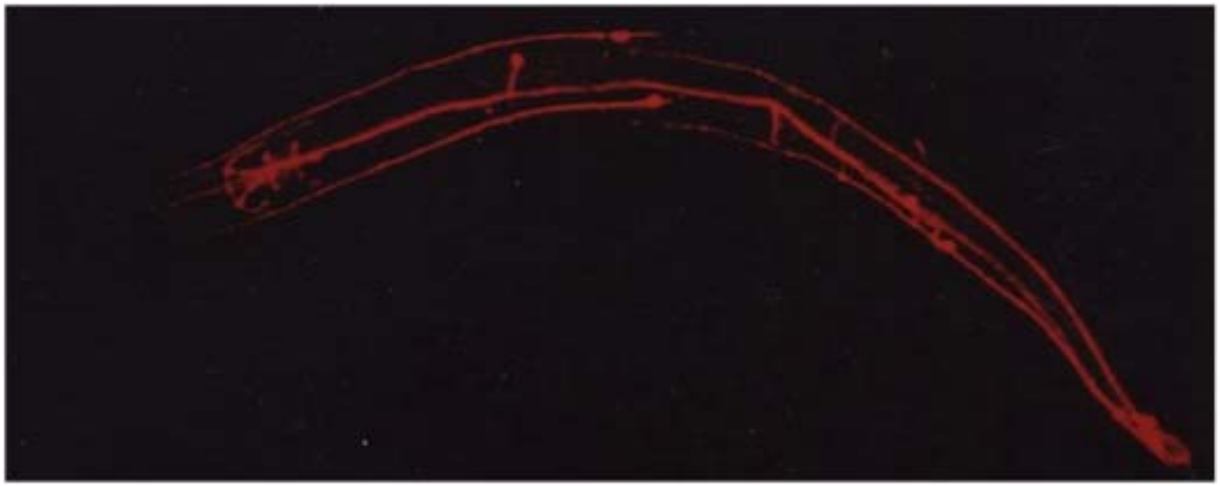
Sydney Brenner Bob Horvitz John Sulston

Caenorhabditis elegans



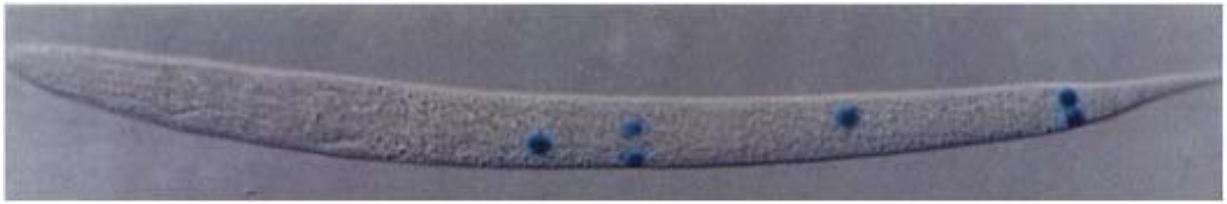
Antibody

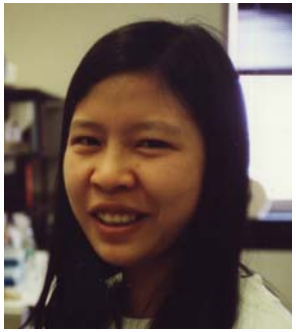
MEC-7



β-galactosidase Activity

mec-9

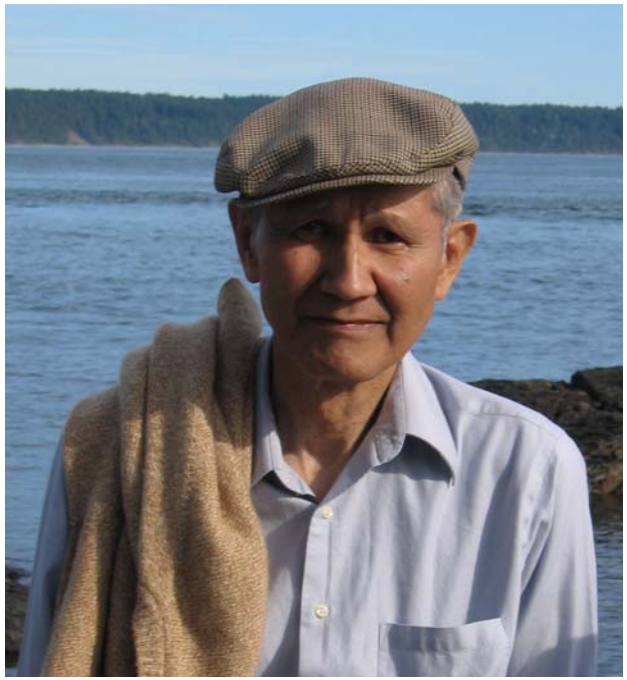




Hongping Du

Paul Brehm

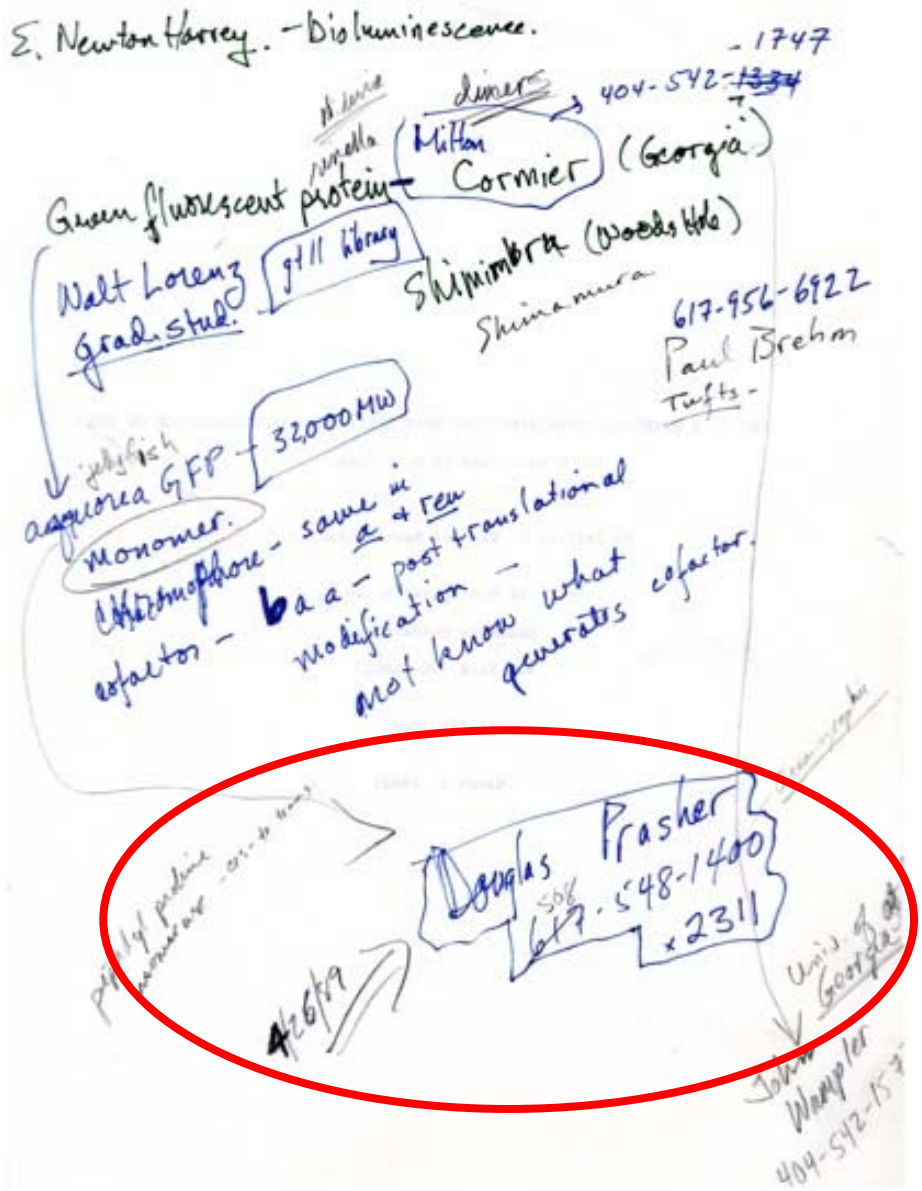
Aequorea victoria



Osamu Shimomura

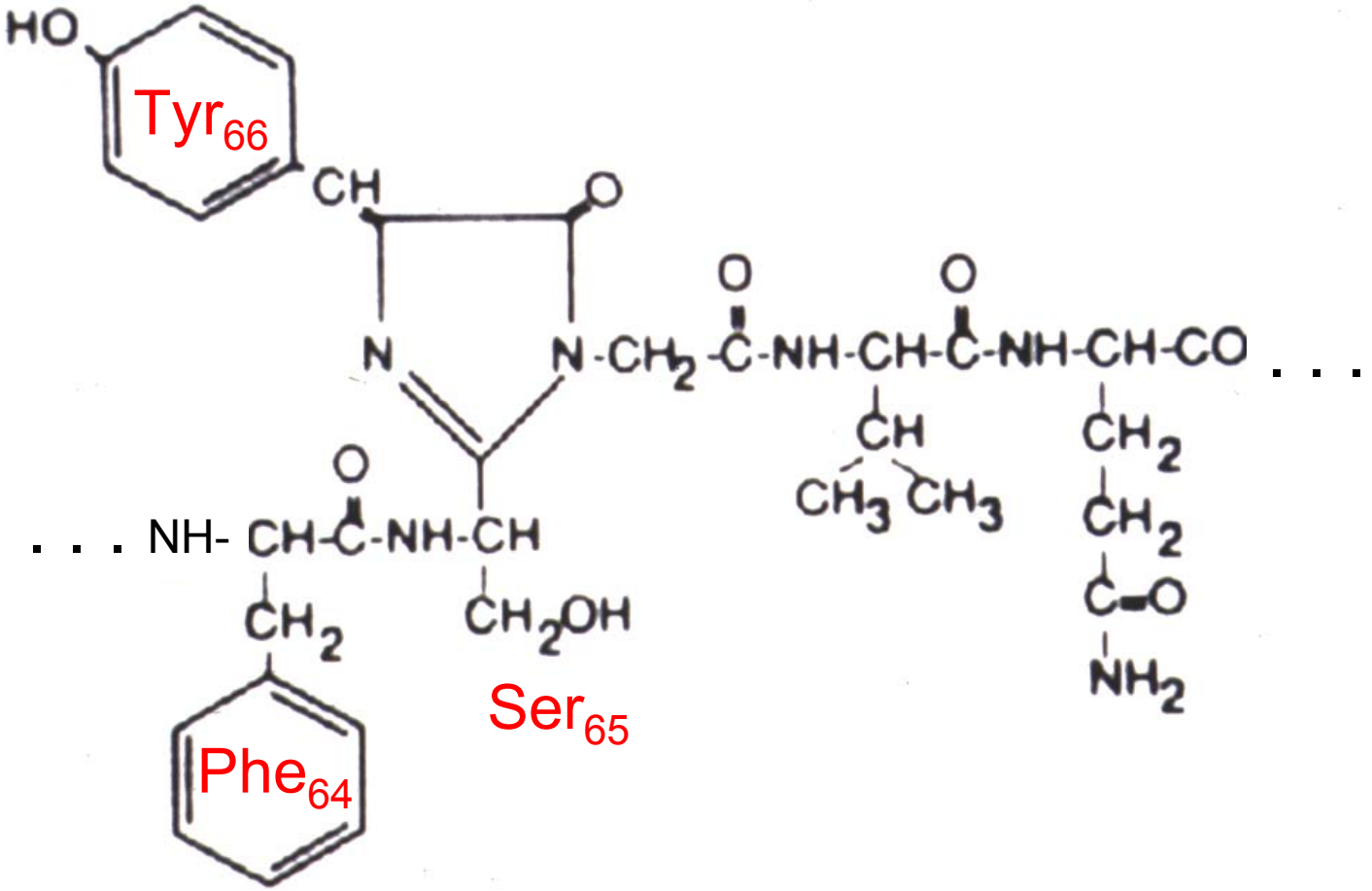
Aequorin + Ca^{++} ⟶ light

Aequorin + Ca^{++} + GFP ⟶ light



Douglas Prasher

The GFP Fluorophore



Gene, 111 (1992) 229–233

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]

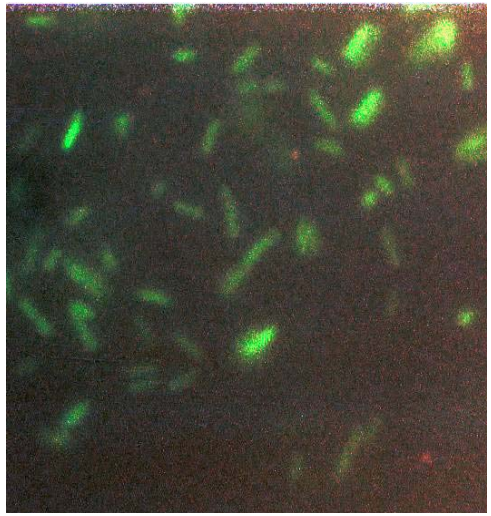
Correspondence to: Dr. D.C. Prasher, Redfield Bldg., Woods Hole Oceanographic Institution, Woods Hole, MA 02543 (U.S.A.)
