over by the progeny of a totipotential stem cell carrying the t(9;22) chromosome translocation (*17*), but the major selective advantage, producing the clinical neoplasms, appears to lie within the myeloid lineage at the granulocyte stage of differentiation. Cells of lineages such as the B and T cells carrying the Philadelphia chromosome are much less expanded, or not at all (*17*).

It is also possible that the levels of *bcl-2* transcripts may be higher in more mature B cells than in the pre–B cells from which they are derived. This possibility can be tested by introducing the activated *bcl-2* gene into B cells at different stages of differentiation, as we have done in the case of the involved c-*myc* gene of Burkitt lymphomas (*18*).

We have shown that the locus for the α chain of the T-cell receptor resides in that region (q11.2) of chromosome 14 that is involved in translocations and inversion in T-cell neoplasms (*19*). It will also be of considerable importance to determine whether the enzymatic system that is involved in the joining of the separated DNA segments coding for the α chain of the T-cell receptor is also directly involved in the chromosomal rearrangements observed in T-cell malignancies.

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Site-Specific Increased Phosphorylation of pp60$^{v\text{-}src}$ After Treatment of RSV-Transformed Cells with a Tumor Promoter

Abstract. *When vole cells that had been transformed by Rous sarcoma virus were treated with the tumor-promoting phorbol ester 12-O-tetradecanoyl-13-acetate (TPA), specific phosphorylation of pp60$^{v\text{-}src}$ was increased. Partial V8 protease mapping indicated that the increased phosphorylation occurred exclusively on serine residues located in the amino terminus of the molecule. Treatment of cells with dimethyl sulfoxide or 4α-phorbol-12,13-didecanoate did not elicit this response. Two-dimensional tryptic phosphopeptide mapping of pp60$^{v\text{-}src}$ immunoprecipitated from untreated and TPA-treated cells indicated that a specific tryptic amino-terminal peptide was hyperphosphorylated.*

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Transformation of cells by Rous sarcoma virus (RSV) is due to the expression of a single viral gene, v-*src* (*1*). The product of this gene is a 60-kilodalton (kD) phosphoprotein termed pp60$^{v\text{-}src}$ (*2*), which contains a protein kinase activity specific for tyrosine residues (*3*). Structural analysis of pp60$^{v\text{-}src}$ by limited proteolysis with V8 protease resolved the molecule into a 34-kD amino-terminal fragment (V1) and a 26-kD carboxyl-terminal fragment (V2) (*4*). Increasing amounts of protease resulted in the further digestion of the amino-terminal 34-kD fragment and the appearance of overlapping 18-kD (V3) and 16-kD (V4) protease cleavage products containing amino-terminal residues (*1*, *4*).

The 34-kD fragment contains mainly phosphoserine residues. The major phosphotyrosine residue is located at position 416 of the 26-kD carboxyl-terminal fragment (*4*, *5*). Minor tyrosine phos-

