P or no hormones were implanted s.c. on days 1 and 10, and tissues were collected 11 days later. Mammary glands were fixed in 10% formalin and stained with hematoxylin as described (*2*). For indirect immunofluorescence, frozen mammary tissue was sectioned at 50-μm thickness, permeabilized with 5% Triton X-100, and fixed in 4% paraformaldehyde. Sections were incubated overnight at 4°C in rabbit polyclonal antiserum to cytokeratin-14 (1:500 dilution; a gift from D. R. Roop, Baylor College of Medicine) containing 1% bovine serum albumin and rat monoclonal IgG to E-cadherin (1:500 dilution; Zymed, San Francisco, CA). Antibodies were visualized with Texas Red–goat antibodies to rabbit IgG (1:200 dilution; Molecular Probe, Eugene, OR) and fluorescein isothiocyanate–goat antibodies to rat IgG (1:400 dilution; PharMingen, San Diego, CA). Serial optical sectioning was performed with a confocal laser-scanning microscope (Multiprobe 2001, Molecular Dynamics). Three-dimensional images were reconstructed with Image Space Software (Molecular Dynamics).

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Oligodendrocyte Precursor Cells Reprogrammed to Become Multipotential CNS Stem Cells

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During animal development, cells become progressively more restricted in the cell types to which they can give rise. In the central nervous system (CNS), for example, multipotential stem cells produce various kinds of specified precursors that divide a limited number of times before they terminally differentiate into either neurons or glial cells. We show here that certain extracellular signals can induce oligodendrocyte precursor cells to revert to multipotential neural stem cells, which can self-renew and give rise to neurons and astrocytes, as well as to oligodendrocytes. Thus, these precursor cells have greater developmental potential than previously thought.

Oligodendrocyte precursor cells (OPCs) are arguably the best-characterized precursors in the mammalian CNS. They arise from multipotential cells in spatially restricted germinal zones and then migrate widely through the developing CNS (*1–3*). After a number of cell divisions, most OPCs terminally differentiate into postmitotic oligodendrocytes (*4, 5*), although some persist in the adult CNS (*6, 7*). Fetal calf serum (FCS) (*8*) and certain cytokines, including some bone morphogenic proteins (BMPs) (*9*), induce OPCs in culture to differentiate into type-2 astrocytes, although there is no convincing evidence that OPCs normally become astrocytes in vivo. Here we show that a 3-day exposure to these signals that induce type-2 astrocytes, followed by culture in basic fibroblast growth factor (bFGF), causes many purified OPCs to revert to a state that resembles that of multipotential CNS stem cells. The reverted cells can self-renew and give rise to neurons and type-1 astrocytes, as well as to oligodendrocytes. These unexpected findings suggest that extracellular signals can reverse glial cell specification and can convert specified precursors into multipotential stem cells.

We purified OPCs from postnatal day 6 (P6) rat optic nerve to greater than 99% purity by sequential immunopanning, as previously described (*10*). We cultured the cells in poly-D-lysine (PDL)–coated culture dishes in serum-free medium containing platelet-derived growth factor (PDGF) but no thyroid hormone (TH), to stimulate their proliferation and to inhibit their differentiation (*11*). After 5 days in culture, we tested their proliferative response to bFGF by removing the PDGF and adding bFGF: >99% of the cells stopped dividing and differentiated into oligodendrocytes within 5 days (not shown), just as they do when PDGF is removed in the absence of bFGF (*11*). This finding attests to the purity of the cultures and indicates that bFGF alone is a poor mitogen in these conditions. When, however, we first induced the OPCs to differentiate into type-2 astrocytes by adding 15% FCS for 3 days (Fig. 1B) and then removed the FCS and PDGF and cultured the cells in bFGF for a further 5 days, >40% of the cells could be labeled by a 6-hour pulse of bromodeoxyuridine (BrdU) (Fig. 1A). Thus, FCS-treated OPCs become responsive to the mitogenic effect of bFGF, a growth factor that is mitogenic for some CNS stem cells (*12*). Epidermal growth factor (EGF), which is also mitogenic for some CNS stem cells (*13*), did not stimulate BrdU incorporation under the same conditions (Fig. 1A). Using this protocol, we could keep the cells proliferating in bFGF, without serum, PDGF, or TH, for many weeks, during which time most of the cells reverted to a bipolar morphology characteristic of perinatal OPCs (*4*) (Fig. 1C). When we cultured the cells in the same way, but in tissue culture dishes without the PDL coating, the cells produced floating neurosphere-like bodies after 10 days (Fig. 1D), just as CNS stem cells do under similar conditions (*13, 14*). Together, these findings raised the possibility that OPCs cultured sequentially in PDGF, FCS, and bFGF may acquire some of the properties of multipotential CNS stem cells.