Tel. (508)457-2000, ext. 2311; Fax (508)457-2195.

λGFP10

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

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

*Eco*RI *Eco*RI



Ghia Euskirchen

Tuesday 13. October 1992

— continued —

Fluorescence Microscopy

- Used 'scope from 368 Eng. Terrace lab with fluorescein block. — Also viewed by Dr. Chuck.
Viewed under oil immersion w/ 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=2hr (after induction) — fluorescing E. coli (strongly) fairly black field

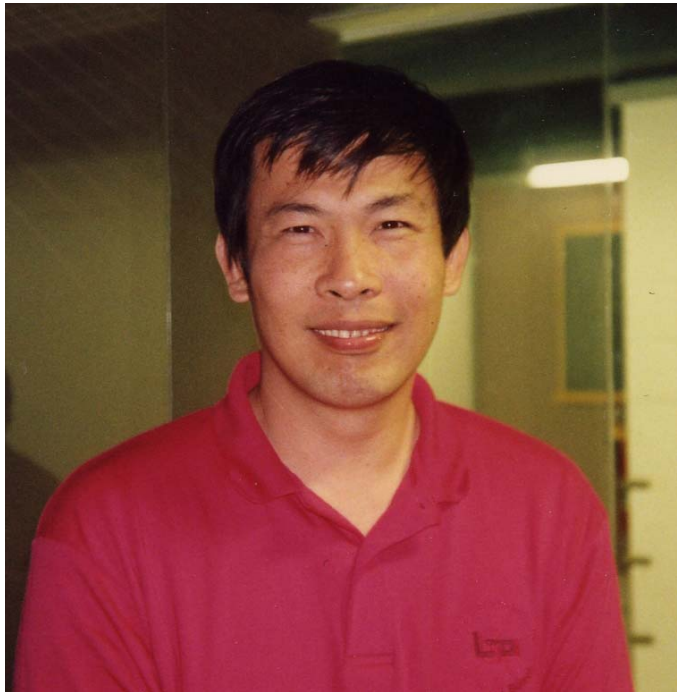
2 t = 0 hr (before induction) — weakly fluorescing E. coli fairly black field

2 t = 2 hr (after induction) — same as # 1 t = 2 hr

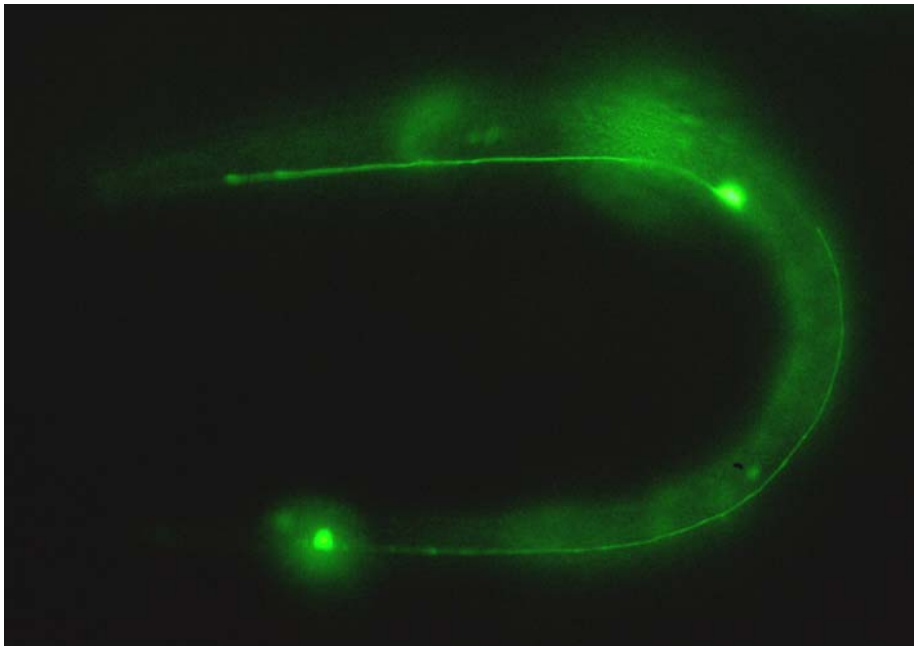
With Vroman's camera,
Kodak EKtar 100 ASA 35 mm
set on 100 ASA

