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
Sydney Brenner  Bob Horvitz  John Sulston

Caenorhabditis elegans
Paul Brehm  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

\[
\begin{align*}
\text{Phe}_{64} & \quad \text{Tyr}_{66} \\
\text{Ser}_{65} & \quad \ldots \quad \text{NH-} \\
\end{align*}
\]
Primary structure of the *Aequorea victoria* green-fluorescent protein

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

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--- continued ---

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 could be seen although the field had a strong greenish cast.

- 1 t=2hr (induction) fluorescent E. coli (strongly)
- 2 t=0 hr (induction) weakly fluorescent E. coli fairly black field
- 2 t=2 hr (after) same as 2 t=2 hr

With Women’s camera,
Kodak Ektar 100 ASA 35mm
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 (see 1+) 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 (ultra). Strong expression is seen in the four embryonic touch cells (the ALM and PHM cells) in ultra animals. Even the terminal branches of these neurons can be followed. Other cells also fluoresce, but less strongly (BDI, FLP, a few cells in the tail, and the AVN and PMV 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 PMV (but not to as great an extent in the PHM cells) was dependent on mec-7. These results are consistent with the previous expression pattern produced by this promoter (Hamburger et al., BBDO J. 11, 2885 (1992); Mclain et al., Development, press) and seem to be equal to our most sensitive method (antibody staining). (The ALM and PHM cells are often displaced anteriorly in ultra 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 507 nm with a shoulder at 530 nm. Because we found that 395 nm light causes a very rapid photobleaching at 470 nm (the fluorescence bleaches, but slowly; there is recovery from photobleaching at both wavelengths), we have tended to use the higher wavelength (470 nm). The standard FITC filter sets provide three wavelengths. For example, we find that appropriate light. However, refinements can be made. For example, we can use at the 470 nm, which would presumably help eliminate the aspects of the autofluorescence from using a xenon rather than a mercury lamp for fluorescence (the output dia at 470 nm with the xenon lamp, but not with the xenon lamp). We have not yet tried high-intensity-light side filters (the autoexcitation 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 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(e121) mutation by mutating a mec-gfp; mec-4(e121) double and looking for the reappearance of fluorescence); 4) to make cells for subsequent isolation and study (e.g. at the same time, or even by to become with Shawn Lockery - who suspected 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 recombination. These are a plasmid or R E derivative (T0459) containing a Ape I - Eco R fragment encoding GFP with an Ape I site S' to the translation and a Bam H site at the termination codon (suggested by Andy Fire) and gfp versions (T0450 - T0453) of the four C. elegans J ac expression vectors (pPD1643, pPD1644, pPD1645, and pPD22.1, respectively) described by Fire et al., Gene 93. If you are interested in obtaining any of these clones, please write (or fax or email) your request (include your FASTAG number; we'd like to know what you are interested in) to Marty Chalfie and he will fax you the necessary Columbia papers to sign (they can be returned by FedEx) and we will try to send out the clones immediately.

The Worm Breeder's Gazette

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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
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

Sarah Chalfie
Implications for \( bcd \) mRNA localization from spatial distribution of \( exu \) protein in \textit{Drosophila} oogenesis

\textit{Nature} \textbf{369}: 400-403, 1994

Shengxian Wang and Tulle Hazelrigg
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

$P_{mec-17gfp}$

Yun Zhang
Co-expression

EGL-44::YFP

EGL-46::CFP

MERGED

Ji Wu
Protein Localization

- MEC-1, MEC-9: Kunitz/EGF proteins
- MEC-5: collagen
- MEC-6: paraoxonase
- MEC-2, UNC-24: PHB-domain proteins
- MEC-7, MEC-12: tubulins

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
The Problem with *C. elegans* Electrophysiology
Cell-specific Electrophysiology

- stimulus probe
- gut
- recording pipette
- glue
- MEC-4
- MEC-6
- MEC-10
- MEC-2

V\text{h}= +6 \text{ mV}

- wild type
- mec-4(u2)
- mec-10(u20)
Non-covalent Reconstitution of GFP

Refining Cell Labeling

mec-3 egl-44

FLP

Intestine
HSN
Other neurons
Hypodermis

ALM
AVM
PVM
PVD
PLM

mec-3

egf-44

Shifang Zhang

\[ P_{mec-3}nzgfp \quad \& \quad P_{egf-44}czgfp \]
The diagram illustrates the expression of the praja-gfp transgene under the control of the P_mec-18 promoter in different developmental stages (L1, L4, Adult) of C. elegans. The expression is compared between Control, Colchicine, and Colchicine + dlk-1 treatments. The diagram also shows the regulatory network involving transcription factors MEC-3, MEC-7 + MEC-12, and microtubules, as well as the involvement of the Homeodomain Transcription Factor and β- and α-tubulin. The diagram includes a pathway indicating the role of DLK-1, MKK-4, PMK-3, and CEBP-1 in the MAPK cascade.
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