To test this possibility, we assessed whether OPCs cultured in this way acquired the ability to produce neurons. We induced purified P6 OPCs to become type-2 astrocytes in 15% FCS and PDGF for 3 days and then cultured them in PDL-coated culture dishes in bFGF, without serum, PDGF, or TH, for 5 days or 1 month. We then stained the cells with neuron-specific monoclonal antibodies that recognize microtubule-associated protein 2 (MAP2), low molecular weight neurofilament protein (NF-L), or middle molecular weight neurofilament protein (NF-M), all of which are widely used as neuronal markers. We also stained the cells with the A2B5 monoclonal antibody (*15*) to identify OPCs (*8*), monoclonal anti-galactocerebroside (GC) antibody (*16*) to identify oligodendrocytes (*17*), and rabbit antibodies to glial fibrillary acidic protein (GFAP) to identify astrocytes (*17, 18*). As shown in Table 1, the proportion of cells that expressed neuron-specific markers greatly increased after 5 days in bFGF and increased two to three times further between 5 days and 1 month in bFGF, and most of these cells were A2B5-negative ($A2B5^-$) (Fig. 1, E to G); at either time point, the proportion of A2B5-positive ($A2B5^+$) OPCs was <40% and the proportions of GC^+ oligodendrocytes and $GFAP^+$ astrocytes were <1%. By contrast, when we stained the cells after 1 month in PDGF or after 3 days in 15% FCS, >80% were $A2B5^+$, less than 5% were $MAP2^+$, and none were $NF\text{-}L^+$ or $NF\text{-}M^+$ (Table 1). Thus, OPCs cultured sequentially in PDGF, FCS, and bFGF can give rise to neurons.

When we cultured cells treated in this way in bFGF for 2 months, many of them remained $MAP2^+$, but <5% now expressed NF-L or NF-M (Fig. 2A). As it was shown previously that PDGF encourages CNS stem cells to develop into neurons (*14, 19*), we

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switched the cells that had been proliferating in bFGF for 2 months to PDGF alone for 2 days and then stained them with the anti-NF antibodies. As shown in Fig. 2A, about 50% of the cells now stained with either anti-NF antibody, suggesting that many of the cells had begun to differentiate into neurons. To confirm this, we maintained the switched cells in PDGF for 1 month and then examined them by reverse transcriptase–polymerase chain reaction (RT-PCR) for the expression of mRNA encoding glutamic acid decarboxylase (GAD), the enzyme responsible for the synthesis of the neurotransmitter GABA (*20*). As shown in Fig. 2B, we could readily detect *GAD65* mRNA in these cells, whereas we could not detect it in either cells switched to PDGF for only 2 days or cells that had been cultured in bFGF alone for 2 months. Thus, PDGF induced the long-term bFGF cells to express NF proteins within 2 days and *GAD65* mRNA within 1 month, supporting previous evidence that it promotes neuronal development in cultures of multipotential CNS stem cells (*14, 19*).

