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 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 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 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 502 nm with a shoulder at 540 nm) (2, 4). Its 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 (8). The mechanisms that produce the dehydrotyrosine and cyclize the poly-

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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. Above, 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 longwave 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 one 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 (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 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 (ALM1, ALM2, 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 *C. elegans* 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/cellulase or 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, and, it should provide an excellent means for monitoring gene expression and protein localization in living cells (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 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 cell.

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**Fig. 1.** Expression of GFP in *E. coli.* The bacteria on the right side of the figure have the GFP expression plasmids were photographed during irradiation with a hand-held 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.
REFERENCES AND NOTES


5. F. G. Prendergast, personal communication.


8. id pGFP10.1 contains the Eco RI fragment encoding the GFP complementary DNA (cDNA) for zpD10 (in pBlues(-)) (Stratagene). The fragment was obtained by amplification with the polymerase chain reaction (PCR) (K. Sakl et al., Science 239, 487 (1988)) with primers flanking the cDNA and subsequent digest with Eco RI. DNA sequencing was performed by the Magic Mini-preps procedure (Promega) and sequenced (after an additional ethanol precipitation) on an Applied Biosystems DNA Sequencer 370A at the DNA Sequencing Facility in Columbia University Physicians and Surgeons. The sequence of the cDNA in pGFP10.1 differs from the published sequence by a change in codon 68 within the coding sequence (GAC to GGC) to code a change that replaces a glutamine residue with arginine. (R. Heim, S. Emr, and R. Tsien, personal communication) first alerted us to a 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 E. coli expression construct was made that purifies a fragment of about 2.5 kDa but lacks the C-terminal 3' fragment of GFP. The construct was expressed as a fusion protein with a photoactivatable suppressor tRNA fungal ribosomal protein L14 (pGFP10-1) that was used to produce the GFP construct. The fusion protein was purified by high-performance liquid chromatography (HPLC) and then characterized by transient transfection in a reporter gene assay in which it was shown to be functional.


10. GFP was purified from 250-mL cultures of BL21 (DE3) Lys S bacterial containing Tu5458; bacteria were grown in LB broth (26) containing ampicillin (100 µg/mL) and 0.8 mM IPTG. Induction was best when IPTG was present continually. Cells were washed in 4 mL of 10 mM tris-HEPES (pH 7.4), 100 mM NaCl, 1 mM MgCl2, and 10 mM dithiothreitol (W. A. J. Bestor, G. F. H. D. P. Bunney, Cell 64, 903 (1991)) and then centrifuged at 10,000 g for 20 s each in 4 mL of the same buffer containing 0.1 mM phenylmethylsulfonyl fluoride, pepstatin A (1 µg/mL), leupeptin (1 µg/mL), and aprotinin (2 µg/mL) for 15 min at 4°C. The supernatant was centrifuged at 30,000 g for 30 min and then treated with three freeze-thaw cycles. The resulting supernatant contained the recombinant GFP from 0.94 to 0.98 mg/mL. The purified recombinant GFP was used for the experiments described above.

11. References and notes continued on page 805.
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—a, b, d, e, and g—have been purified to homogeneity from the budding yeast *Saccharomyces cerevisiae* and have been identified as counterparts of human-rat factors TFIIE-e, TFIIH-δ, TFIID-τ, TFIIB-α, and TFIIFI-βy, 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 d (TFIID), is followed by factor e (TFIIB), and then by polymerase and the remaining factors (6, 9). Factors b (TFIIE), e, and g (TFIIFH), 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 d (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 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...