You can observe a lot by watching.

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
Ultramicroscope by Richard Zsigmondy, Chemistry, 1925

Phase Contrast Microscope by Frits Zernike, Physics, 1953

Large-Array Radio Telescopes by Martin Ryle, Physics, 1974

X-ray Crystallography by William Bragg, Physics, 1915

Electron Microscope by Ernst Ruska, Physics, 1986

Scanning Tunneling Microscope by Heinrich Rohrer, Physics, 1986

Computer Assisted Tomography by Allan Cormack, Physiology or Medicine, 1979

Magnetic Resonance Imaging by Paul Lauterbur and Peter Mansfield, Physiology or Medicine, 2003
Sydney Brenner  Bob Horvitz  John Sulston

*Caenorhabditis elegans*
β-galactosidase Activity

Antibody

MEC-7

β-galactosidase Activity

mec-9

Hongping Du
Aequorin + Ca\textsuperscript{++}

Aequorea victoria

Osamu Shimomura

\[\text{Aequorin} + \text{Ca}^{++} \rightarrow \text{light}\]

\[\text{Aequorin} + \text{Ca}^{++} + \text{GFP} \rightarrow \text{light}\]
Douglas Prasher
The GFP Fluorophore

\[ \text{Phe}_{64} \text{Tyr}_{66} \ldots \text{NH}^- \]

\[ \ldots \text{NH}^- \text{CH} \cdot \text{CH} \cdot \text{CH} \cdot \text{CH} \cdot \text{CH} \cdot \text{CO} \ldots \]

\[ \text{Phe}_{64} \text{Ser}_{65} \]
Primary structure of the *Aequorea victoria* green-fluorescent protein

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

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

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

- Used ‘scope from 368’ Eng. Terrace lab with
  fluorescent block. - Also viewed by Ding & Chuck.
Viewed under oil immersion of 100x objective.

E. coli from Ding untreated
no auto fluorescence
could be seen although the
field had a strong greenish cast

# 1 t=2 hr (after induction)
fluorescent 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 Woman’s camera,
Kodak Ektar 100 ASA 35 mm
set on 100 ASA

1st group of exposures were the untreated E. coli from Ding
2nd group ∼ # 30 were # 2 t = 2 hr
3rd group (few?) were # 2 t = 0 hr

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

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-7::gfp 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 (ultr). Strong expression is seen in the four embryonic touch cells (the ALM and PVM cells) in ultr animals. Even the terminal branches of these neurons can be followed. Other cells also fluoresce, but less strongly (RDV, PDP, a few cells in the tail, and the AVN and FMN touch cells). Two additional cells in the tail also show fairly strong fluorescence; by the projection of their processes, these appear to be the ALM cells. The staining of the ALM, AVN and FMN (but not to as great an extent in the PVM cells) was dependent on mec-7. These results are consistent with the previous expression pattern produced by this promoter (Hasegawa et al., EMBO J. 11, 2685 (1992); McKiernan et al., Development, in press) and seem to be equal to our most sensitive method (antibody staining). (The ALM and PVM cells are often displaced anteriorly in ultr animals, but not in the strain; 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 at 395 nm (the fluorescence bleaches, but slowly; there is recovery from photobleaching at both wavelengths), we have tended to use the higher-wavelength light. The standard FITC filter sets provide the best wavelength to use. However, refinements can be made. For example, we find that a high-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 have not seen any improvement. We haven't yet looked at cirl-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 of the lamp is at 370 nm with the xenon 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 for 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 so changes in expression, e.g., through cell division); 2) to examine the outgrowth and migration of cells in situ; 3) to look for mutations that change the pattern of expression (e.g., looking for revertants of the degeneration-causing sec-4(e1621) mutation by mutating a mec-gfp; sec-4(e1621) double and looking for the reappearance of fluorescence :=(1); 4) to back-cross cells for subsequent isolation and study (in e.g., work we have to do soon with Shawn Locke - 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 ph useful K ES (+) derivative (T6865) containing a Apse I - Bgl I fragment encoding GFP with an Apse I site 5' to the translation start and a Bam I site at the translation codon (suggested by Andy Fire) and gfp versions (T6860, T6861) of the four C. elegans lacZ expression vectors (pGPa6, 43, pGPa3, 39, pGPa2, 0, and pGPa2, 1, respectively) described by Fire et al., Gene 93. If you are interested in obtaining any of these clones, please write to a befox or email your request (include your FAX number, we would like to know what you are interested in). The above should be faxed to Marty Chalfie and he will send you the necessary Columbia papers to sign (they can be returned by FAX) and we will try to send out the clones immediately.

The Worm Breeder's Gazette

The Worm Jean Sequins Project

Volume 13 No. 1
October 1, 1993
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
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

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
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

Tyr_{66} Emission

Ser_{65} Excitation
Papers Using Green Fluorescent Protein

![Graph showing the number of papers using green fluorescent protein over the years from 1990 to 2006. The number of papers increases significantly from 1990 to 2006.]
The First Human GFP Transgenic?

Ang Lee
Gene Expression

$P_{mec-17\text{gfp}}$

Yun Zhang
Co-expression

EGL-44::YFP

EGL-46::CFP

MERGED

FLP

FLP

FLP

Ji Wu
Protein Localization

mec-4::yfp

Dattananda Chelur
Mutant Screens and Characterization

Wild Type

unc-51

mec-7

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

Refining Cell Labeling

- ALM
- AVM
- PVM
- PVD
- PLM
- Intestine
- HSN
- Other neurons
- Hypodermis

mec-3  egl-44

$P_{mec-3n zgfp} \& P_{egl-44c zgfp}$

Shifang Zhang

Chuck Ma
**Gene Expression Analysis**

- **Control**
- **Colchicine**
- **dlk-1**
- **dlk-1 + Colchicine**

**Gene Expression Patterns**

- **L1**
- **L4**
- **Adult**

**Gene Networks**

- **CEBP-1**
- **PMK-3**
- **M KK-4**
- **DLK-1**

**Molecular Components**

- **MEC-3**
- **MEC-7 + MEC-12**
- **β-tubulin**
- **α-tubulin**
- **μ-tubulin**

**Transcription Factors**

- **Homeodomain Transcription Factor**
- **bZip Transcription Factor**

**Signaling Pathways**

- **MAPK**
- **MAPKK**
- **MAPKKK**

**Plots**

- **P_mec-18**
- **pra ja gfp**

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