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Disruption of the Human SCL Locus by "Illegitimate" V-(D)-J Recombinase Activity

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A fusion complementary DNA in the T cell line HSB-2 elucidates a provocative mechanism for the disruption of the putative hematopoietic transcription factor *SCL*. The fusion cDNA results from an interstitial deletion between a previously unknown locus, *SIL* (*SCL* interrupting locus), and the 5′ untranslated region of *SCL*. Similar to 1;14 translocations, this deletion disrupts the *SCL* 5′ regulatory region. This event is probably mediated by V-(D)-J recombinase activity, although neither locus is an immunoglobulin or a T cell receptor. Two other T cell lines, CEM and RPMI 8402, have essentially identical deletions. Thus, in lymphocytes, growth-affecting genes other than immune receptors risk rearrangements.

THE *SCL* (ALSO CALLED *TCL5* AND *tal*-1) gene is a member of the family of genes defined by a primary amino acid motif consisting of a basic domain (B) NH_2-terminal to a helix-loop-helix (HLH) structure (*1, 2*). This family of proteins participates in either the growth or differentiation of the tissues in which its members are expressed. In many cases the B-HLH motif confers both cell type–specific DNA binding as well as protein-protein dimerization capability on the encoded gene product (*3*). *SCL* was originally identified at the site of a 1;14 translocation associated with the development of a hematopoietic "stem" cell leukemia in a 16-year-old male (*4*). The *SCL* transcript is in developing human fetal liver and adult regenerative bone marrow, but it is not in more mature cells of the lymphoid or myeloid lineages. Because of its (i) association with a stem cell leukemia, (ii) pattern of expression, and (iii) identification as an HLH family member, it probably is a hematopoietic transcription factor involved in early hematopoiesis. Other 1;14 translocations involving the *SCL* locus have been characterized (*2, 5*) and are associated with the development of both stem cell and less mature T cell leukemias. All of the translocation breakpoints either structurally or functionally eliminate a portion of the 5′ untranslated region of the *SCL* message (*6*).

While studying the *SCL* transcript, we used the anchored polymerase chain reaction (PCR) technique (*7*) to clone cDNA from the T cell line HSB-2 (*8*). Sequence analysis of a subset of the HSB-2 cDNA clones revealed a novel 5′ exon. One of these clones was used as a probe in a ribonuclease (RNase) protection assay and was completely protected in the HSB-2 cell line (Fig. 1A). In some other tissues and cell lines, including those that do not express *SCL*, only the novel exon was protected, suggesting that the HSB-2 cDNAs were unlikely to represent a normal, common *SCL* variant. We used the same probe to screen a human bone marrow cDNA library. Two nonoverlapping sets of clones were obtained. One set belonged to *SCL*, and the other set contained the novel 5′ exon of the HSB-2 cDNA, no *SCL* homology, and was otherwise unique. The cDNA identified by the novel 5′ exon hybridized to a 5.5-kb mRNA from thymus and the T cell line SUPT1 (Fig. 1B), neither of which express the normal 5-kb *SCL* message. Thus, in the cell line HSB-2, two normally distinct transcripts, one of which was derived from *SCL*, had apparently become fused. The bulk of *SCL*, including the complete *SCL* coding sequence, had been retained in the fusion message, but the *SCL* 5′ end had been replaced by an exon that was normally found in a distinct locus (Fig. 2). We called this newly identified region "SIL" for SCL interrupting locus. As with *SCL*, *SIL* is conserved cross-species (*9*). We have not found any significant identity between *SIL* sequences and any gene sequence previously submitted to GenBank. It is unlikely that a chimeric *SIL/SCL* protein is formed, because *SIL* joins *SCL* in the *SCL* 5′ untranslated region upstream of an in-frame TAA stop codon that precedes the initiation ATG in the *SCL* message.