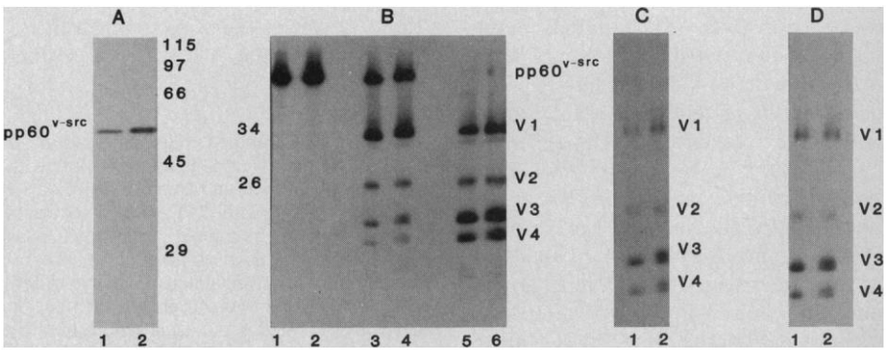

Fig. 1. Partial protease analysis of pp60$^{v\text{-}src}$ from TPA- and DMSO-treated cells. (A) The cells were European field vole *(Microtus agrestis)* cells transformed by the Schmidt-Ruppin strain (subgroup D) of RSV (clone I–T; originally provided by A. J. Faras, University of Minnesota Medical School), hereafter referred to as IT cells. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5 percent calf serum. The cells were labeled for 4 hours in phosphate-free DMEM containing 5 percent calf serum and [^{32}P]orthophosphate (1 mCi/ml). Cells were then treated with TPA in DMSO (100 ng/ml), 4α-PDD in DMSO (100 ng/ml), or DMSO alone for 20 minutes. The final concentration of DMSO in each case was 0.2 percent. The pp60$^{v\text{-}src}$ was immunoprecipitated with monoclonal antibody EB7DE61 as described (*2*). Immunoprecipitates were fractionated on 10 percent polyacrylamide–SDS gels (*16*); the gel was dried and autoradiographed with Cronex 4 x-ray film and Lightening-Plus intensifying screens. (Lane 1) IT cells treated with DMSO; (lane 2) cells treated with TPA. The numbers on the right indicate the position of molecular weight standards in kilodaltons. (B) ^{32}P-labeled pp60$^{v\text{-}src}$ was purified from DMSO- or TPA-treated cells by immunoprecipitation and two cycles of SDS-PAGE and analyzed by partial V8 protease analysis as described (*4*, *17*). (Lanes 1, 3, and 5) DMSO-treated cells; (lanes 2, 4, and 6) TPA-treated cells. (Lanes 1 and 2) No enzyme; (lanes 3 and 4) 5 ng of enzyme; (lanes 5 and 6) 50 ng of enzyme. The separation gel was a 16-cm 15 percent polyacrylamide–SDS gel, and electrophoresis was stopped as soon as the bromophenol blue dye reached the bottom. Numbers on the left refer to molecular weight in kilodaltons. (C) Same as lanes 5 and 6 in (B), except that electrophoresis was continued at 30 mA for 1 hour after the dye reached the bottom of the gel. (Lane 1) DMSO-treated cells, 50 ng of enzyme; (lane 2) TPA-treated cells, 50 ng of enzyme. (D) Cells were labeled with [^{32}P]orthophosphate and treated with TPA or 4α-PDD for 20 minutes as above. The pp60$^{v\text{-}src}$ was immunoprecipitated and analyzed by partial V8 protease digestion as described in (C). (Lane 1) 4α-PDD-treated cells, 50 ng of enzyme; (lane 2) TPA-treated cells, 50 ng of enzyme.