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

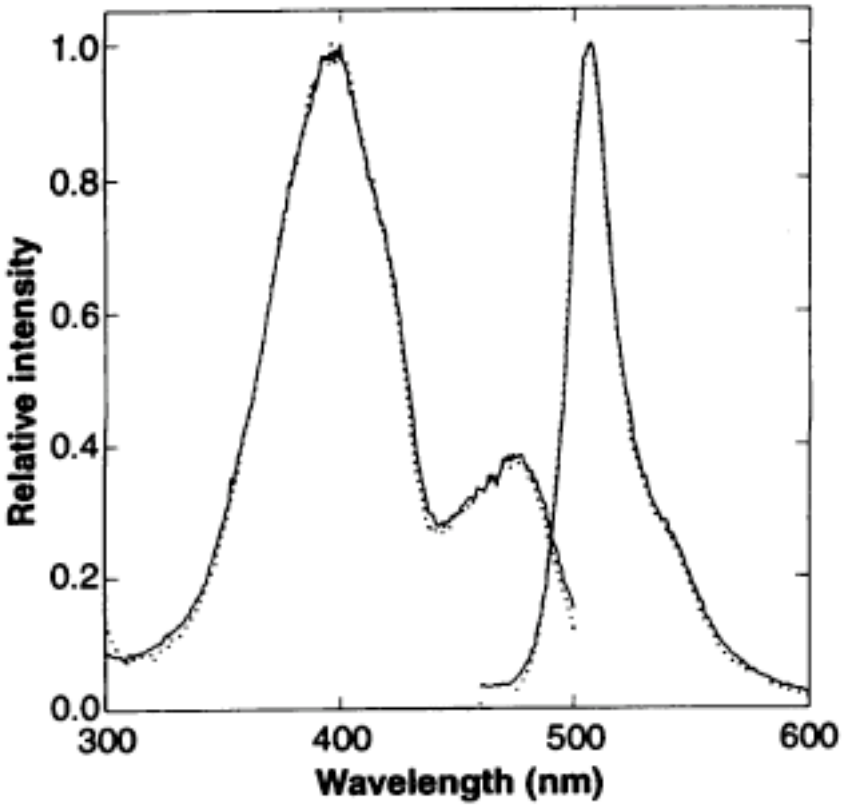
* For auto exposure time which was ~ 60 sec, cells had completely BLEACHED.



Yuan Tu



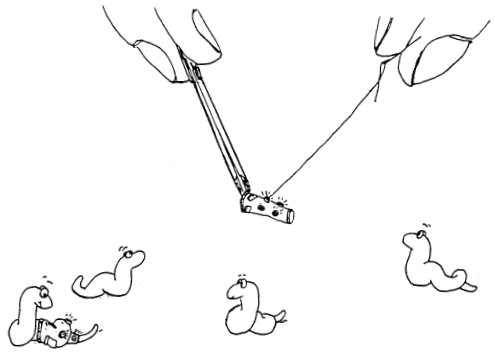
Bill Ward


1.0
0.8
0.6
0.4
0.2
0.0
Relative intensity
300
400
500
600
Wavelength (nm)

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, PLP, 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. The standard FITC filter sets provide the appropriate light. 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 pBluescript II KS (+) derivative (TU#65) containing a *Kpn* I - *Eco*R 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.

~~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

Tulle Hazelrigg

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

Shengxian Wang and Tulle Hazelrigg

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

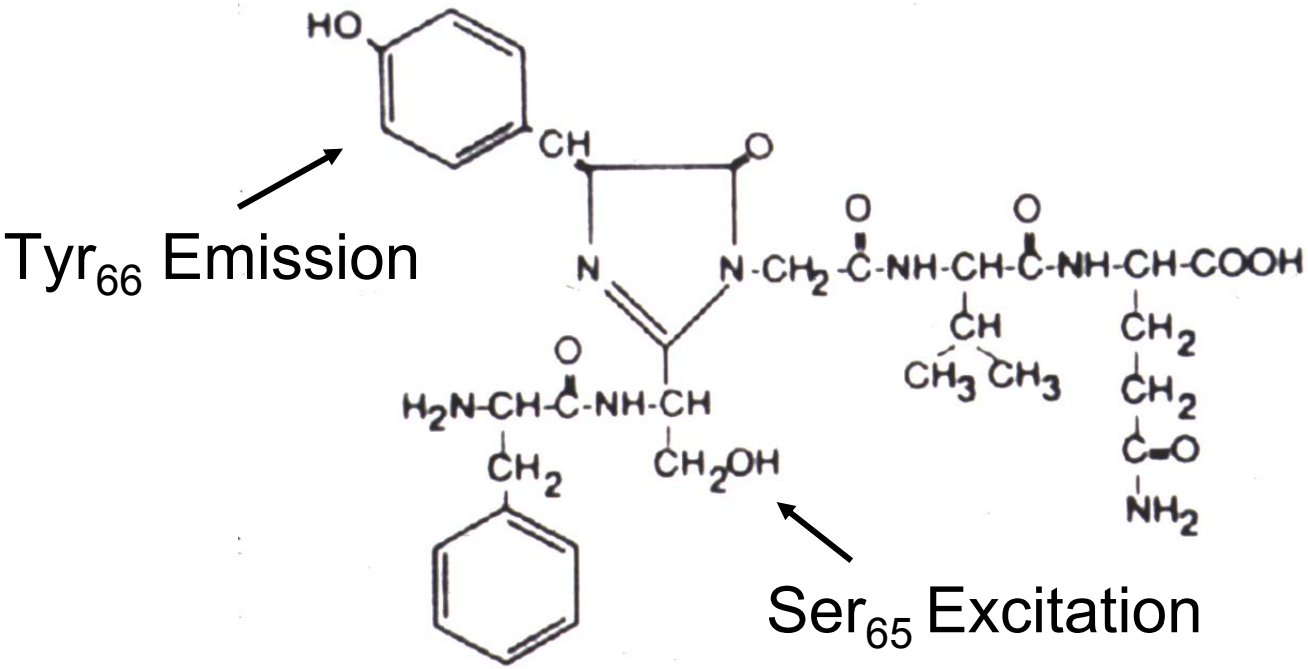
Shengxian Wang

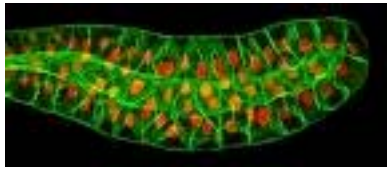
Advantages of GFP as a Biological Marker

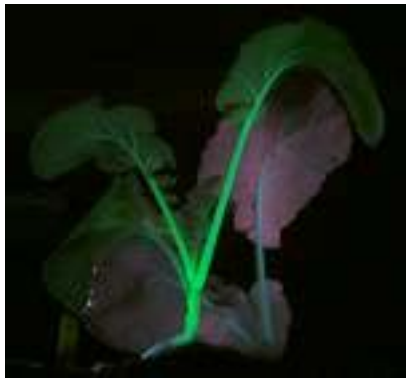
1. Heritable
2. Relatively Non-invasive
3. Small and Monomeric
4. Visible in Living Tissues