To determine whether any of the cells grown in bFGF for 2 months could still differentiate into oligodendrocytes, we removed the bFGF and added PDGF and TH to encourage oligodendrocyte development (*11, 14*). After 5 days, we stained the cells for GC. Whereas less than 2% of the cells maintained in bFGF (or PDGF) for 2 months were GC^+, about 30% of the cells switched to PDGF plus TH for 5 days were GC^+ (Fig. 2C). The GC^+ cells displayed the characteristic morphology of oligodendrocytes and were NF^- (Fig. 2E). Thus, many of the cells cultured in bFGF for 2 months could develop into oligodendrocytes when switched to PDGF and TH. Fewer than 10% of the cells became oligodendrocytes when switched from bFGF to bFGF plus TH (Fig. 1C).

To determine whether any of the cells grown in bFGF for 2 months could differentiate into type-1 astrocytes (*21*), we removed the bFGF, added PDGF and BMP-2 plus BMP-4 (BMPs) for 5 days, and then stained the cells for GFAP (*18*). BMPs have been shown to promote both type-1 and type-2 astrocyte development (*9, 22*). Whereas none of the cells growing in bFGF for 2 months were $GFAP^+$, about 10% became $GFAP^+$ after 5 days in PDGF plus BMPs, and about 3% did so after 5 days in bFGF plus BMPs (Fig. 2D). Some of the $GFAP^+$ cells had the process-bearing morphology of type-2 astrocytes (*8, 21*) (Fig. 2F), although most had the fibroblast-like morphology of type-1 astrocytes (*21*) (Fig. 2, G to J). The latter, but not the former, $GFAP^+$ cells were stained weakly with the monoclonal anti–Ran-2 antibody (*23*) (Fig. 2I), which stains type-1 but not type-2 as-

