with no more cell death than previously reported (*6*). Since the H9 line was derived by infection of target HT cells with pooled AIDS patient material (*6*), different proviruses with variant transcriptional patterns might be expressed in different H9 populations. Alternatively, the results we report could reflect differences in RNA analysis. Our poly(A)$^+$ RNA's have been subjected to electrophoresis through long 1 percent agarose gels (migration of bromophenol blue dye = 18 cm) at 40 to 50 V to allow clear separation of large RNA species such as the 5.5- and 5.0-kb transcripts. RNA's of this size are not clearly resolved in the analysis of H4 cells (*9*).

Although the Northern blot hybridizations shown above demonstrate that the 5.5- and 5.0-kb AIDS RV RNA's contain LTR, "A," *env*, and "B" region sequences, such an analysis can only approximate the actual mRNA structure. For example, the 5.5- and 5.0-kb mRNA's hybridize to probe 4 DNA (Figs. 1 and 2) but not to probe 3. This implies that the splice acceptors for these mRNA's must lie between the 3′ end of probe 3 (position 4156) and the 3′ end of probe 4 (position 5134). In preliminary S1-nuclease experiments (*10*), we have identified a major splice acceptor 5′ to the "A" region at 4914 bp that could generate an mRNA of approximately 5.0 kb that would be in the proper reading frame for translation of "A" sequences. In addition, we have identified a second splice acceptor site at 4542 bp which could serve as an acceptor for the 5.5-kb RNA. Complementary DNA clones of these novel mRNA's will allow detailed study of their structure. The potential function of an "A" region gene product (or products) remains obscure. The *trans*-activating protein of the AIDS RV has recently been shown to be encoded by a doubly spliced mRNA (*9*) that contains 215 bp of coding sequence entirely 3′ to the "A" open reading frame (positions 5376 to 5590). Furthermore, deletion of the "A" coding region does not abolish the *trans*-activation property of the AIDS RV (*11*). Thus "A" does not encode the *trans*-activating property nor does it specify any known viral structural protein. Site-specific mutagenesis of "A" region sequences as well as expression of cDNA clones may elucidate the function of this novel retroviral gene.

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19. We thank S. McCoy and S. Phillips for preparation of virus-infected cells, R. Willey and R. Rutledge for subcloning and isolation of viral DNA segments, J. Sears for assistance in preparation of figures, and S. Rosenfeld and H. Jordan for editorial assistance.

9 April 1985; accepted 6 August 1985

The t(14;18) Chromosome Translocations Involved in B-Cell Neoplasms Result from Mistakes in VDJ Joining

Abstract. *In this study, the joining sequences between chromosomes 14 and 18 on the 14q$^+$ chromosomes of a patient with pre–B-cell leukemia and four patients with follicular lymphoma carrying a t(14;18) chromosome translocation were analyzed. In each case, the involved segment of chromosome 18 has recombined with the immunoglobulin heavy-chain joining segment (J_H) on chromosome 14. The sites of the recombination on chromosome 14 are located close to the 5′ end of the involved J_H segment, where the diversity (D) regions are rearranged with the J_H segments in the production of active heavy-chain genes. As extraneous nucleotides (N regions) were observed at joining sites and specific signal-like sequences were detected on chromosome 18 in close proximity to the breakpoints, it is concluded that the t(14;18) chromosome translocation is the result of a mistake during the process of VDJ joining at the pre–B-cell stage of differentiation. The putative recombinase joins separated DNA segments on two different chromosomes instead of joining separated segments on the same chromosome, causing a t(14;18) chromosome translocation in the involved B cells.*