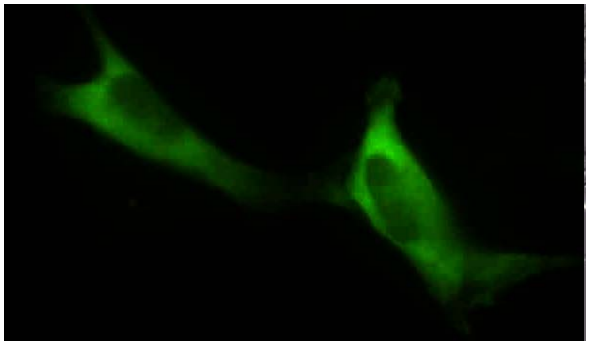
Improving GFP

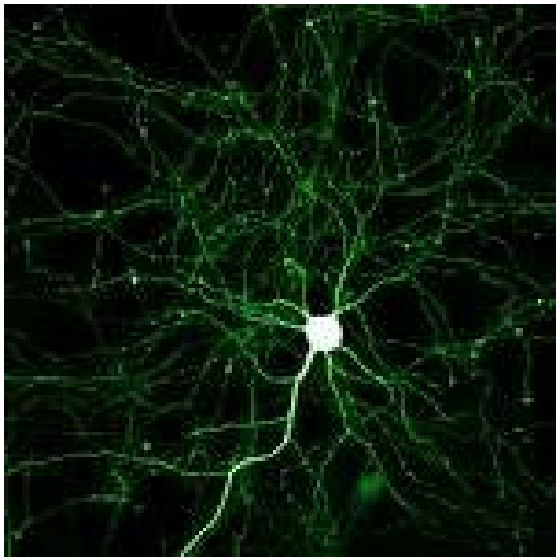
Roger Tsien

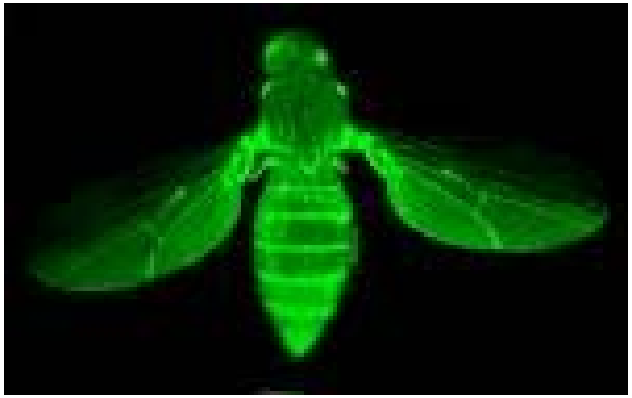


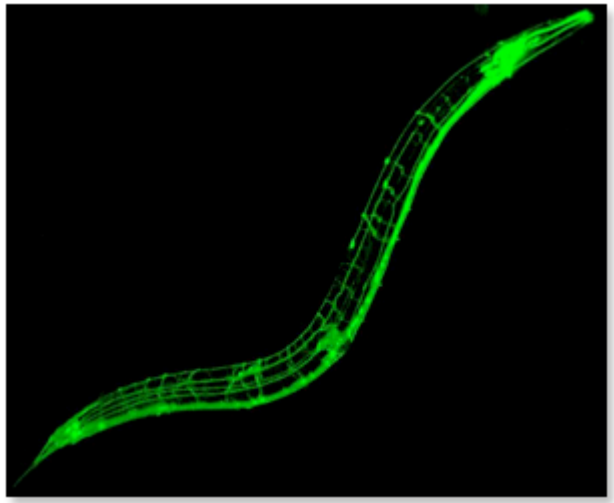




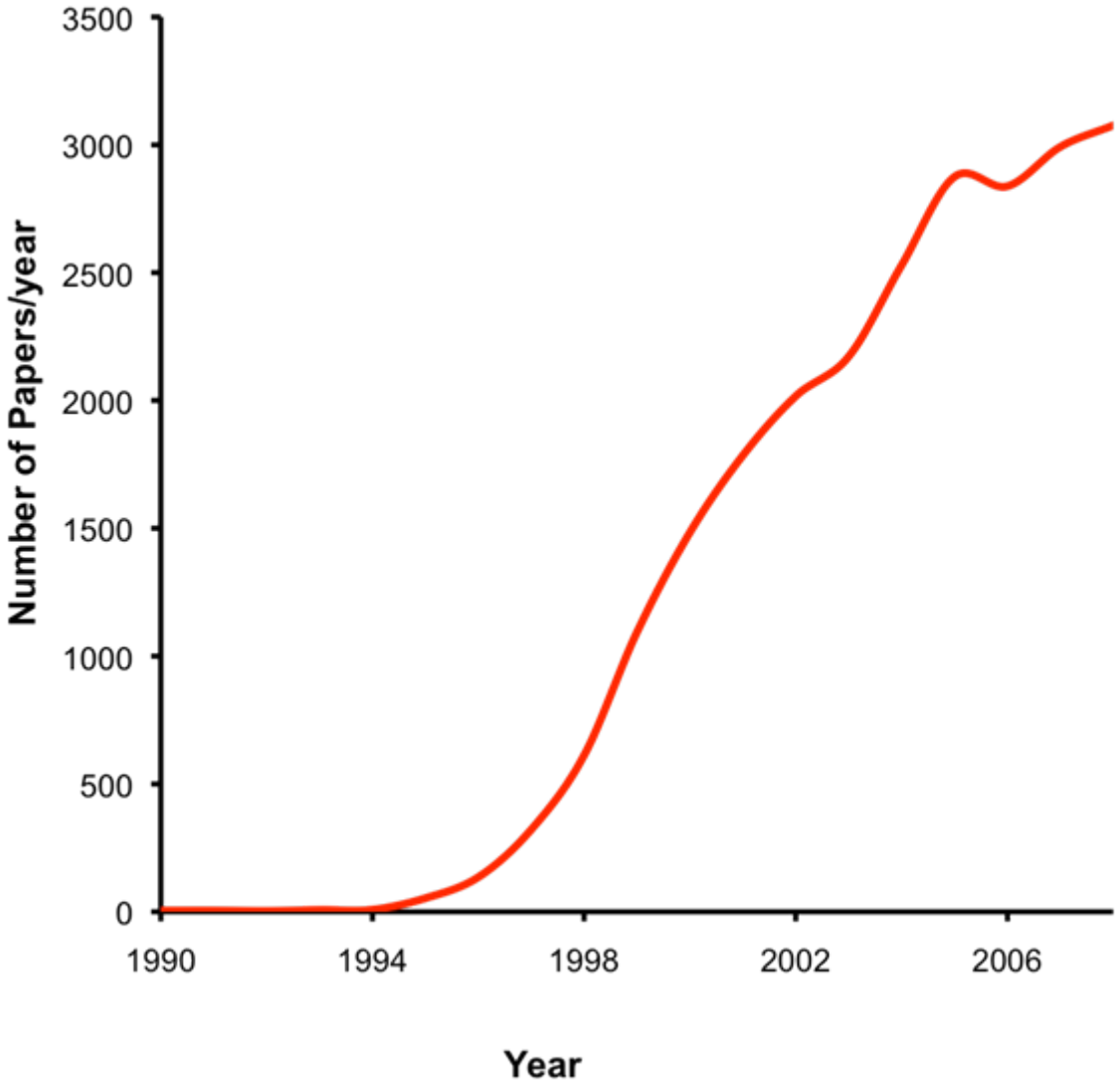








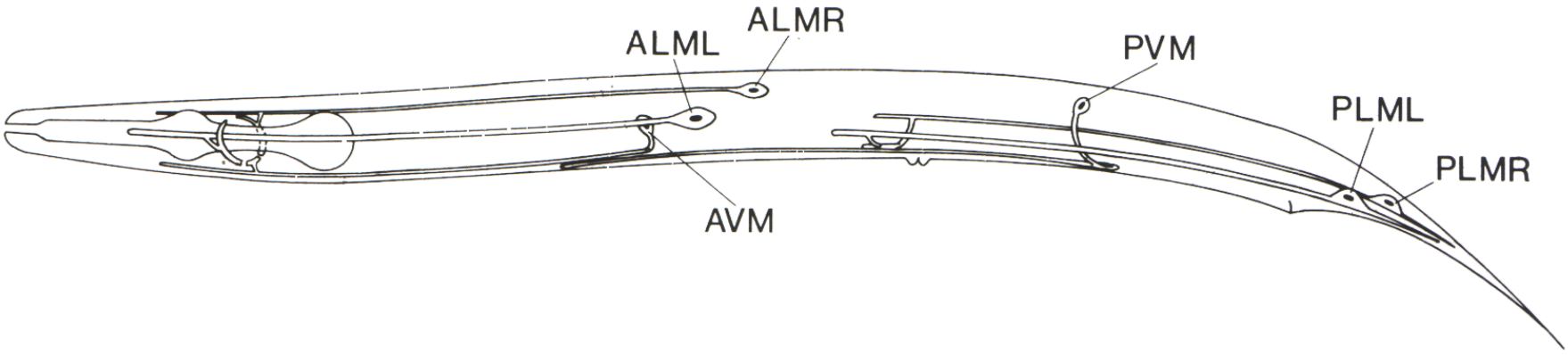
Papers Using Green Fluorescent Protein

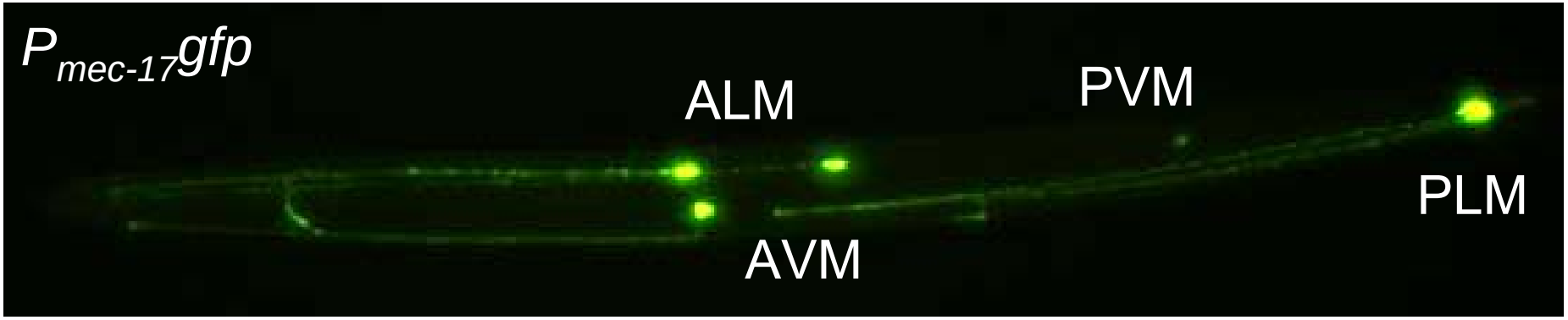


The First Human GFP Transgenic?