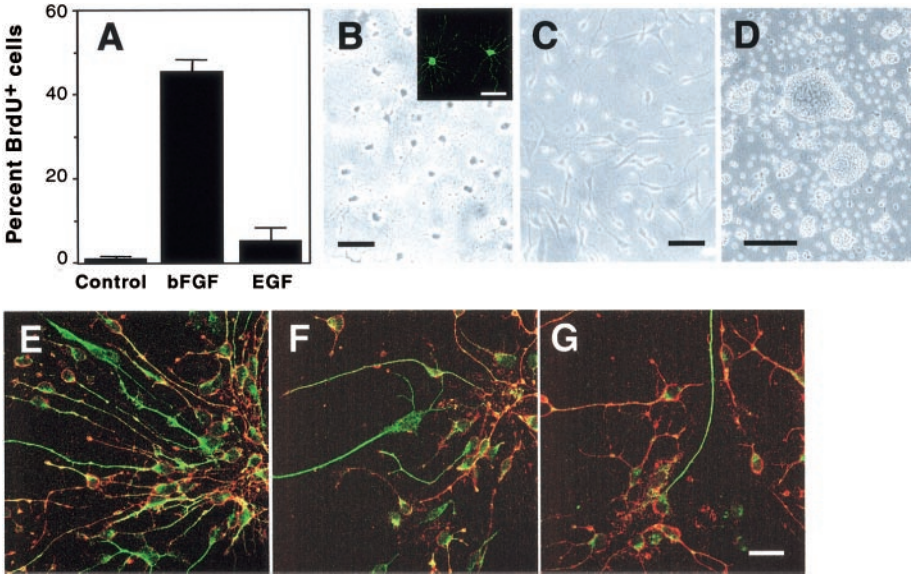

Fig. 1. Characteristics of cells derived from type-2 astrocytes. (**A**) Purified P6 OPCs were cultured in PDGF (10 ng/ml) for 5 days, in 15% FCS and PDGF for 3 days, and then in either bFGF (20 ng/ml) or EGF (20 ng/ml) for 5 days; BrdU (20 μM) was added for the last 6 hours. The proportions of $BrdU^+$ cells are shown as means ± SD of three cultures. (**B**) OPCs were cultured in PDGF and then 15% FCS and PDGF as in (A) and then immunostained for GFAP. They are shown after fixation by phase contrast and fluorescence (inset). (**C**) OPCs were cultured as in (A) and photographed while alive after 7 days in bFGF. (**D**) OPCs were cultured as in (A), except they were cultured for 10 days in bFGF on a plate without the PDL coating and photographed as in (C). (**E** to **G**) OPCs were cultured as in (A) and after 5 days in bFGF they were double-labeled with A2B5 antibody (red) and for the neuronal antigens MAP2 (E), NF-M (F), or NF-L (G), all shown in green. All experiments reported in this paper were repeated at least three times with similar results (*30*). Scale bar, 50 μm in (B) and (C), 300 μm in (D), and 25 μm in (E) to (G).

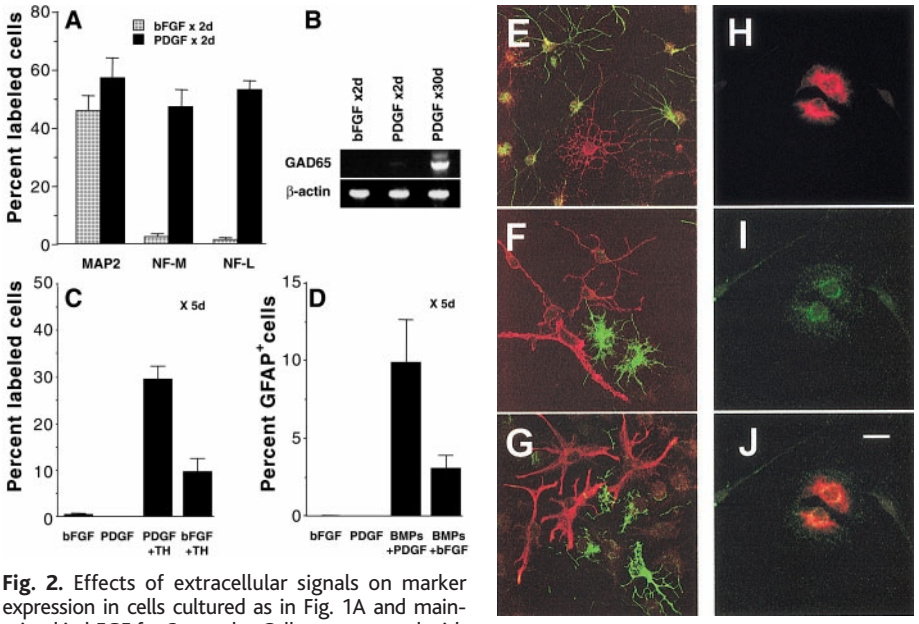

Fig. 2. Effects of extracellular signals on marker expression in cells cultured as in Fig. 1A and maintained in bFGF for 2 months. Cells were treated with additional signaling molecules for 2, 5, or 30 days. (**A**) Cells were treated with bFGF or PDGF for 2 days before they were immunostained for MAP2 or NFs. (**B**) Cells were treated with bFGF for 2 days or with PDGF for either 2 days or 30 days before RT-PCR analysis for *GAD65* mRNA. (**C**) Cells were treated for 5 days with bFGF, PDGF, PDGF plus TH (triiodothyronine, 30 ng/ml), or bFGF plus TH before immunostaining for GC. (**D**) Cells were treated for 5 days with bFGF, PDGF, PDGF plus BMPs (BMP2 and BMP4, each at 10 ng/ml), or bFGF plus BMPs before immunostaining for GFAP. (**E** to **J**) Fluorescence micrographs of cells treated for 5 days with PDGF and TH (E) or PDGF and BMPs (F to J) before immunostaining as indicated: GC in (E), red; NFs in (E) and (G), green; A2B5 in (F), red; GFAP in (F), green, and in (G) and (H), red; and Ran-2 in (I), green. (J) is the fused image of (H) and (I). The same field of cells is shown in (H) to (J). Scale bar, 25 μm.