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Reciprocal chromosomal translocations involving the heavy-chain locus on chromosome 14 (*1*, *2*) have been observed in human B-cell neoplasms (*3*, *4*) and in Burkitt lymphomas (*5*). In diffuse B-cell lymphomas and in chronic lymphocytic leukemias of the B-cell type carrying the t(11;14)(q13;q32) chromosome translocation (*4*, *6*), the *bcl*-1 locus, normally located at band q13 of chromosome 11, translocates to the heavy-chain locus on chromosome 14 (*7*). In follicular lymphomas with the t(14;18) translocation, the *bcl*-2 gene (which is normally located at band q21 of chromosome 18) translocates to the heavy-chain locus (*8*, *9*). The translocation results in enhanced expression of the *bcl*-2 gene (*9*). By analyzing the joining between chromosomes 11 and 14 on the 14q$^+$ chromosome of two independent cases of chronic lymphocytic leukemia of the B-cell type with the t(11;14) chromosome translocation, we discovered that the breakpoints on chromosome 14 involved the J_H (J, joining; H, heavy) region at the sites where D (diversity) segments recombine with J_H DNA segments to generate a productively rearranged heavy-chain gene (*10*).

In this study, we have characterized the chromosome breakpoints in a pre–B-cell leukemia (380) (*8*, *11*) and in four independent follicular lymphomas carrying a t(14;18) chromosome translocation to investigate the molecular mechanisms involved in the t(14;18) chromosome translocation. We have previously described the cloning of the joining between chromosomes 14 and 18 in a pre–B-cell leukemia carrying a t(14;18) chromosome translocation (*8*), and the con-

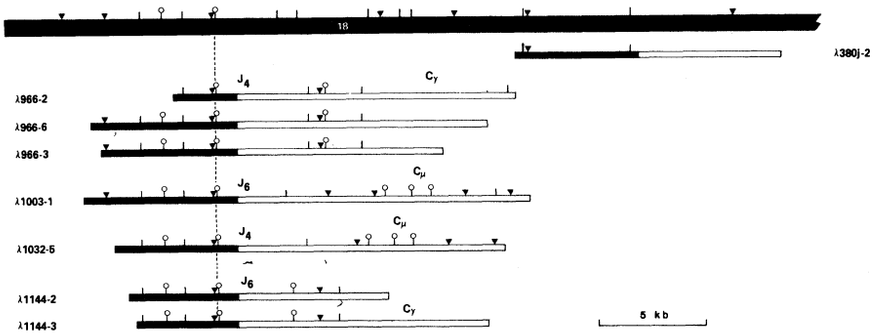

Fig. 1. Restriction maps of the regions surrounding the breakpoints of the t(14;18) chromosome translocation in follicular lymphomas. Top bar shows chromosome 18. The horizontal bars under chromosome 18 represent insert DNA's of the recombinant clones containing the breakpoints of t(14;18) translocation from leukemia 380 cells and four follicular lymphoma DNA's. The filled and open segments represent chromsome 18 and 14 sequences, respectively. The breakpoints were mapped by comparing the detailed restriction maps of breakpoint-containing clones and corresponding normal chromosome 18 clones that were previously isolated (*9*). The restriction enzyme sites of Hind III, Sst I, and Eco RI are shown by |, ▼, and ?, respectively.

struction of λ phage libraries from neoplastic specimens of four patients with follicular lymphoma (FL) (FL 966, 1003, 1032, and 1144) was carried out as described (*9*). Their DNA's were partially digested with Sau 3A and fractionated by sucrose gradient centrifugation. Then, 15- to 23-kilobase (kb) DNA fragments were pooled and ligated with λ EMBL3A phage vector DNA (*7, 8*). After in vitro packaging, we screened approximately 200,000 recombinant clones from each follicular lymphoma DNA library with a *bcl*-2 DNA probe, a DNA fragment of chromosome 18 between the Hind III and the Sst I site closest to the breakpoints (Fig. 1).