To analyze the formation of this fusion gene, we digested HSB-2 genomic DNA with Bam HI, Eco RI, or Hind III and probed with probe 2.2XX (Fig. 3A). Rearranged bands were seen in all three digests (the Hind III digest is shown in the left panel of Fig. 3B). These rearrangements are not seen in the SB cell line (*10*), a B lymphoblastoid line derived from the same patient as HSB-2 (*11*). Thus, the rearrangements are neither polymorphisms nor constitutional rearrangements. Using an *SIL*

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probe, we found identical rearrangements on all three of the above digests, indicating the rearrangement had made these formerly discrete loci contiguous. Two other T cell acute lymphoblastic leukemic (ALL) lines, CEM (*12*) and RPMI 8402 (*13*), had rearrangements indistinguishable from HSB-2 (Fig. 3B). That all three of these lines were unrelated despite the similarity of their *SCL/SIL* rearrangements was confirmed by their completely different rearrangements of the T cell receptor–β (TCR-β) (Fig. 3B). Thus, these three cell lines had independently undergone similar site-specific rearrangements of *SCL* and *SIL*. In addition, the same type of *SCL/SIL* fusion transcript identified in HSB-2 was seen in CEM and RPMI 8402 (*9*). These were three of the first cell lines with T cell properties established in continuous culture (*13*); perhaps this *SIL/SCL* rearrangement contributed to the relative ease of development of these three cell lines.

Despite this common rearrangement, these three lines do not share a consistent karyotypic abnormality of *SCL* on chromosome 1, band p33 (*11, 13*). Thus, the common rearrangement event might not be detected by cytogenetic studies. We therefore attempted to link *SIL* to *SCL* by pulsed-field gel (PFG) analysis (Fig. 3C). The Southern blot analysis of a Not I digest of unrearranged genomic DNAs shows an identical band of less than 260 kb when probed with either *SIL* or *SCL*. An Sal I digest (*9*) also showed a comigrating band of approximately 400 kb. Thus, the rearrangement was an interstitial deletion of chromosome 1 of less than 260 kb.

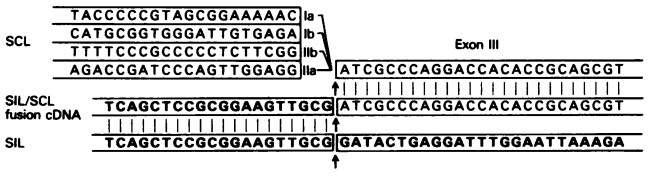

Fig. 2. Nucleotide sequence of *SCL*, *SIL*, and *SIL/SCL* fusion cDNA. Sequencing of both strands was done with USB Sequenase enzymes and protocols. *SCL* sequences are in capital letters; specific *SCL* exons are indicated with roman numerals. *SIL* sequences are in bold type. Identical nucleotide sequences are indicated by vertical lines. Exon splice junctions are indicated by arrows.

We used PCR to gain insight into the mechanism of the common rearrangement event that had occurred in these three T cell lines. Germline (unrearranged) *SIL* genomic clones from a placental library were compared to the amplified PCR-genomic *SIL/SCL* rearrangements from HSB-2, CEM, and RPMI 8402. These fragments were sequenced within the region involved in the breakage and rejoining event (Fig. 4). As anticipated from the Southern blot analysis, the breakpoints in all three lines occurred within eight nucleotides of each other, confirming the site specificity of the event. The primary DNA sequence of these germline and rearranged loci supported a V-(D)-J recombinase mechanism of DNA rearrangement. Immediately 5′ of the breakpoint in *SCL* is a heptamer sequence, GGCTGTG, a five of seven match with the V-(D)-J recombinase consensus signal sequence, CAC(T/A)GTG; the two inconsistent nucleotides are furthest from the actual breakpoint. Twenty-three bases 5′ of the heptamer is a nonamer sequence, TTTTCCTTA, consistent in spacing and primary sequence with the V-(D)-J recombinase signal. Immediately 3′ of the breakpoint in *SIL* is the heptamer CACTCTG, a five of seven match with the consensus. No convincing nonamer sequence was found 12 bases downstream of this heptamer.