Table 1. Neuronal and glial markers in OPCs in various conditions. Purified P6 OPCs were cultured in PDGF for 30 days, in PDGF for 5 days, and then in PDGF and 15% FCS for 3 days to induce them to differentiate into type-2 astrocytes, or in PDGF for 5 days, PDGF and 15% FCS for 3 days, and then in serum-free medium containing bFGF alone for either 5 or 30 days.

Markers	PDGF × 30 days (%)	PDGF + 15% FCS × 3 days (%)	bFGF × 5 days (%)	bFGF × 30 days (%)
A2B5	89 ± 4	83 ± 6	38 ± 5	32 ± 3
GC	5 ± 0.4	0	0	0.7 ± 0.2
GFAP	0	79 ± 4	0	0
MAP2	1 ± 2	4 ± 1	38 ± 4	62 ± 5
NF-M	0	0	12 ± 2	37 ± 3
NF-L	0	0	10 ± 4	32 ± 1

Table 2. Evidence that BMP-2 plus BMP-4 can substitute for FCS in allowing neuronal development. Purified P6 OPCs were cultured in PDGF for 5 days, in PDGF and either FCS or BMPs for another 3 days, and then in serum-free medium with bFGF alone for 7 days.

Markers	15% FCS (%)	BMP2 + BMP4 (%)
A2B5	28 ± 5	21 ± 2
GC	0	0
GFAP	1 ± 0.6	1 ± 0.9
MAP2	72 ± 4	73 ± 2
NF-M	24 ± 3	14 ± 6
NF-L	21 ± 6	12 ± 4

trocytes in optic nerve cultures (*21*). None of the $GFAP^+$ cells were stained with the A2B5 monoclonal antibody (Fig. 2F), which stains the majority of type-2 astrocytes induced by FCS in optic nerve cultures (*8, 21*). Thus, at least some of the $GFAP^+$ cells seemed to be type-1 astrocytes. The $GFAP^+$ cells also did not stain with the anti-NF antibodies (Fig. 2G). Thus, the OPC-derived cells maintained in bFGF for 2 months could give rise to both oligodendrocytes and type-1 astrocytes, as well as to neurons.

To determine whether individual cells in cultures maintained in bFGF for 2 months were multipotential and able to give rise to neurons, oligodendrocytes, and astrocytes, we cultured them in bFGF in 96-well culture plates at an average density of one cell per well. After 2 to 3 weeks, about 10% of the wells contained a colony of cells, most of which probably derived from a single cell. We then cultured the cells from individual colonies on several PDL-coated glass coverslips in 24-well Falcon dishes (Falcon, Franklin Lakes, New Jersey) for 5 days in PDGF, TH, and BMPs and then stained them with various combinations of anti-NF, anti-GFAP, and anti-GC antibodies. In all cases, the individual colonies produced NF^+ cells, $GFAP^+$ cells, and GC^+ cells, and the cells stained with one antibody were not stained with the other antibodies (not shown). Thus, at least some of the cells cultured for 2 months in bFGF could give rise to neurons, astrocytes, and oligodendrocytes and therefore resembled multipotential CNS stem cells.

To determine whether BMPs could substitute for FCS in allowing OPCs to respond to bFGF by proliferating and reverting to a multipotential state, we cultured P6 OPCs in PDGF for 5 days, added BMPs for three more days, and then cultured them for a further 7 days in bFGF alone. Almost all of the cells became $GFAP^+$ type-2 astrocytes after 3 days in the BMPs, and these cells proliferated in the presence of bFGF alone. Moreover, more than 10% became NF^+ after 7 days in bFGF (Table 2). Thus, BMPs could substitute for FCS in these respects.