DNA from clones λ966-2, -3, and -6, λ1003-1, λ1032-5, λ1144-2, and λ1144-3 hybridized with the J_H specific probe pHj (*8, 9*), confirming the involvement of the J_H region of the heavy-chain locus in the t(14;18) chromosome translocation of follicular lymphoma. We have mapped the breakpoints (Fig. 1) and determined the nucleotide sequences surrounding the breakpoints and the corresponding sequences on normal chromosome 18 (Fig. 2). We have also determined the nucleotide sequences surrounding the joining between chromosomes 14 and 18 in the pre–B-cell leukemia 380 and the corresponding sequences of normal chromosome 18. The breakpoints in all four follicular lymphomas we examined were clustered and were located within 104 base pairs (bp) of each other (Fig. 2) on chromosome 18. This region is separated from the chromosome 18 breakpoint in leukemia 380 cells by approximately 18.5 kb (Fig. 1). The DNA sequences beyond the points where the breakpoint sequences started to diverge from chromosome 18 sequences were identical or almost identical to that of one of the J_H segments of the heavy-chain locus. The t(14;18) chromosome translocation of leukemia 380 cells involved the J_5 segment, that of FL 966 and FL 1032 involved the J_4 segment, and that of FL 1003 and FL 1144 involved the J_6 segment (Fig. 2). The DNA sequences 3′ to the J_4 segment of FL 966 and FL 1032 show considerable differences from the published J_4 3′ flanking sequences (*12*) but are identical to those we determined by the analysis of the t(11;14) chromosome breakpoints in two cases of chronic lymphocytic leukemia of the B-cell type (CLL) (*10*). It is possible that the discrepancy may be due to genetic polymorphism, although such sequences were identical in all cases we examined (*10*).

As in the cases of t(11;14) chromosome translocations in CLL, the breakpoints on chromosome 14 are located close to the 5′ end of each J_H segment, at the position where the D segments are recombined with the J_H segments during DJ joining to produce an active heavy-chain gene. This observation suggests that the enzyme system responsible for VDJ joining may be involved in the t(14;18) chromosome translocation as well as in the t(11;14) chromosome translocation in CLL (*10*). The possibility of the involvement of the VDJ joining en-

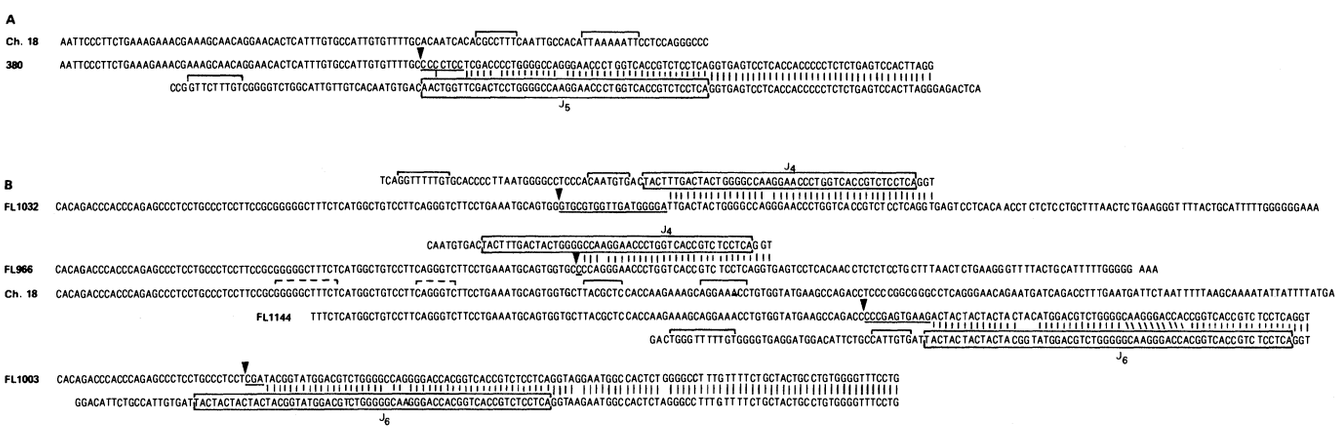

Fig. 2. Nucleotide sequences of the breakpoints of t(14;18) translocation and of corresponding normal chromosome 18. DNA sequences were determined by chemical degradation (*10*). The breakpoint sequences are aligned so that the chromosome 18–derived sequences match the corresponding normal chromosome 18 sequences. The triangles represent the regions where DNA sequences of the leukemia 380 cells and the four follicular lymphomas start to diverge from normal chromosome 18 sequences. The identical nucleotides between the breakpoint sequences and J_H segment sequences (boxed) are shown by vertical lines. N region sequences are underlined. The heptamer-nonamer signal and signal-like sequences are shown by the brackets. DNA sequences of chromosome 18 that are very similar to the heptamer-nonamer signal sequences 5′ to the D segment (*10*) are shown by the broken brackets.