Ang Lee

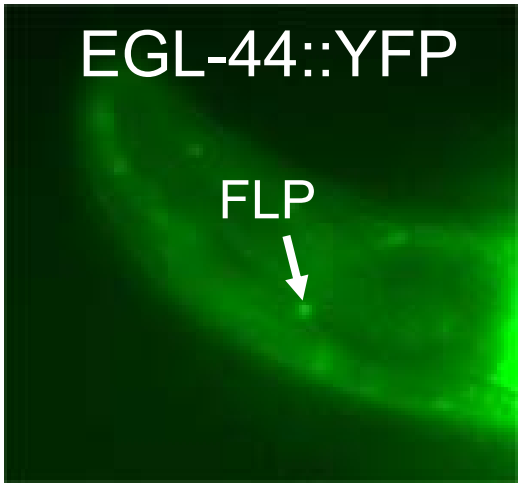
Gene Expression

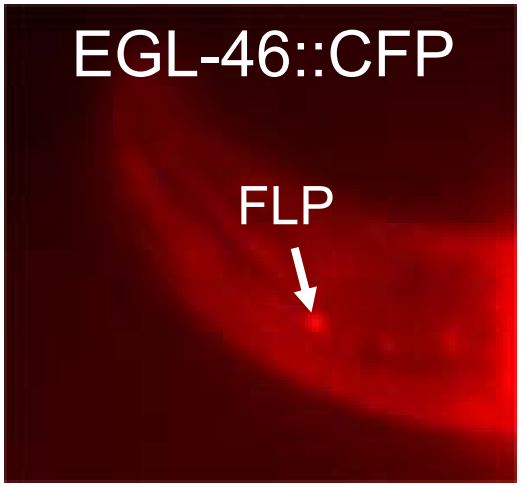


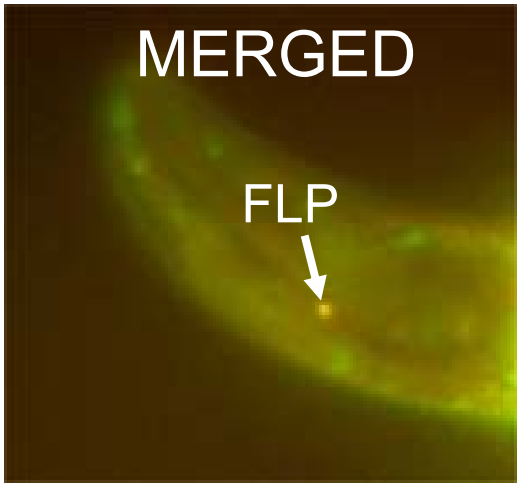


Yun Zhang

Co-expression

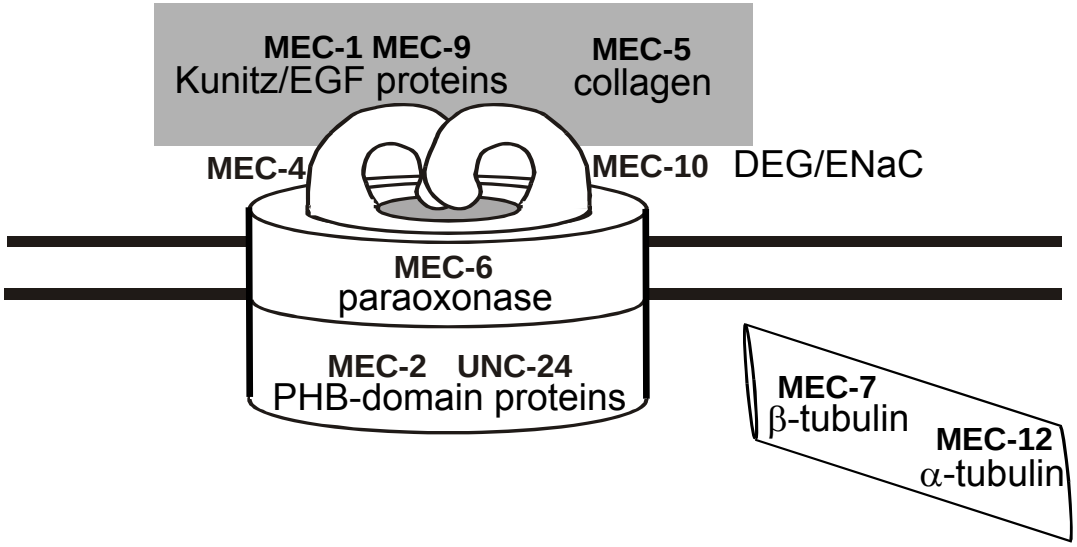






Ji Wu

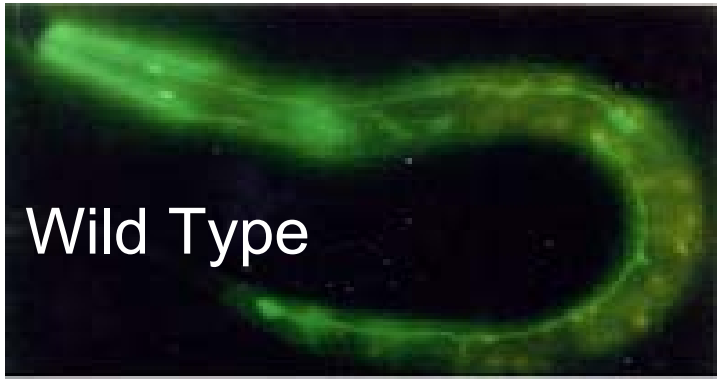
Protein Localization

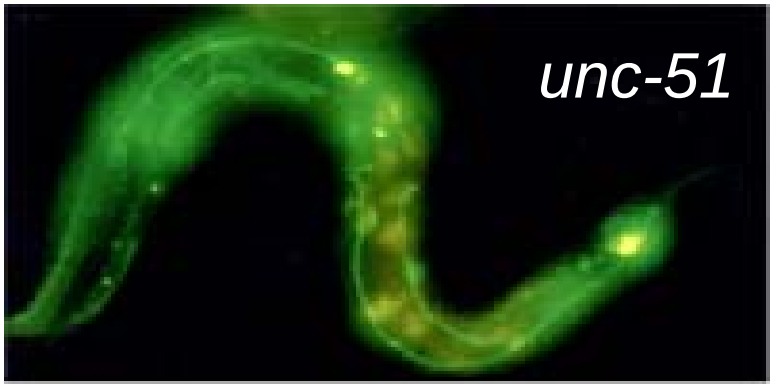


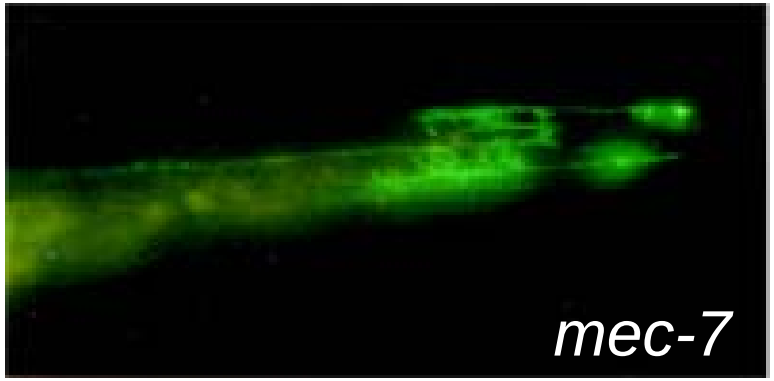
mec-4::yfp

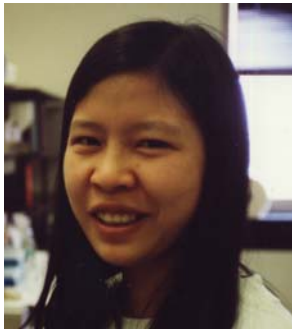
Dattananda Chelur

Mutant Screens and Characterizstion





