sample is extracted from the ampoule and cleaned of the surrounding excess impregnant by standard mechanical polishing techniques. In this way, we prepared nanowires of various metals (In, Sn, and Al) and semiconductors (Se, Te, GaSb, and Bi$_2$Te$_3$) (Fig. 2).

The nanowire composites create substantial electric field patterns over the sample surface. We used a scanning probe microscope to measure electric fields at the surface of a nanocomposite. In a NanoScope (Digital Instruments, Santa Barbara, California) scanning force microscope, the sample is mounted with conductive epoxy to a metal holder and is held at a few volts relative to a conductive cantilever tip that is grounded. The metal-coated, etched, single-crystal silicon tip has a radius of curvature of about 5 nm. The tip is set to oscillate at a frequency near its resonance frequency (78 kHz). When the cantilever encounters a vertical electric field gradient, the effective spring constant is modified, shifting its resonance frequency. By recording the amplitude of the cantilever oscillations while scanning the sample surface, we obtain an image that reveals the strength of the electric force gradient (13, 14).

The image, however, may also contain topographical information; it is difficult to separate the two effects. This is circumvented by taking measurements in two passes over each scan line (15). On the first pass, a topographical image (Fig. 3A) is taken with the cantilever tapping the surface, and the information is stored in memory. On the second pass, the tip is lifted to a selected separation between the tip and local surface topography (typically 20 to 200 nm), such that the tip does not touch the surface. By using the stored topographical data instead of the standard feedback, we can keep the separation constant. In this second pass, cantilever oscillation amplitudes are sensitive to electric force gradients without being influenced by topographical features (Fig. 3B). This two-pass measurement process is recorded for every scan line, producing separate topographic and electric force images. From these images, contours of electric force gradient (Fig. 3C) can be drawn.

The amplitude of the cantilever oscillations is very large for small lift heights, and the images fade at separations larger than 80 nm. This is consistent with previous reports of a strong dependence of the tip-surface force on the vertical separation (13). More work needs to be done to understand this quantitatively. Note that some of the nanowires that appear in the topographic image are missing from the electric field image (Fig. 3). This is because either electrical contact to these nanowires has failed or interrupted electrical conduction along the wire length has been interrupted. The scanning force technique thus provides a unique way of mapping the electrical properties of nanocomposites.

Applications of the metal nanowire composites include high-density electrical multiplexing and high-resolution plates for transferring a two-dimensional charge distribution between microelectronic devices. The semiconductor nanowires can be used in photodetector arrays of high spatial resolution, where each wire acts as a pixel of submicrometer dimensions. Also, with the application of the injection technique to ultrasmall channel insulators (channel diameter less than 50 nm) (16, 17), nanowire arrays can be made for fundamental studies of a variety of phenomena, such as quantum confinement of charge carriers and mesoscopic transport.

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**Green Fluorescent Protein as a Marker for Gene Expression**

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A complementary DNA for the Aequorea victoria green fluorescent protein (GFP) produces a fluorescent protein when expressed in prokaryotic (Escherichia coli) or eukaryotic (Caenorhabditis elegans) cells. Because exogenous substrates and cofactors are not required for this fluorescence, GFP expression can be used to monitor gene expression and protein localization in living organisms.

Light is produced by the bioluminescent jellyfish Aequorea victoria when calcium binds to the photoprotein aequorin (1). Although activation of aequorin in vitro or in heterologous cells produces blue light, the jellyfish produces green light. This light is the result of a second protein in A. victoria that derives its excitation energy from aequorin (2), the green fluorescent protein (GFP).