Nontemplated nucleotides ("N" regions) were found at each *SIL/SCL* junction, three in HSB-2, seven in CEM, and nine in RPMI 8402. In CEM and RPMI 8402 there is evidence of "nibbling" away of DNA at the junction point, never amounting to more than ten nucleotides from either *SIL* or *SCL*. The established hallmarks of the immunoglobulin (Ig) or TCR recombinase system include site specificity organized around signal sequences, N-region addition, and nibbling at the coding sequence junctions (*14*). These criteria are fulfilled in the rearrangement events analyzed here. TCR gene analysis independently confirms that the recombinases have been active in these cell lines (Fig. 3B) (*15*). Although there is extensive data on intralocus Ig and TCR gene rearrangement (*16, 17*), interlocus rearrangement between Ig or TCR loci (*18, 19*), and recombinase-mediated rearrangement between an Ig or TCR locus and putative growth affecting genes (*20*), these data show V-(D)-J recombinase-mediated rearrangements that occurred naturally between two loci, neither one of which is an Ig or TCR. A related rearrangement may have been seen (*21*) under imposed selective pressure. In the presence of the recombinase system, genomic instability may be more widespread than thought, and such rearrangements may escape detection by conventional cytogenetics. Since chromatin accessibility can often serve as a marker of gene activation in a differentiated cell (*17, 22*), an implication of the *SIL/SCL* rearrangements is that these two loci were "accessible" (*23*) at the time that the recombinase was active in these cells.

It is possible that *SIL* and *SCL* represent genomic regions that normally undergo gene rearrangements analogous to Ig and TCR, but we have no evidence of this. Normal peripheral blood lymphocytes (PBLs) or PBLs from a patient with ataxia-

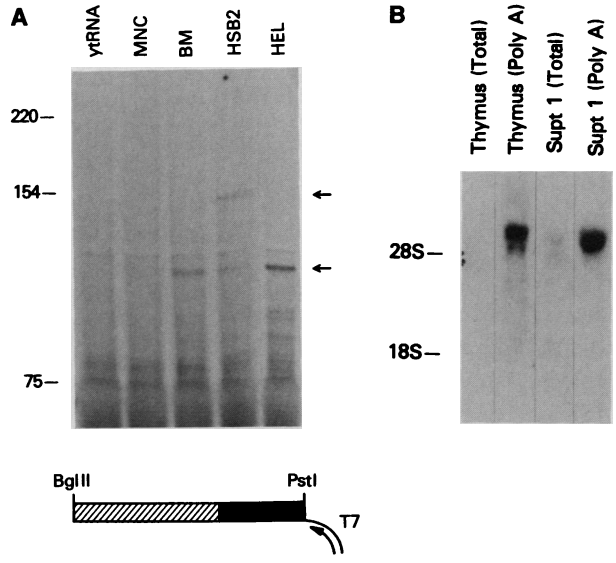

Fig. 1. (**A**) A plasmid that contained the novel exon derived from HSB-2 was used as a template to synthesize a radiolabeled antisense cRNA, with Promega Riboprobe reagents and protocols. Known *SCL* sequence (37 bp) is boxed; the 112-bp novel exon is represented by slashed lines. By standard techniques (*24*), the radiolabeled antisense cRNA (2×10^5 cpm) was hybridized to test RNA (30 μg of total RNA) at 50°C for 16 hours. The products were digested with RNase A, treated with proteinase K, extracted with phenol-chloroform, and separated on a 6% acrylamide–7M urea denaturing gel. Size standards were ^{32}P-labeled pBR322 fragments that had been digested with Hinf I and Eco RI. A band of 112 nucleotides can be seen in the bone marrow, HEL (*25*), and HSB-2 lanes, indicating protection of the novel exon only, while a band of 149 nucleotides representing full-length protection (of both the novel exon and known *SCL* sequences) is seen in HSB-2 only. (**B**) A 1.1-kb Kpn I–Eco RI fragment of *SIL* was labeled by nick-translation (*26*) and hybridized overnight at 42°C to a Northern blot (*26*) of RNA from thymus or the T cell line SUPT1 (*27*). Either total RNA (10 μg) or polyadenylated [poly(A$^+$)] selected RNA (2 μg) were loaded. The blot was washed three times at room temperature in 2× saline sodium citrate (SSC), 0.1% SDS, and two times at 52°C in 0.1× SSC, 0.1% SDS. A single band, enriched in the poly(A$^+$) fraction, can be seen at approximately 5.5 kb.

telangiectasia (a condition with increased recombinase-mediated interlocus rearrangements) (*19*) had no *SIL/SCL* rearrangements by PCR analysis. It is provocative, however, that this region of *SCL* has such a good heptamer, 23-bp spacer, and nonamer.