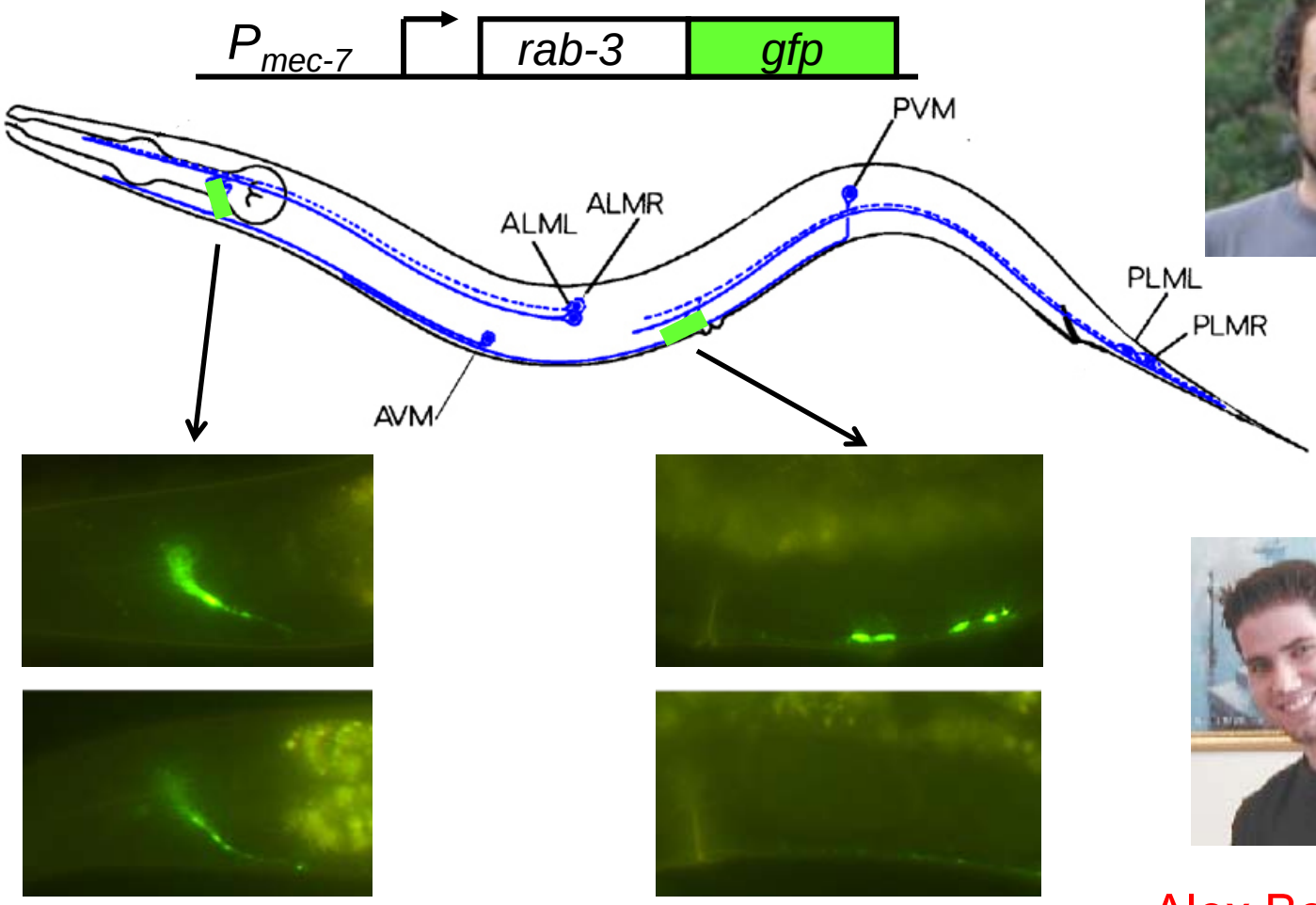




Hongping Du

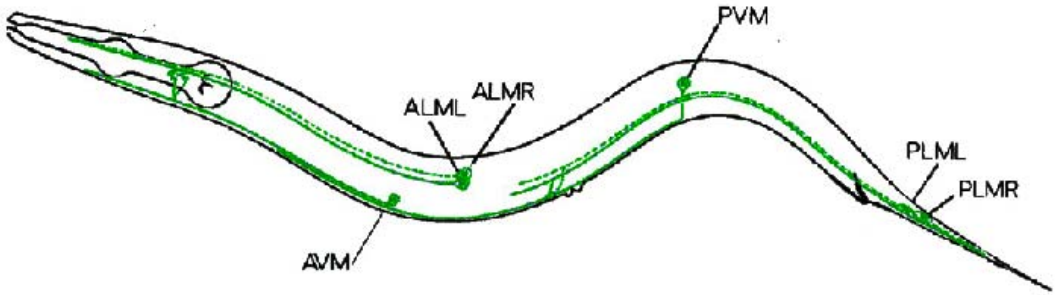
Visualizing Synapses

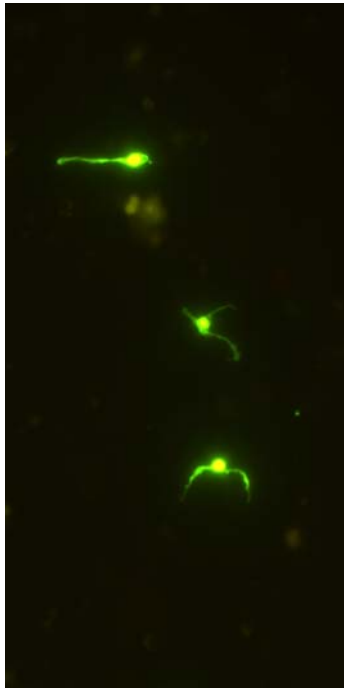
Mike Nonet



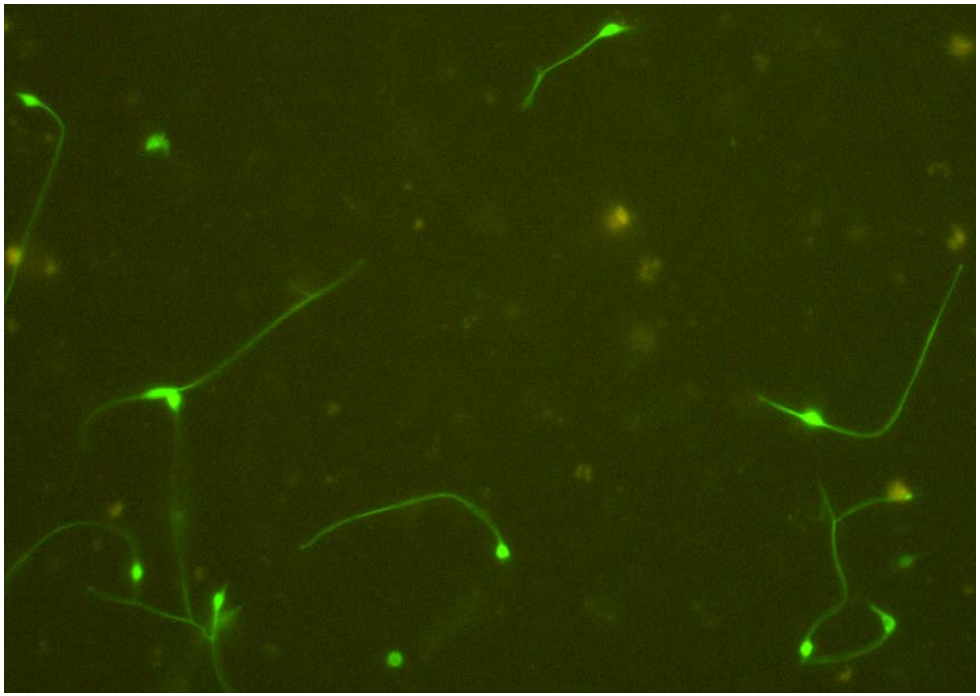
Alex Bounoutas

Cell Isolation





FLP neurons

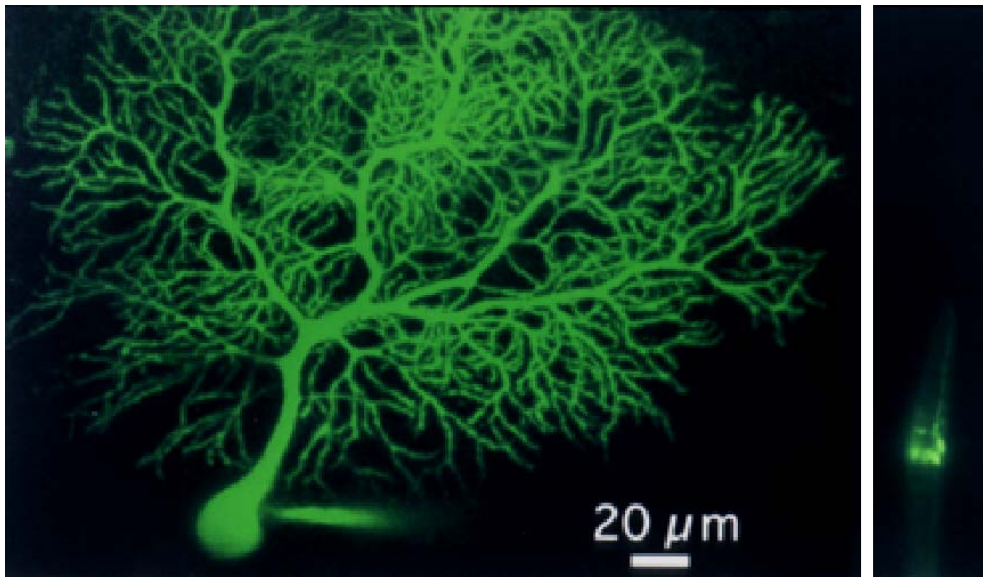


Touch neurons

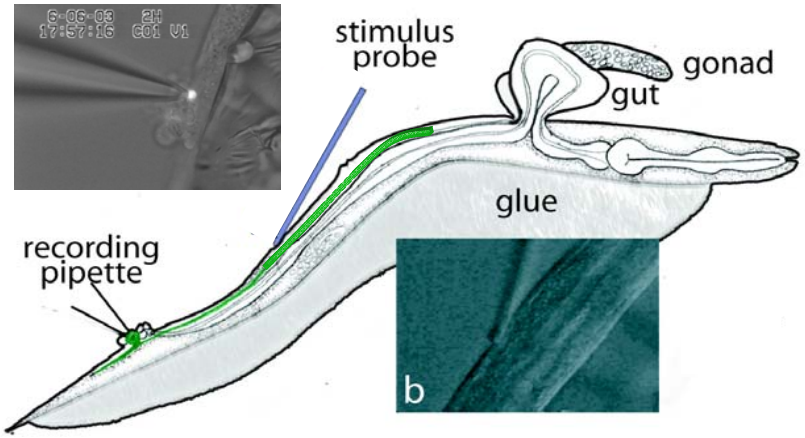
Irini Topalidou

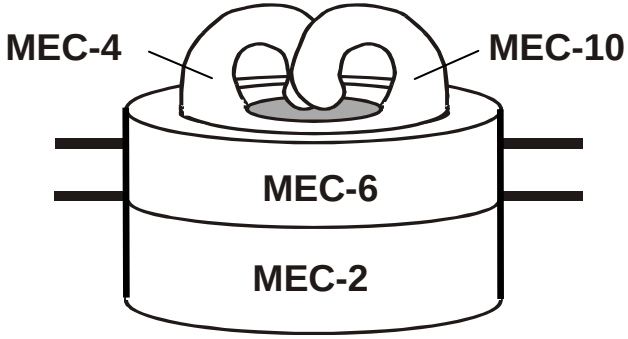
Yun Zhang

The Problem with *C. elegans* Electrophysiology

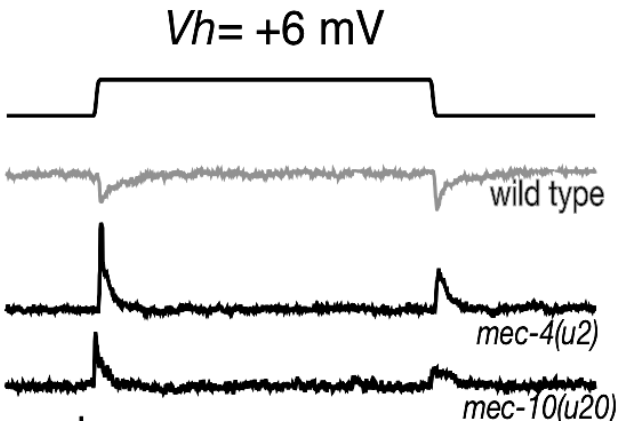