Purified GFP, a protein of 238 amino acids (3), absorbs blue light (maximally at 395 nm with a minor peak at 470 nm) and emits green light (peak emission at 509 nm with a shoulder at 540 nm) (2, 4). This fluorescence is very stable, and virtually no photobleaching is observed (5). Although the intact protein is needed for fluorescence, the same absorption spectral properties found in the denatured protein are found in a hexapeptide that starts at amino acid 64 (6, 7). The GFP chromophore is derived from the primary amino acid sequence through the cyclization of serine-dehydrotyrosine-glycine within this hexapeptide (7). The mechanisms that produce the dehydrotyrosine and cyclize the poly-
peptide to form the chromophore are unknown. To determine whether additional factors from A. victoria were needed for the production of the fluorescent protein, we tested GFP fluorescence in heterologous systems. Here, we show that GFP expressed in prokaryotic and eukaryotic cells is capable of producing a strong green fluorescence when excited by blue light. Because this fluorescence requires no additional gene products from A. victoria, chromophore formation is not species-specific and occurs either through the use of ubiquitous cellular components or by autocatalysis.

Expression of GFP in Escherichia coli (8) under the control of the T7 promoter results in a readily detected green fluorescence (9) that is not observed in control bacteria. Upon illumination with a long-wave ultraviolet (UV) source, fluorescent bacteria were detected on plates that contained the inducer isopropyl-β-D-thiogalactoside (IPTG) (Fig. 1). Because the cells grew well in the continual presence of the inducer, GFP did not appear to have a toxic effect on the cells. When GFP was partially purified from this strain (10), it was found to have fluorescence excitation and emission spectra indistinguishable from those of the purified native protein (Fig. 2). The spectral properties of the recombinant GFP suggest that the chromophore can form in the absence of other A. victoria products.

Transformation of the nematode Caenorhabditis elegans also resulted in the production of fluorescent GFP (11) (Fig. 3). GFP was expressed in a small number of neurons under the control of a promoter for the mec-7 gene. The mec-7 gene encodes a β-tubulin (12) that is abundant in six touch receptor neurons in C. elegans and less abundant in a few other neurons (13, 14). The pattern of expression of GFP was similar to that detected by MEC-7 antibody or from mec-7-lacZ fusions (13–15). The strongest fluorescence was seen in the cell bodies of the four embryonically derived touch receptor neurons (ALML, ALMR, PLML, and PLMR) in younger larvae. The processes from these cells, including their terminal branches, were often visible in larval animals. In some newly hatched animals, the PLM processes were short and ended in what appeared to be prominent growth cones. In older larvae, the cell bodies of the remaining touch cells (AVM and PVM) were also seen; the processes of these cells were more difficult to detect. These postembryonically derived cells arise during the first of the four larval stages (16), but their outgrowth occurs in the following larval stages (17), with the cells becoming functional during the fourth larval stage (18). The fluorescence of GFP in these cells is consistent with these previous results: no fluorescence was detected in these cells in newly hatched or late first-stage larvae, but fluorescence was seen in four of ten late second-stage larvae, all nine early fourth-stage larvae, and seven of eight young adults (19). In addition, moderate to weak fluorescence was seen in a few other neurons (Fig. 3) (20).

Like the native protein, GFP expressed in both E. coli and C. elegans is quite stable (lasting at least 10 min) when illuminated with 450- to 490-nm light. Some photo-bleaching occurs, however, when the cells are illuminated with 340- to 390-nm or 395- to 440-nm light (21).

Several methods are available to monitor gene activity and protein distribution within cells. These include the formation of fusion proteins with coding sequences for β-galactosidase, firefly luciferase, and bacterial luciferase (22). Because such methods require exogenously added substrates or cofactors, they are of limited use with living tissue. Because the detection of intracellular GFP requires only irradiation by near UV or blue light, it is not limited by the availability of substrates. Thus, it should provide an excellent means for monitoring gene expression and protein localization in living cells (23, 24). Because it does not appear to interfere with cell growth and function, GFP should also be a convenient indicator of transformation and one that could allow cells to be separated with fluorescence-activated cell sorting. We also envision that GFP can be used as a vital marker so that cell growth (for example, the elaboration of neuronal processes) and movement can be followed in situ, especially in animals that are essentially transparent like C. elegans and zebrafish. The relatively small size of the protein may facilitate its diffusion throughout the cytoplasm of extensively branched cells like neurons and glia. Because the GFP fluorescence persists after treatment with formaldehyde (9), fixed preparations can also be examined. In addition, absorption of appropriate laser light by GFP-expressing cells (as has been done for Lucifer Yellow-containing cells) (25) could result in the selective killing of the cells.
REFERENCES AND NOTES