The *SIL/SCL* rearrangements disrupt the normal *SCL* transcript unit by disrupting a predominant *SCL* transcription start site and its related TATA promoter sequence (Fig. 4). The rearrangements are therefore reminiscent of the 1;14 translocations (*2, 5, 6*) in which the normal transcriptional regulation of the *SCL* gene is abrogated by the introduction of sequences from TCR-δ. All *SCL* disruptions alter the transcription unit and are observed in cells with deregulated growth and malignant potential, perhaps providing some selective advantage and possibly transforming potential to such cells. The existence of such similar and precise rearrangements further suggests that the particular deregulating effect of the *SIL/SCL* fusion may be important. Whereas this might only be a reflection of the proximity or accessibility of *SIL*, it could also reflect a contribution to growth deregulation caused by the regulatory influence of *SIL* or the disruption of the normal *SIL* product itself. The *SIL/SCL* fusion is not restricted to T cell lines. We have observed an identical *SIL/SCL* rearrangement in the leukemic cells of an adolescent male with T cell ALL (*9*).

It has long been speculated that genes other than Ig or TCR might use the V-(D)-J mechanism of gene rearrangement. Our observations support the concept that other differentially expressed loci can be targets for the V-(D)-J recombinase system. At times, the result of that targeting can be of consequence to the subsequent growth and development of the cells in which it occurs.

Note added in proof: Since the submission of this manuscript, Brown *et al.* (*30*) have reported genomic rearrangement consistent with *SIL/SCL* fusion in 13 of 50 children with T cell ALL.

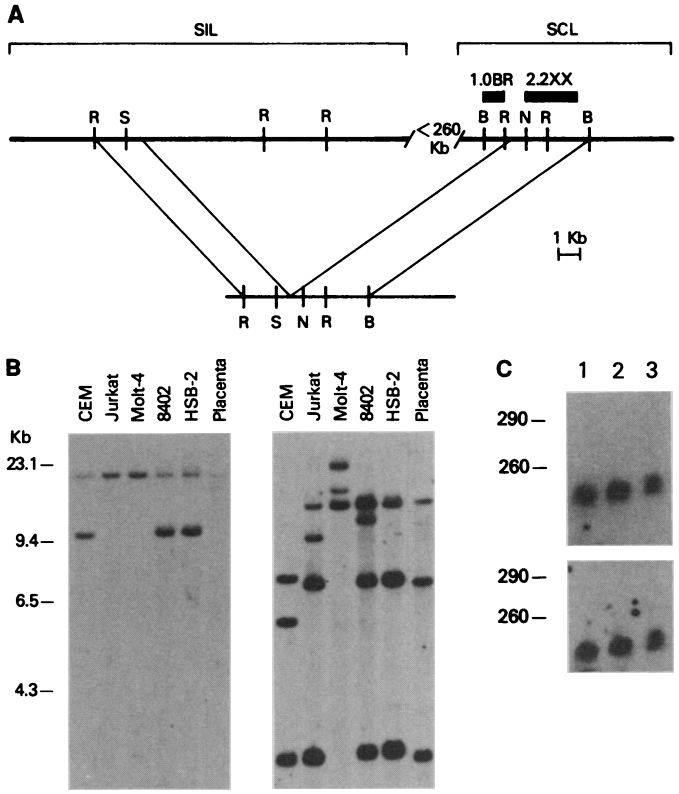

Fig. 3. (A) Genomic restriction map showing relevant portions of *SIL*. The interstitial deletion juxtaposes the two loci as shown. Restriction sites: S, Sst II; N, Not I; R, EcoRI; B, Bam HI (not all Sst II and Bam HI sites are shown). (**B**) Genomic Southern blot showing *SCL* rearrangement. (Left) Genomic DNA (10 μg) (*11, 13, 28*) was digested to completion with Hind III, size-fractionated on a 0.8% agarose gel, and transferred to a nylon membrane [Genescreen plus (Du Pont)]. The membrane was hybridized to *SCL* probe 2.2 XX and washed as in Fig. 1. A germline band at 21 kb is seen in all lanes; an additional rearranged band of approximately 10 kb is seen in the HSB-2, CEM, and RPMI 8402 lanes. (Right) The same genomic Southern blot was stripped and rehybridized to TCR-β constant region probe (*29*). Distinct rearranged bands can be seen in CEM, Jurkat, MOLT-4, and RPMI 8402; HSB-2 is germline. (**C**) Pulsed-field gel Southern blot analysis. Lanes 1 to 3, peripheral blood lymphocytes. (Top) Viable cells (5×10^5) were embedded in low-melt agarose and digested to completion with Not I. The DNA was size-fractionated on a 0.8% agarose gel with an LKB Pulsaphor with 170 V, 45-s pulses. Size markers were yeast chromosome DNA. Southern transfer and hybridization were as in Fig. 2. Hybridization to an *SCL* probe (1.0 BR) immediately 5′ of a Not I site showed a single band of <260 kb in all lanes. (Bottom) The blot was stripped and allowed to decay. After autoradiography the blot was rehybridized to the *SIL* probe described in Fig. 1B to confirm there was no residual signal. Again, a single band of <260 kb was seen. Overlaying the two autoradiographs showed the <260- kb band to be identical.