Cell-specific Electrophysiology



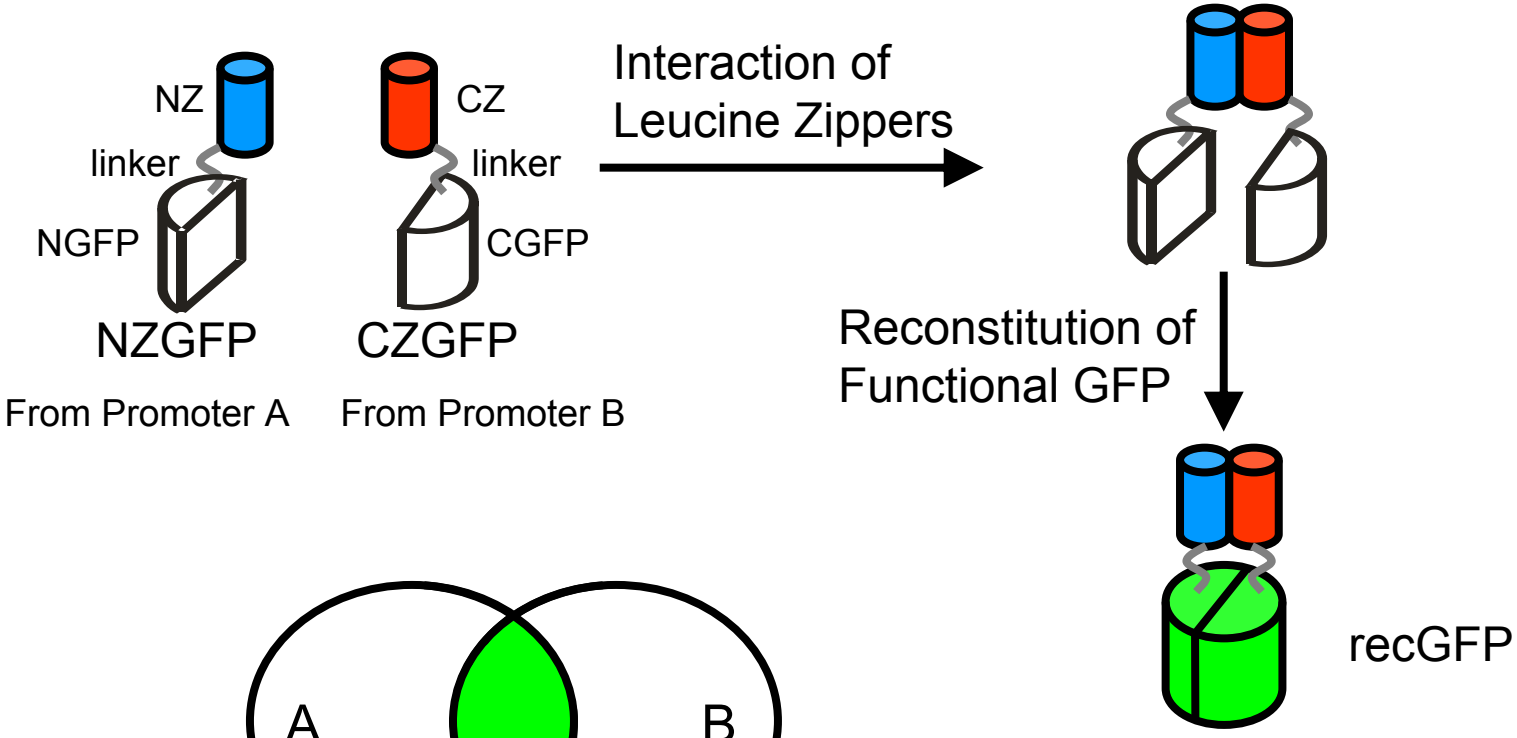


Bob O'Hagan



Miriam Goodman

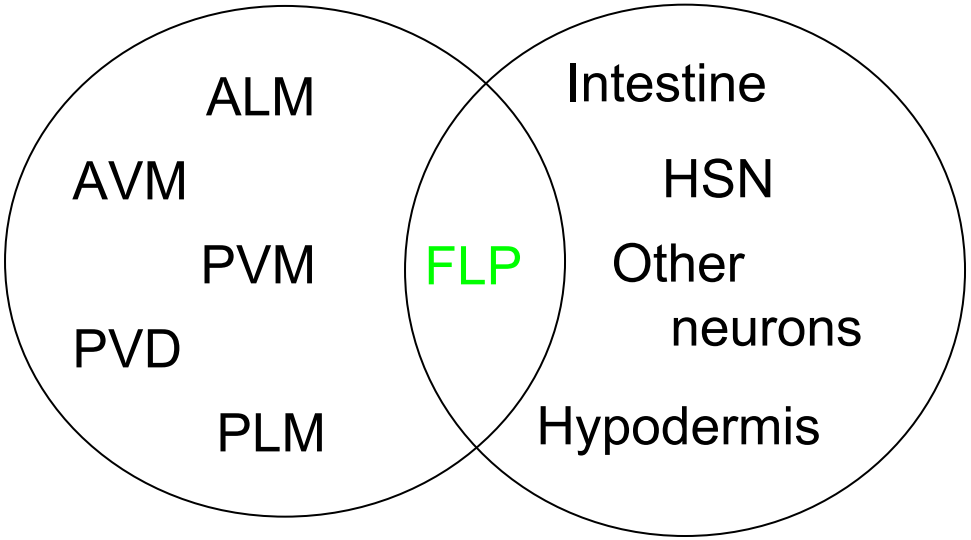
Non-covalent Reconstitution of GFP

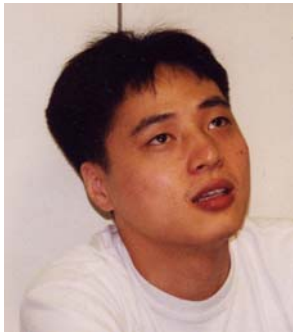


Lynne Regan

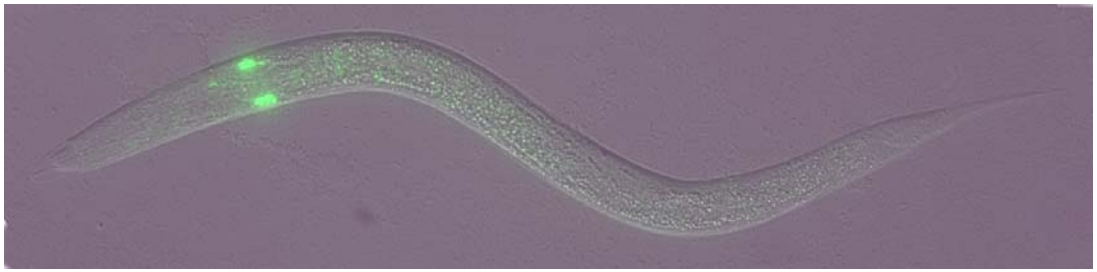
Indraneel Ghosh, Andrew D. Hamilton, and Lynne Regan (2000) Antiparallel Leucine Zipper-Directed Protein Reassembly: Application to the Green Fluorescent Protein. *J. Am. Chem. Soc.* **122:** 5658–5659.