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8. Plasmid pGFP10.1 contains the Eco RI fragment encoding the GFP complementary DNA (cDNA) for logP10 (3) in pBluescript) (Stratagene). The fragment was obtained by amplification with the polymerase chain reaction (PCR) (R. K. Saki et al., Science 239, 487 (1988)) with primers flanking the Eco RI cloning sites and subsequent digestion with Eco RI. DNA was ligated with the Magic Mini-prep procedure (Promega) and sequenced (after an additional ethanol precipitation) on an Applied Biosystems DNA Sequencer 373A at the DNA Sequencing Facility at Columbia University College of Physicians and Surgeons. The sequence of the cDNA in pGFP10.1 differs from the published sequence by a change in codon 80 within the coding sequence. The sequence was chosen because it changes a glutamine residue with arginine. [R. Heim, S. Emr, and R. Tissen (personal communication) first alerted us to this possible sequence change in this clone and independently noted the same change.] This replacement has no detectable effect on the spectral properties of the protein (Fig. 2). An Eco RI expression construct was made by inserting the PCR fragment into a fragment with an Nhe I site at the start of translation and an Eco RI site 5' to the termination signal of the GFP coding sequence from pGFP10.1. The 5' primer was ACAAGAAGCTCAGAAGAAGAAGAC and the 3' primer was the T3 primer (Stratagene). The Nhe I–Eco RI fragment was ligated into the similarly cut vector pET3a [A. H. Rosenberg et al., Gene 56, 125 (1987)] by standard methods (26). The resulting codon sequence contains an Ala for the initial GFP Met, which becomes the second amino acid in the polyepitope. The Eco RI site in pET3a is in the KpnI site of the pBluescript) (Stratagene) vector. 
9. Wild-type and were grown and genetic strains were constructed according to S. Brenner [Genetics 77, 71 (1974)]. The plasmid pGFP10.1 was used as a template for PCR (with the S primer: 5'-GACCAAGATGAGTAAG and the T3' primer) to generate a fragment with a 5' Nhe I site (at the start of translation) and a 3' Eco RI site (3' of the termination codon). This DNA was cut to produce an Nhe I–Eco RI fragment that was ligated into plasmid pPD 16.51 (12, 27), a vector containing the promoter of the C. elegans mec-7 gene. Wild-type C. elegans were transformed by connecting this DNA (TU646) and the DNA for plasmid pRF4, which contains the dominant rol-6 (sul06) mutation, into adult gonads as described [C. Mello, J. K. Stinchcomb, W. Ambros, EMBO J. 10, 3959 (1991)]. A relatively stable line was isolated (TU1710), and the DNA it carried was integrated as described by Mitani et al. (15) to produce the integrated elements TU3 and TU4 (in strains TU1754 and TU1755, respectively). Living animals were mounted on agar (or agarose) pads and viewed with 10 mM NaN3 as an anesthetic (28) (another nematode anesthetic, propoxycam, quenched the fluorescence) and examined with either a Zeiss or Nikon microscope. C. elegans, a long-pass emission filter works best because the animal’s intestinal autofluorescence (which increases as the animal matures) appears yellow (with band-pass emission filters it appears green and obscures the GFP fluorescence). Because much more intense fluorescence was seen in uls4 than in uls3 animals (for example, it was often difficult to see the processes of the ALM and PLM cells in uls3 animals when the worms were illuminated with a mercury lamp), the former were used in the observations reported here. The general pattern of cell body fluorescence was the same in both strains and in the parental, nonintegrated strain (fluorescence in this strain was observed with 150-mg streptomycin and in the uls4 animals). The uls4 animals, however, did show an unusual phenotype: both the