To test the possibility that the multipotential cells in our bFGF-stimulated cultures may have derived from a small population of CNS stem cells contaminating our initial OPC population, we cultured purified OPCs for 5 days in PDGF and then on dishes without the PDL coating for 2 weeks in bFGF alone. We cultured the neurosphere-like bodies that formed under these conditions on PDL-coated coverslips in PDGF alone for 4 days and then stained the cells for GC and the neuronal markers: >90% of the cells were GC^+ oligodendrocytes, and no cells were $MAP2^+$ or NF^+ (not shown). As CNS stem cells normally produce neurons rather than oligodendrocytes in these conditions (*14*), we conclude that our initial OPC population was not contaminated with CNS stem cells.

Taken together, our findings indicate that OPCs are not irreversibly committed to forming oligodendrocytes, type-2 astrocytes, or adult OPCs (*24*). Sequential exposure to FCS (or BMPs) and bFGF induces them to revert to a state that resembles that of multipotential CNS stem cells. OPCs in the adult CNS may also be able to revert in this way, as it was shown previously that cultures of adult rat optic nerve treated with bFGF can generate neurons (*25*). Our findings may have relevance for normal neural development, as BMPs are expressed in the ventricular zone of the developing rodent brain (*26*), and some $GFAP^+$ cells in the subventricular zone can behave like multipotential CNS stem cells (*27*). There is increasing evidence that stem cells generally have a broader developmental potential than previously thought and that local environmental cues normally restrict this potential in tissues [reviewed in (*28*)]. Adult hemopoietic stem cells, for example, can be reprogrammed to behave like embryonic hemopoietic stem cells if exposed to a fetal environment (*29*). The use of extracellular signal molecules to reprogram specified precursor cells in culture to become multipotential stem cells may prove useful for cell therapy, as specified precursors are generally more abundant and easier to purify than multipotential stem cells.

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20. Cells were harvested by trypsinization, and poly(A)$^+$ RNA was prepared using a QuickPrep Micro mRNA Purification kit (Pharmacia Biotech, Uppsala, Sweden); 1.5 μg of partially purified poly(A)$^+$ RNA was reverse-transcribed in 33 μl of reaction mixture, using a First-Strand cDNA Synthesis kit (Pharmacia Biotech). The RT-PCR reaction was carried out in 50 μl of reaction mixture that contained 3 μl of cDNA as template, 1 pmol of the specific oligonucleotide primer pair, and 1.25 units of *Taq* DNA polymerase. Cycle parameters for GAD65 cDNA were 30 s at 94°C, 30 s at 62°C, and 2 min at 72°C for 35 cycles. The cycle parameters for β-actin cDNA were 15 s at 94°C, 30 s at 53°C, and 1 min at 72°C for 25 cycles. The identity of the amplified products was checked by digestion with appropriate restriction enzymes. The following oligonucleotide DNA primers were synthesized: For rat GAD65, the 5′ primer was 5′-TCTTT-TCTCCTGGTGGTGCC-3′ and the 3′ primer was 5′-CCCCAAGCAGCATCCACAT-3′. For rat β-actin, the 5′ primer was 5′-TGGAATCCTGTGGCATCC-3′ and the 3′ primer was 5′-TCGTACTCCTGCTTGCTG-3′.
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Scanometric DNA Array Detection with Nanoparticle Probes