Refining Cell Labeling



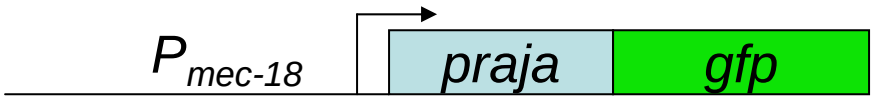


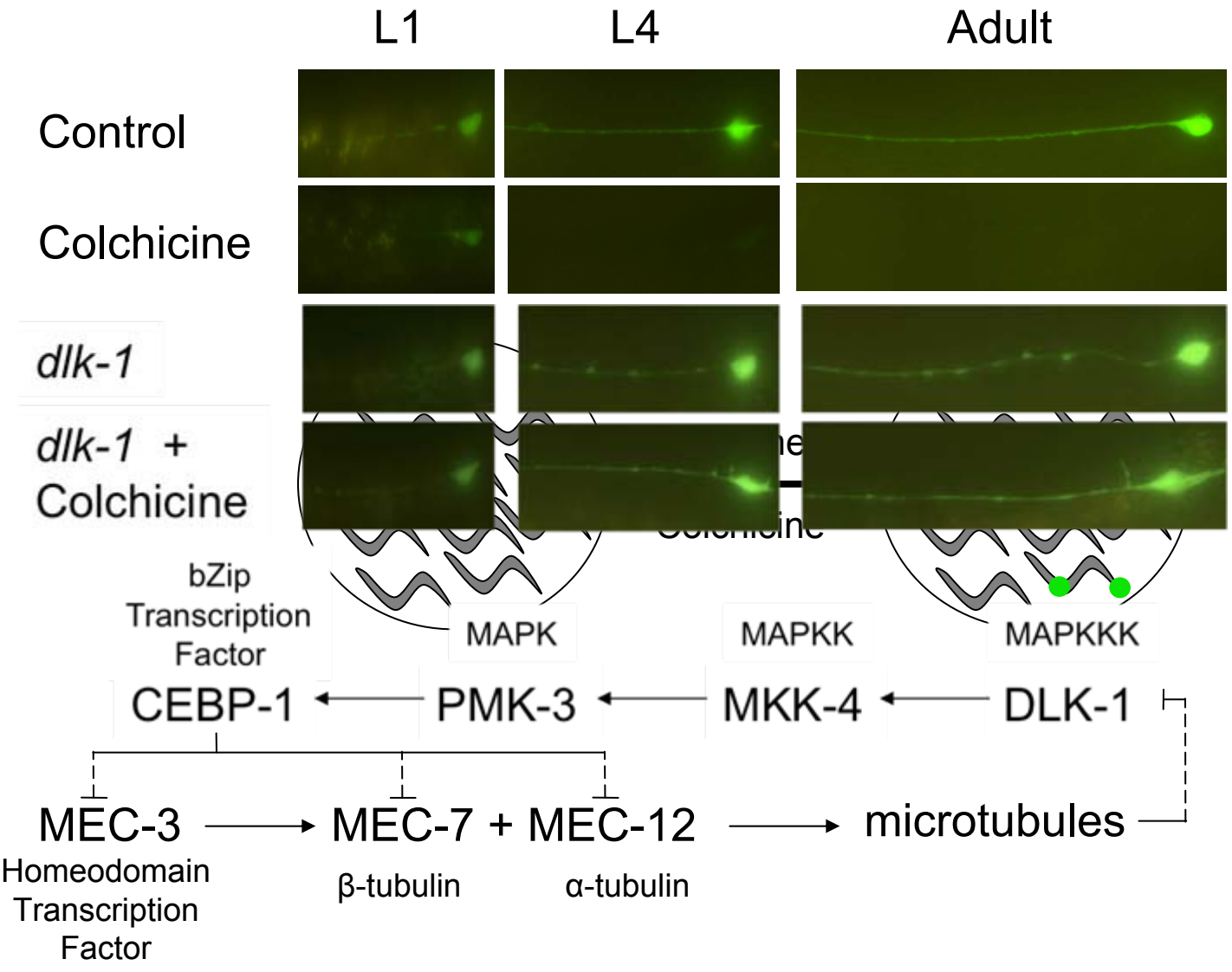
Shifang Zhang



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

Chuck Ma

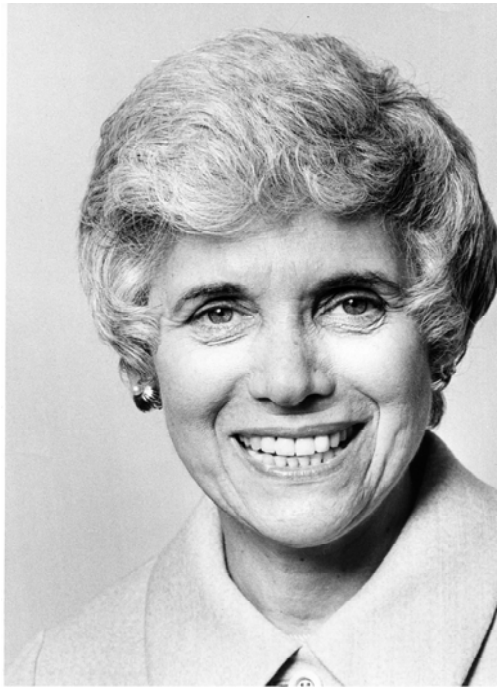




Chuck Ma

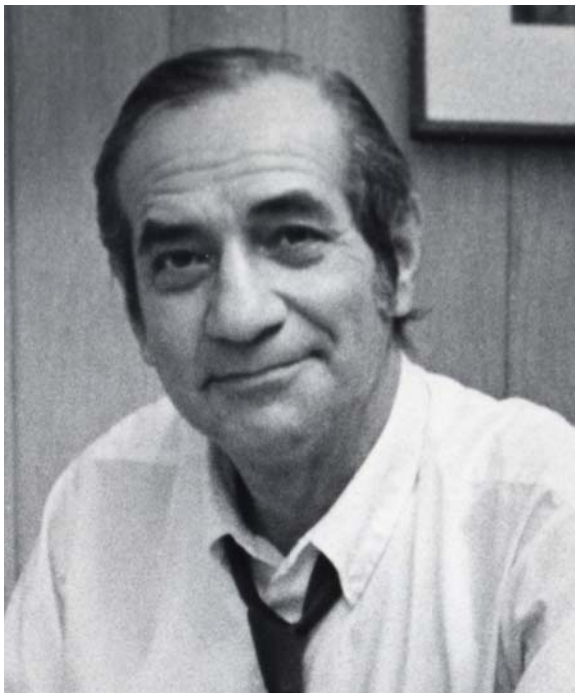
Leslie Emtage

Alex Bounoutas



Vivian Chalfie

Madeline Friedlen



Eli Chalfie

Postdoctoral Scientists

Anahita Amiri
Guy Caldwell
Lucinda Carnell
Anne Chambers
Dattananda Chelur
Brian Coblitz
Monica Driscoll
Chip Ferguson
Nikolaos George
Miriam Goodman
Jang Hee Hahn
Chris Li
Charles Ma
Chris Martin
Shohei Mitani
Erich Schwarz
Anne Toker
Irini Topalidou
Millet Treinin
Bill Walthall
Jeff Way
Eve Wolinsky

Graduate Students

Alex Bounoutas
Andrea Calixto
Xiaoyin Chen
Yushu Chen
Hongping Du
Anne Duggan
Lesley Emtage
Glen Ernstrom
Ghia Euskirchen
Jaime García-Añoveros
Guoqiang Gu
Mingxia Huang
Siavash Karimzadegan
Charles Keller
John Kratz
Joy Liang
Shujun Luo
Cathy Savage
Jim Taub
Ji Wu
Yi-Chun Wu
Ding Xue
Shifang Zhang
Yun Zhang

Undergraduates

Macy Au
Paul Auwaeter
Peggy Brickman
Kate Brauman
John Byun
Alex Chang
Victor Chang
Iris Chin
Bradley Collins
Michael Dreyfuss
Angela Georgopoulos
Rachel Goldstein
Judith Green
Peter Homer
Rafaz Hoque
James Hudspeth
Shari Jawetz
Paul Josephson
Eric Kanter
Lexy Kovach
Joe Lau
Sam Lee
Lorraine Lothringer
David Meshoulam
Jeremy Mindich
Mariya Rosenblit
Julie Rosenthal
Aaron Scheffler
Shai Shaham
Jay Srinivasan
Leslie Vosshall
Molly Weiner
Chris William

Technicians

Margaret Barnes
Elke Bergholz
Lei Chen
Ellen Dean
Nora Hom
Maud Kinnell
Connie Mauoka
Evelyn Reilly
Yuan Tu
Yingzi Xue
Andrea Yao

Faculty Visitors

Christian Neri
Jonathan Rothblatt