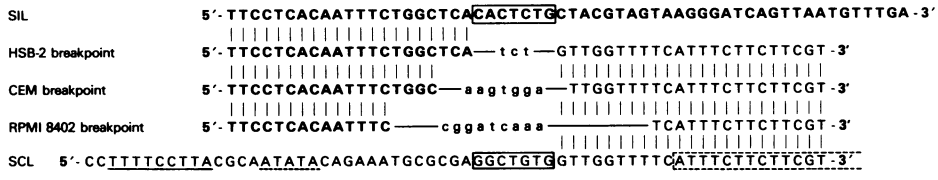

Fig. 4. Nucleotide sequence of germline *SIL*, germline *SCL*, and the breakpoints from HSB-2, CEM, and RPMI 8402. *SCL* sequences are in capital letters; *SIL* sequences are in bold type. Identical nucleotide sequences are indicated by vertical lines. Nongermline-encoded nucleotides at the *SIL/SCL* breakpoints (presumably added by the mechanism for "N-region" addition) are indicated in lowercase type. Heptamer sequences are boxed, and a nonamer sequence 23 bp upstream of the *SCL* heptamer is underlined. *SCL* exon Ib (*5*) is indicated by a dashed box with its TATA motif 30 bases upstream underlined with dashes. Germline *SCL* and *SIL* sequence was obtained from plasmid subclones of genomic phage clones. The genomic rearrangements were cloned using a 5′ *SIL* oligonucleotide, 5′-GCTCCTACCCTGCAAACAGA-3′, and a 3′ *SCL* oligonucleotide, 5′-ATTTAGAGAGACCGGCCCCTCTGAATAGG-3′, to amplify 1 μg of genomic DNA. PCR amplification was accomplished with 250 ng of each primer, 2.5 U of Taq polymerase (Cetus) in 100 μl of 200 μM each deoxynucleoside triphosphate, 50 mM KCl, 10 mM tris (pH 8.3), 1.5 mM $MgCl_2$, and 0.01% gelatin, with 30 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 6 min. The PCR products were then subcloned into the Not I and Sst II sites for pBluescript II (Stratagene), and at least three independent subclones of each rearrangement were sequenced.

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7. A modification of the technique described by M. A. Frohman, M. K. Dush, and G. Martin [*Proc. Natl. Acad. Sci. U.S.A.* **85**, 8998 (1988)] was used. Briefly, total cellular RNA (10 μg) from HSB-2 was annealed to an SCL-specific primer 5′-GGCATATTTAGAGAGACCG-3′, and first-strand cDNA synthesis was carried out with Moloney murine leukemia virus reverse transcriptase (BRL). First-strand cDNA purification and tailing with deoxyadenosine triphosphate was performed as described (M. A. Frohman, M. K. Dush, G. Martin, above). PCR [R. K. Saiki *et al.*, *Science* **230**, 1350 (1985)] amplification was accomplished with a 5′ oligonucleotide, 5′-GACTCGACTCGACATCGATTTTTTTTTTTTTTTT-3′, containing Sal I, Cla I, and Xho I sites, and a "nested" SCL-specific 3′ oligonucleotide, 5′-ACCGGCCCCTCTGAATAGG-

3'. Thermal cycling was carried out for 40 cycles (95°C, 45 s; 50°C, 1 min; 72°C, 2 min). The PCR products were then subcloned into the Pst I and Cla I sites of pSP 73 (Promega).
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31. We thank G. Reaman, Children's Hospital National Medical Center, for providing tissue samples from patients with T cell ALL. We also thank M. Fourcault for expert technical assistance.