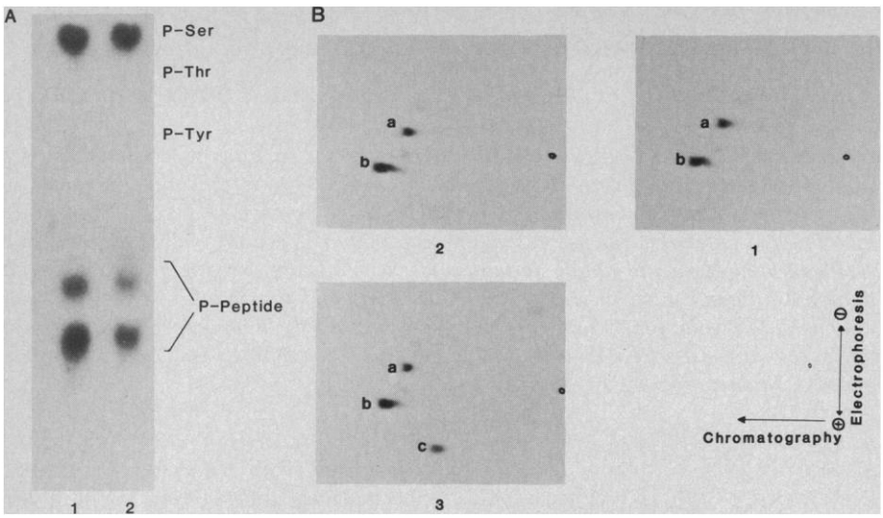

Fig. 2. Two-dimensional tryptic mapping and phosphoamino acid analysis of pp60$^{v\text{-}src}$. (A) pp60$^{v\text{-}src}$ was purified from DMSO- and TPA-treated cells by immunoprecipitation and two cycles of polyacrylamide gel electrophoresis. The gel slices containing pp60$^{v\text{-}src}$ were excised and digested with V8 protease (500 ng). The resulting fragments were separated on a preparative 15 percent polyacrylamide–SDS gel. The V3 and V4 bands were localized by autoradiography, eluted, and subjected to phosphoamino acid analysis as described (*4*). (Lane 1) V3 plus V4 from DMSO-treated cells; (lane 2) V3 plus V4 from TPA-treated cells. (B) The V3 plus V4 fragments of pp60$^{v\text{-}src}$ were purified from cells treated with DMSO (1), 4α-PDD (2), or TPA (3). The material was digested with TPCK-treated trypsin (TPCK, L-1-tosylamide-2-phenylethylchloromethyl ketone) and analyzed by chromatography and electrophoresis on plastic-backed cellulose sheets (*3*).

phorylation sites have also been identified in the amino-terminal half of the pp60$^{v\text{-}src}$ molecule; however, these appear to be short-lived (*6*). Previous results indicated that phosphorylation of serine residues in the 34-kD fragment of pp60$^{v\text{-}src}$ occurred in an adenosine 3′,5′ monophosphate (cyclic AMP)–dependent fashion (*4*), whereas phosphorylation of tyrosine residues at both amino and carboxyl terminals may be the result of autophosphorylation (*7, 8*).

We investigated alterations in the phosphorylation of pp60$^{v\text{-}src}$ after treatment of RSV-transformed cells with the tumor-promoting phorbol ester 12-*O*-tetradecanoyl-13-acetate (TPA). The TPA receptor has been identified as an 80-kD membrane protein with protein kinase activity that is activated by TPA in vitro (*9*). Our results indicate that treatment of RSV-transformed vole cells with TPA increases the phosphorylation of a serine residue located in the amino terminus of the molecule.

Figure 1A shows a sodium dodecyl sulfate (SDS)–polyacrylamide gel analysis of pp60$^{v\text{-}src}$ immunoprecipitated from RSV-transformed vole cells (IT cells) that were labeled for 4 hours with [^{32}P]orthophosphate and then treated for 20 minutes with either dimethyl sulfoxide (DMSO) or TPA. The phosphorylation of pp60$^{v\text{-}src}$ increased 50 percent when cells were treated with TPA. Enhanced labeling of the V3 and V4 fragments indicated that this increased phosphate incorporation occurred at the amino end of the molecule (Fig. 1B and Table 1). When these gels were run for longer times, the V3 and V4 amino-terminal fragments from the TPA-treated cells were clearly resolved into two doublets (lane 2 in Fig. 1C). The doublets were not as well resolved when the V8 fragments were analyzed under our standard conditions (lane 6 in Fig. 1B).

Table 1. TPA-induced phosphorylation of pp60$^{v\text{-}src}$. IT cells were labeled for 4 hours with [^{32}P]orthophosphate and treated with DMSO or TPA for 20 minutes. Detergent extracts were prepared, a sample from each was precipitated with trichloroacetic acid, and radioactive counts were determined. No difference between the two samples in the specific activity of labeled protein, as determined by protein content, were observed. Equal amounts of protein were immunoprecipitated, and the precipitates were fractionated on an SDS–polyacrylamide gel. Supernatants were precipitated again to ensure antibody excess. No additional pp60$^{v\text{-}src}$ was detected. pp60$^{v\text{-}src}$ was localized by autoradiography. The bands were excised and quantified by scintillation spectrometry. The samples were further analyzed by partial V8 protease as described in Fig. 1. The fragments were localized by autoradiography, excised, and counted by scintillation spectrometry. The V2 band was taken as the carboxyl end of the molecule, and the V1, V3, and V4 bands were pooled and counted as the amino end of the molecule.