ALM and PLM touch cells were often displaced anteriorly. The mature cells usually had processes in the correct positions, although occasional cells had abnormally projecting processes. These cells could be identified as touch receptor cells because the fluorescence was dramatically decreased in a gene that specifies touch cell fate (13, 15, 19, 28). Expression of mec-7 is reduced in the ALM touch cells of the head (but not as dramatically in the PLM touch cells) (13, 15). We find a similar change of GFP expression in a mec-3 mutant background for both uls3 and uls4. Thus, GFP accurately represents the expression of these cep-1–touch receptor neurons that the reduced staining in uls3 animals and the misplaced cells in uls4 animals are results of either a shift in the activity or the amount or position of the integrated DNA.
10. We used a variety of microscopes (Zeiss Axio-phot, Nikon Microphot FXA, and Olympus BH2–RFC and BX50) that were equipped for epifluorescence microscopy. Usually, filter sets for fluorescence illumination were used (for example, the Zeiss filter set used a BP450-490 excitation filter, 510-nm dichroic, and either a GFP or a routine emission filter), although for some experiments filter sets that excited at lower wavelengths were used (for example, the Zeiss filter set with BP395-440 and LP470 filters and a combination with BP490-500 and LP400 filters with a 395-nm dichroic). In some instances, a xenon lamp appeared to give a more intense fluorescence than a mercury lamp when cells were illuminated with light around 470 nm, although usually comparable. No other attempts were made to enhance the signal (for example, with low-intensity light cameras), although such enhancement may be useful in some instances. Prominent among these was that the native protein was fluorescent after glutaraldehyde fixation (W. W. Ward, unpublished data). S. Wang and T. Hazeldigg (personal communication) (23) have found that GFP fusion proteins in Drosophila melanogaster are fluorescent after formaldehyde fixation. We have confirmed that fluorescence persists after formaldehyde fixation with animals and with recombinant GFP isolated from E. coli. However, the chemicals in nail polish, which is often used to seal cover slips, did appear to interfere with the C. elegans GFP fluorescence.
11. Photobleaching with 395- to 440-nm light is further accelerated as the animals mature. In the presence of 10 mM NaN3, which is used as a C. elegans anesthetic (11). However, when cells in C. elegans have been photobleached, some recovery is seen within 10 min. Further investigation is needed to determine whether this recovery represents de novo synthesis of GFP. Rapid photobleaching (complete within a minute) of the green product was also seen when C. elegans was illuminated with 340- to 390-nm light. Unlike the photobleaching with 395- to 440-nm light, which abolished fluorescence produced by the 340- to 390-nm light, photobleaching with 340- to 390-nm light did not appear to affect the fluorescence produced by 395- to 490- or 450- to 490-nm light. Indeed, the fluorescence produced by 450- to 490-nm light appeared to be more intense after brief photobleaching by 340- to 390-nm light. This selective photobleaching may be a reflection of the production of more than one fluorescent product in the animal. These data on GFP fluorescence within E. coli and C. elegans are in contrast to preliminary studies that suggest that the isolated rat liver E. coli proteins are very photostable. We do not know whether this in vivo sensitivity to photobleaching is a normal feature of the jellyfish protein (the fluorescence in this case was observed with 150-mg streptomycin) or results from the absence of a necessary posttranslational modification unique to A. victoria.