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D_1 and D_2 Dopamine Receptor–Regulated Gene Expression of Striatonigral and Striatopallidal Neurons

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The striatum, which is the major component of the basal ganglia in the brain, is regulated in part by dopaminergic input from the substantia nigra. Severe movement disorders result from the loss of striatal dopamine in patients with Parkinson's disease. Rats with lesions of the nigrostriatal dopamine pathway caused by 6-hydroxydopamine (6-OHDA) serve as a model for Parkinson's disease and show alterations in gene expression in the two major output systems of the striatum to the globus pallidus and substantia nigra. Striatopallidal neurons show a 6-OHDA–induced elevation in their specific expression of messenger RNAs (mRNAs) encoding the D_2 dopamine receptor and enkephalin, which is reversed by subsequent continuous treatment with the D_2 agonist quinpirole. Conversely, striatonigral neurons show a 6-OHDA–induced reduction in their specific expression of mRNAs encoding the D_1 dopamine receptor and substance P, which is reversed by subsequent daily injections of the D_1 agonist SKF-38393. This treatment also increases dynorphin mRNA in striatonigral neurons. Thus, the differential effects of dopamine on striatonigral and striatopallidal neurons are mediated by their specific expression of D_1 and D_2 dopamine receptor subtypes, respectively.

THE BASAL GANGLIA ARE A MAJOR brain system through which the cerebral cortex affects behavior. Processing of cortical input in the striatal portion of the basal ganglia is modulated by dopaminergic input from the substantia nigra (*1*). This dopaminergic modulation has profound effects on behavior. Reduction in striatal dopamine levels, as in Parkinson's disease, results in reduced movement (*2*), whereas excessive dopaminergic action within the striatum produces involuntary dyskinetic movements (*1*). Dopamine differentially regulates the two major output pathways from the striatum, one to the globus pallidus (the external segment of the globus pallidus in primates) and the other to the substantia nigra and entopeduncular nucleus (the internal segment of the globus pallidus in primates) (*1, 3*). These output pathways arise from the major postsynaptic target of dopamine in the striatum, the medium spiny neurons (*4*), which constitute more than 90% of the striatal neuronal population. Although these neurons often provide collaterals to more than one nucleus, the target of the main axon collateral distinguishes two subpopulations of approximately equal numbers of striatopallidal neurons and striatonigral neurons (the entopeduncular nucleus and substantia nigra are here considered part of the same extended nuclear complex) (*5*). All of the medium spiny neurons utilize γ-aminobutyric acid (GABA) as a neurotransmitter (*6*), but the two subpopulations contain different neuropeptides. Striatopallidal neurons contain enkephalin, whereas striatonigral neurons contain substance P and dynorphin (*7, 8*). These peptides are differentially regulated by dopamine. Reduction of dopamine input to the striatum results in increased expression of enkephalin (*9, 10*) and a reduction in substance P (*11*). Conversely, increases in dopamine receptor stimulation result in increases in both substance P and dynorphin levels (*12*). These actions of dopamine may be differentially mediated by D_2 and D_1 dopamine receptors (*13*). Both D_1 and D_2 dopamine receptors act via G proteins; D_1 receptors activate and D_2 receptors inhibit adenylyl cyclase activity in striatal neurons (*14*). Indirect evidence suggests that D_1 and D_2 dopamine receptors are differentially expressed in striatonigral and striatopallidal neurons (*15*).

In a first experiment, striatonigral and striatopallidal neurons were characterized by their expression of D_1 and D_2 dopamine receptor subtypes and the peptides dynorphin, substance P, and enkephalin with in situ hybridization histochemistry (ISHH) combined with retrograde labeling of striatonigral neurons (Fig. 1). Oligonucleotide probes (48 bases in length) complementary to the mRNA encoding the D_1 and D_2 dopamine receptors and the peptides dynorphin, substance P, and enkephalin (*16*) were labeled with ^{35}S-labeled deoxyadenosine monophosphate (dAMP) tails of approximately 25 bases in length (*17*) and used for ISHH labeling of striatal sections from animals that had received an injection of fluorogold into the substantia nigra (*18*). Approximately 43% of the striatal cells were retrogradely labeled with fluorogold (194 of 452 cells). Fluorogold-labeled striatonigral neurons were often labeled with the D_1 probe (149 of 173 D_1 cells), the dynorphin

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