	DMSO-treated (count/min)	TPA-treated (count/min)	Increase
pp60$^{v\text{-}src}$	9176	13,774	50
Amino end	2563	4,670	82
Carboxyl end	802	812	0

The biologically inactive phorbol analog 4α-phorbol-12,13-didecanoate (4α-PDD) had no effect on the phosphorylation of pp60$^{v\text{-}src}$ as assayed by partial V8 protease mapping, whereas TPA treatment resulted in the V3 and V4 fragments migrating as well-resolved doublets (lane 2 in Fig. 1D). Phosphoamino acid analysis of the V3 and V4 fragments from TPA-treated cells indicated that the increased phosphorylation occurred on serine residues (Fig. 2A).

The possibility that TPA treatment resulted in the phosphorylation of a new site on pp60$^{v\text{-}src}$ was examined. The V3 and V4 protease fragments purified by preparative SDS–polyacrylamide gel electrophoresis (SDS-PAGE) were eluted and subjected to two-dimensional tryptic phosphopeptide analysis. Treatment with TPA resulted in the increased phosphorylation of a major tryptic peptide (spot c in Fig. 2B3), which under these exposure conditions was not visible in maps of pp60$^{v\text{-}src}$ obtained from cells treated with DMSO or 4α-PDD (Fig. 2B, 1 and 2). Longer exposure of these maps revealed that phosphorylation of this tryptic peptide occurred in these cells, although at a much lower level than in cells treated with TPA. A second tryptic peptide of pp60$^{v\text{-}src}$, which is phosphorylated at a much lower level than peptide C, can be seen migrating toward the cathode in the V3 plus V4 maps of pp60$^{v\text{-}src}$ from TPA-treated cells (Fig. 2B3). This observation was not limited to IT cells. Treatment of RSV-transformed BALB/c cells with TPA had the same effect on the V3 and V4 fragments of pp60$^{v\text{-}src}$ as it did in the RSV-transformed vole cells.

The mediator of the phosphorylation that occurs in response to TPA may be protein kinase C, a phospholipid and Ca^{2+}-activated kinase (*10*). Phosphorylation of the receptor for epidermal growth factor (EGF) increased when cells were treated with TPA. Treatment of A431 cells with TPA and EGF resulted in the phosphorylation of two threonine residues within the EGF receptor molecule. This phosphorylation event modulates both the EGF binding and protein kinase activity of the EGF receptor (*11*). Addition of protein kinase C to purified A431 membranes resulted in the phosphorylation of the same threonine residues in the EGF receptor as was seen in intact cells (*11*).

The increased phosphorylation of pp60$^{v\text{-}src}$ in TPA-treated cells parallels that observed for the EGF receptor, with the exception that we have consistently observed a low degree of phosphorylation of peptide C in untreated cells. Our results suggest that an interaction (direct or indirect) may take place between pp60$^{v\text{-}src}$ and the phorboid receptor kinase. The use of purified components in an in vitro reaction will determine whether protein kinase C can phosphorylate pp60$^{v\text{-}src}$.

