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, Te, and Al) and semiconductors (Se, Sn, and BiAs) (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 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 multifeedthroughs 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.

REFERENCES AND NOTES


Green Fluorescent Protein as a Marker for Gene Expression

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A complementar DNA for the Aequorea victoria green fluorescent protein (GFP) produces a fluorescent product 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 photobleaching 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 zebra fish. 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.

Fig. 1. Expression of GFP in E. coli. The bacteria on the right side of the figure have the GFP expression plasmid. Cells were photographed during irradiation with a handheld-long-wave UV source.

Fig. 2. Excitation and emission spectra of E. coli-generated GFP (solid lines) and purified A. victoria L form GFP (dotted lines).

Fig. 3. Expression of GFP in a first-stage C. elegans larva. Two touch receptor neurons (ALMR and PLMR) are labeled at their strongly fluorescing cell bodies. Processes can be seen projecting from both of these cell bodies. Halos produced from the out-of-focus homologs of these cells on the other side of the animal are indicated by arrowheads. The thick arrow points to the nerve ring branch from the ALMR cell (out of focus); thin arrows point to weakly fluorescing cell bodies. The background fluorescence is the result of the animal's autofluorescence.
5. F. G. Prendergast, personal communication.
8. Plasmid pGPF10.1 contains the Eco RI fragment encoding the GFP complementary DNA (cDNA) for the pRFP1 (3 in pEB8+) (Stratagene). The fragment was obtained by amplification with the primerase chain reaction (PCR) ([R. K. Saki et al., Science 239, 487 (1988)] with primers flanking the Eco RI sites and subsequent digestion with Eco RI. DNA was prepared by the Magic Mini-prep procedure (Promega) and sequenced (after an additional ethanol precipitation) on an Applied Biosystems DNA Sequencer 373A at the DNA Sequencing Core Laboratory of the Columbia College of Physicians and Surgeons. The sequence of the cDNA in pGPF10.1 differs from the published sequence by a change in codons 80 within the coding region of the GAG sequence to CCA, a change that replaces a guanine residue with arginine.
9. [R. Helm, G. L. Lanini, et al., Science 239, 487 (1988)] prepared by the Magic Mini-prep procedure (Promega) and sequenced (after an additional ethanol precipitation) on an Applied Biosystems DNA Sequencer 373A at the DNA Sequencing Core Laboratory of the Columbia College of Physicians and Surgeons. The sequence of the cDNA in pGPF10.1 differs from the published sequence by a change in codons 80 within the coding region of the GAG sequence to CCA, a change that replaces a guanine residue with arginine.
10. GFP was purified from 250-mL cultures of BL21 (DE3) Lys S bacteria containing pTU458; bacteria were grown in LB broth (26) containing ampicillin (100 µg/mL) and 0.8 mM IPTG. Induction was best when IPTG was present continuously. Cells were washed in 4 mL of 10 mM tris-HCl (pH 7.4), 100 mM NaCl, 1 mM MgCl2, and 10 mM dithiothreitol ([A. Kerrong, W. G. Druny, Cell 64, 903 (1991)] and then centrifuged for 20 min at 10,000 rpm. The supernatant was centrifuged for 1 h at 30,000 rpm to remove bacterial debris. The GST fusion proteins in Drosophila melanogaster are fluorescent after formaldehyde fixation. We have confirmed that fluorescence persists after formaldehyde fixation with wild-type 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
11. The fluoresence was not a mercury lamp when cells were illuminated with light around 470 nm, although we are using comparable light. No other attempts were made to enhance the signal (for example, with low-intensity light cameras), although such enhancement may be useful in some applications. Preliminary experiments showed that the native protein was fluorescent after glu-taraldehyde fixation (W. W. Ward, unpublished data).
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15. We used a variety of microscopes (Zeiss Axiosphot, Nikon Microphot FXA, and Olympus BH-2, RCF, and BX50) that were equipped for epifluorescence microscopy. Usually, filter sets for fluorescence of the same wavelength were used (for example, the Zeiss filter set used a BP450-490 excitation filter, 510-nm dichroic, and either a BGP 490 (CG4 filter) or a BP510 and LP520 filters with a BP480/500 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 we are using comparable light. No other attempts were made to enhance the signal (for example, with low-intensity light cameras), although such enhancement may be useful in some applications. Preliminary experiments showed that the native protein was fluorescent after glutaraldehyde fixation (W. W. Ward, unpublished data).
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or from nonspecific damage within the cells.


23. R. Heim, S. Emr, and R. Tsien (personal communication) have found that GFP expression in Saccharomyces cerevisiae can make the cells strongly fluorescent without causing toxicity. S. Wang and T. Hazelbrog (personal communication) have found that both COOH-terminal and NH2-terminal protein fusions with GFP are fluorescent in D. melanogaster. L. Banin and F. McKeon (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 plasmid pBR KS (+) derivative (TUA#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 gp2 expression vectors (TUA#60 to TUA#63) of the four C. elegans lacZ expression vectors (pPD16.43, pPD21.28, pPD22.04, and pPD22.11, respectively) as described (27) except that the Kpn I fragment containing the SV40 nuclear localization signal.


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 NP640 to D.C.P.

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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 factors TFIH and TFIIF, could be exchanged individually but could not 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 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-RNAP TFIE-ε, TFIH-δ, TFIID-δ, TFIIF-α, and TFIIF-β, respectively (1–8). Because these factors assemble at a promoter in a complex with RNA polymerase II, 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 δ (TFIID), is followed by factor ε (TFIIE), and then by polymerase and the remaining factors (6, 9). Factors β (TFIH), ε, and γ (TFIIF), however, bind directly to polymerase II, and as many as four of the five factors may assemble with the polymerase in a holoenzyme (10) before promoter binding. There are a couple of limitations implicit in these findings: The functional significance of interactions revealed by binding is questionable because only a few percent of initiation complexes give rise to transcripts, and there is little indication of the roles of the various interactions in the initiation process.

We have used a functional approach to analyze general transcription factor interactions on the basis of the ability of factors to be exchanged between transcription systems. Exchange between S. cerevisiae and mammalian systems is of interest because of a marked difference in location of the transcription start site, 40 to 120 base pairs downstream of the TATA box in the former versus about 30 base pairs in the latter (11). The TATA-binding component (TBP) of factor δ (TFIID) is functionally interchangeable between S. cerevisiae and humans (4, 12, 13), but the transcription start site remains characteristic of the particular transcription system, irrespective of the source of TBP. The factor or factors responsible for start site selection could not be identified by this approach because neither of the other factors nor the polymerase proved interchangeable between S. cerevisiae and higher eukaryotic systems. We decided to use a Schizosaccharomyces pombe system because of its similarity to higher eukaryotes in the location of RNA polymerase II transcription start sites and its closer evolutionary relation to S. cerevisiae. Initiation from S. pombe promoters occurs about 30 base pairs downstream of the TATA box, and initiation from mammalian promoters introduced in S. pombe occurs at the same sites as in mammalian cells (14).

We have described the derivation of a chromatographic fraction from S. pombe that, upon addition of TBP, will support promoter-dependent RNA polymerase II transcription.

Fig. 1. Factor e of S. pombe copurifies with a 35-kD polypeptide cross-reactive with human TFIIF antisera. (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) using purified anti-human 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.

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