23. R. Heim, S. Emr, and R. Tsien (personal communication) have found that GFP expression in Saccharomyces cerevisiae can make the cells fluorescent without causing toxicity. S. Wang and T. Hazelrigg (personal communication) have found that both COOH-terminal and NH2-terminal protein fusions with GFP are fluorescent in D. melanogaster. L. Lanini and F. McKeeon (personal communication) have expressed a GFP protein fusion in mammalian (COS) cells.

24. We have generated several other plasmid constructions that may be useful to investigators. These include a His6 derivative (TU#65) containing a Kpn I-Eco RI fragment encoding GFP with an Age I site 5' to the translation start and a Bam I site at the termination codon. Also available are GFP versions (TU#60 to TU#63) of the four C. elegans lacZ expression vectors (pD164.43, pD221.28, pD222.04, and pD222.11, respectively) as described (27) except that the Kpn I fragment containing the SV40 nuclear localization signal.


RNA Polymerase II Initiation Factor Interactions and Transcription Start Site Selection

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An RNA polymerase II transcription system was resolved and reconstituted from extracts of Schizosaccharomyces pombe. Exchange with components of a Saccharomyces cerevisiae system was undertaken to reveal the factor or factors responsible for the difference in location of the transcription start site, about 30 base pairs and 40 to 120 base pairs downstream of the TATA box in S. pombe and S. cerevisiae, respectively. Two components, counterparts of human transcription factor IIF (TFIIF) and TFIIH, could be exchanged individually between systems without effect on the start site. Three components, counterparts of human TFIIB, TFIIE, and RNA polymerase II, could not be exchanged individually but could be swapped in the pairs TFIIE-TFIIF and TFIIB-RNA polymerase II, which demonstrates that there are functional interactions between these components. Moreover, exchange of the latter pair shifted the starting position, which shows that TFIIB and RNA polymerase II are solely responsible for determining the start site of transcription.

Synthesis of mRNA in eukaryotes requires RNA polymerase II and accessory factors, some of which are general and act at most, if not all, promoters, and others of which confer specificity and control. Five general factors—α, β, δ, ε, and γ—have been purified to homogeneity from the budding yeast Saccharomyces cerevisiae and have been identified as counterparts of human-rat factors TFIIE-ε, TFIIH-δ, TFIID-τ, TFIIB-α, and TFIIF-β, respectively (1–8). Because these factors assemble at a promoter in a complex with RNA polymerase II, the interactions among them are assumed to be important for the initiation of transcription.

Most studies of general factor interactions have focused on binding (8). The results have shown that the order of assembly of the initiation complex on promoter DNA begins with factor d (TFIIF), is followed by factor e (TFIIB), and then by polymerase and the remaining factors (6, 9). Factors b (TFIIE), α, and γ (TFIIF), however, bind directly to

Fig. 1. Factor e of S. pombe copurifies with a 35-kD polypeptide cross-reactive with human TFIIF antiserum. (A) Assay of fractions (2 μl) from HAP (16) for pTFe activity. Assays were performed with the complete S. pombe system (16), except for the omission (first lane) of pTFe. (B) Immunoblot analysis of fractions (40 μl) from acetic acid precipitation, 12% SDS-polyacrylamide gel electrophoresis, and blotting onto nitrocellulose were followed by successive incubations with polyclonal human TFIIF antiserum (1:300) for 18 hours at 4°C and with goat monoclonal antibody to rabbit (1:2000) for 1 hour at 24°C as described (26). The 35-kD polypeptide (indicated by arrow) was the only cross-reactive species seen when smaller amounts of protein were loaded. Lane 1 contained 10 ng of human TFIIF.


29. We are indebted to A. Duggan and D. Xue for technical suggestions, to L. Kerr and P. Presley at the Marine Biological Laboratories at Woods Hole for help with microscopy, to M. Cutler and R. Ludescher for assistance in obtaining the excitation and emission spectra, to A. Fire for suggestions on vector construction, and to the colleagues listed in (6) and (23) for permission to cite their unpublished research. Supported by NIH grant GM31997 and a McKnight Development Award to M.C. and by American Cancer Society grant NP540 to D.C.P.

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