Phosphorylation and dephosphorylation regulate the activity of numerous enzymes (*12*). Phosphorylation of tyrosine residues in the amino-terminal half of pp60$^{v\text{-}src}$ increases the protein kinase activity of pp60$^{v\text{-}src}$ (*8, 13*). Removal of the major phosphorylated tyrosine residue of pp60$^{v\text{-}src}$ does not affect the protein kinase activity or the transformation ability of the molecule in vitro (*14*). However, cells transformed by the mutated gene were able to form tumors only in immunodeficient mice (*15*). Our results suggest that phosphorylation of pp60$^{v\text{-}src}$ at serine residues can occur in a cyclic AMP–independent (in addition to a cyclic AMP–dependent) fashion. Phosphorylation of pp60$^{v\text{-}src}$ at this additional serine site could affect the enzyme in a number of ways, including alteration of enzymatic activity, substrate specificity, or subcellular localization. Preliminary immune-complex tyrosine kinase assays in which casein or angiotensin are used as substrates have revealed no overall difference in the kinase activity of pp60$^{v\text{-}src}$ immunoisolated from control cells and from TPA-treated cells. However, these assays do not rule out small differences in enzyme specificity or kinetic parameters. The effects of phosphorylation on the enzymatic activity of pp60$^{v\text{-}src}$ are not yet clear.

Note added in proof: TPA treatment of normal uninfected cells resulted in the hyperphosphorylation of pp60$^{c\text{-}src}$ at a novel serine residue (*18*).

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Abnormal Visual Pathways in Normally Pigmented Cats That Are Heterozygous for Albinism

Abstract. *The various forms of albinism affect about one in 10,000 births in the United States. An additional 1 to 2 percent of the population has normal pigmentation but is heterozygous and carries a recessive allele for albinism. The retinogeniculocortical pathways were studied in normally pigmented cats that carry a recessive allele for albinism. The cats exhibited abnormalities in their visual pathways similar to those present in homozygous albinos. These results imply that visual anomalies like those found in albinos may be present in 1 to 2 percent of the human population.*

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Over the past 20 years the abnormal retinogeniculocortical pathways in albino mammals have been described in detail (*1–7*). In mammals homozygous for the albino gene, an abnormally large proportion of the axons of retinal ganglion cells originating in the temporal retina cross to the contralateral side of the brain. As a result, in albinos both the visual thalamus [the dorsal lateral geniculate nucleus (LGNd)] and the visual cortex (areas 17 and 18) contain an abnormally large representation of the contralateral temporal retina and, therefore, the ipsilateral visual field (*2–4, 7, 8*).

We report here evidence that normally pigmented heterozygous cats with one homozygous tyrosinase-negative albino parent and one normally pigmented parent (obligate heterozygotes) have visual system abnormalities similar to those in homozygous albinos. The widely held belief that visual system abnormalities are restricted to mammals that are obviously hypopigmented and homozygous for albinism must therefore be reevaluated.

The cats used in this study were bred in our colony originating from albino cats with no measurable tyrosinase activity. These albino cats are presumed to be homozygous for a *c*-locus tyrosinase-

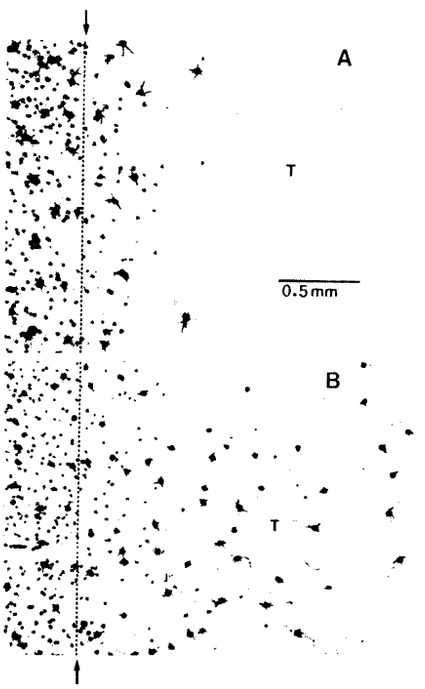

Fig. 1. Photomicrographs showing the contralateral retina of a homozygous, normally pigmented cat (A) and that of a heterozygote for albinism (B). Both animals received injections of HRP unilaterally into the LGNd. The position of the vertical meridian is indicated (arrows). The presence of many labeled cells in the contralateral temporal retina (T) of the heterozygote indicates that an abnormally large proportion of retinal ganglion axons cross at the optic chiasm and project to the opposite side of the brain.