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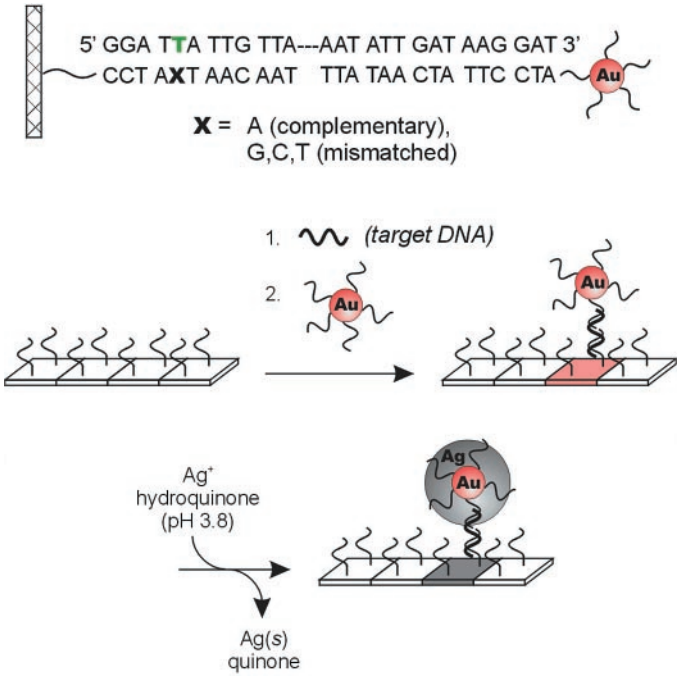
A method for analyzing combinatorial DNA arrays using oligonucleotide-modified gold nanoparticle probes and a conventional flatbed scanner is described here. Labeling oligonucleotide targets with nanoparticle rather than fluorophore probes substantially alters the melting profiles of the targets from an array substrate. This difference permits the discrimination of an oligonucleotide sequence from targets with single nucleotide mismatches with a selectivity that is over three times that observed for fluorophore-labeled targets. In addition, when coupled with a signal amplification method based on nanoparticle-promoted reduction of silver(I), the sensitivity of this scanometric array detection system exceeds that of the analogous fluorophore system by two orders of magnitude.

Sequence-selective DNA detection has become increasingly important as scientists unravel the genetic basis of disease and use this new information to improve medical diagnosis and treatment. Commonly used heterogeneous DNA sequence detection systems, such as Southern blots and combinatorial DNA chips, rely on the specific hybridization of surface-bound, single-strand capture oligonucleotides to complementary targets. Both the specificity and sensitivity of these assays are dependent on the dissociation properties of capture strands hybridized to perfect and to mismatched complements. Recently, we developed a nanoparticle-based detection scheme that uses two gold particle probes with covalently bound oligonucleotides that are complementary to a target of interest (*1*–*3*). When encountering target strands, these particle probes are polymerized and form network structures composed of thousands of particles. In addition, the polymerization process is accompanied by a red-to-blue color change, providing a means of detection. These network structures exhibit exceptionally sharp melting profiles; the full width at half-maximum (FWHM) for the first derivatives of these melting transitions is as low as 2°C. Sharp melting transitions allow one to differentiate a perfectly complementary target strand from a strand with a single base mismatch, regardless of position on a 24-nucleotide sequence. The sharp melting in this nanoparticle network system as compared with normal DNA duplex melting as monitored by ultraviolet (UV)–visible absorption at 260 nm derives, in part, from: (i) a cooperative effect due to the multiple duplex interconnects between particles in the network structure, and (ii) the monitoring of a nanoparticle optical signature that is sensitive to interparticle distance and particle aggregate size rather than a DNA base signature. Here we report that the use of single-nanoparticle probes in recognizing DNA segments immobilized on a chip affords substantially sharper and higher temperature melting profiles than those obtained with analogous, conventional fluorophore-based systems. This observation, combined with (i) the development of a quantitative signal amplification method based on nanoparticle-promoted reduction of silver(I) and (ii) the use of a conventional flatbed scanner as a reader, have allowed us to develop a new "scanometric" chip-based detection system for DNA that has single mismatch selectivity and a sensitivity that, at present, is 100 times greater than that of conventional, analogous fluorescence-based assays as monitored by confocal fluorescence microscopy.

Gold nanoparticles modified with oligonucleotides (*3*) were used to indicate the presence of a particular DNA sequence hybridized on a transparent substrate in a three-component sandwich assay format (Scheme 1). In a typical experiment, target-active substrates were fabricated by attaching 3′-thiol–modified capture oligonucleotides to the surface of float glass microscope slides (Fisher Scientific, Pittsburgh, Pennsylvania) according to procedures from the



Scheme 1.

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