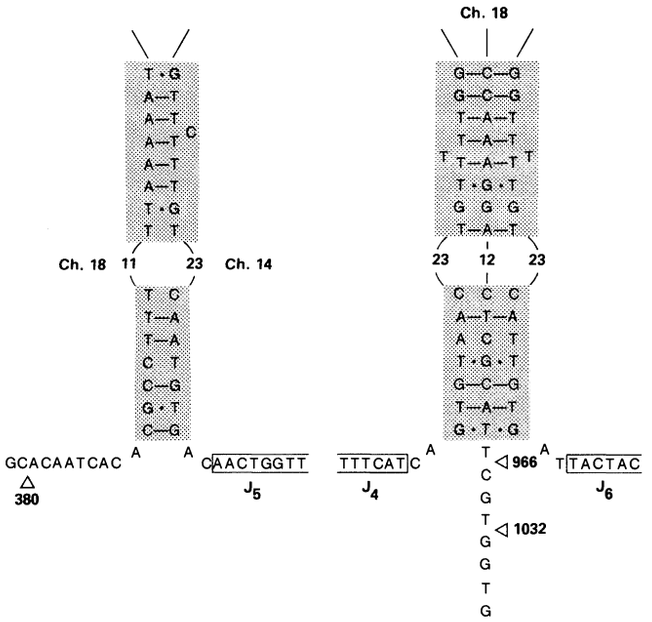

Fig. 3. Possible secondary structure of chromosome 18 close to the t(14;18) breakpoint and of the J_H segment of chromosome 14. The heptamer-nonamer signal sequences of J_H segments and the signal-like sequences of chromosome 18 are drawn in secondary structures.

zyme in the t(11;14) chromosome translocation was further strengthened by the finding of a heptamer-nonamer signal-like sequence with a 12-nucleotide spacer in close proximity to the breakpoint on chromosome 11 (*10*). The chromosome 18 regions close to the breakpoints of follicular lymphomas and pre–B-cell leukemia 380 contain sequences very similar to the heptamer-nonamer sequences for the VDJ joining enzyme (Figs. 2 and 3).

It has been proposed that the VDJ joining enzyme selects heptamer-nonamer signal sequences with a 12-bp spacer and heptamer-nonamer signal sequences with a 23-bp spacer as joining partners for DJ or VDJ joining (*13*). The J_H segment has a heptamer-nonamer signal sequence with a 23-bp spacer while the signal sequence on chromosome 18 is separated by an 11- or 12-bp spacer (Fig. 3). This suggests that the t(14;18) translocations of follicular lymphomas and pre–B-cell leukemia and the t(11;14) translocation of CLL are mediated by the VDJ joining enzyme and occur at the pre–B-cell stage of differentiation. The DNA region close to the breakpoints on chromosome 18 of follicular lymphomas also shows a DNA sequence similar to the heptamer-nonamer signal sequences 5′ to the D segments (Fig. 2). This signal sequence has a 23-bp spacer and is separated by 21 bp from the other heptamer-nonamer signal sequence mentioned above. Thus, this region of chromosome 18 is similar to the region of the heavy-chain locus that carries D segments.

As shown in Fig. 1, λ966 and λ1144 hybridized with the C_γ probe (pOMB) (*5*) and λ1003 and λ1032 hybridized with a C_μ probe (pCμ 1.2) (*5, 7*). All joining regions we sequenced showed extra nucleotides at joining sites between chromosomes 14 and 18 (Fig. 3). Such extra nucleotides were not derived from these two chromosomes (Fig. 2). These extra nucleotides may represent N regions, stretches of extra nucleotides that are added presumably by the enzyme terminal transferase at the pre–B-cell stage of differentiation (*10, 14*).

Thus, our data indicate that the t(14;18) chromosome translocations occur because of mistakes during DJ joining. While normally the putative recombinase recombines separated segments of DNA on the same chromosome, in very rare occasions it may join segments of DNA on different chromosomes recognized on the basis of their signal sequences for VDJ joining. We have not observed any case in which the *bcl*-2 locus is translocated to a variable-region gene (V_H). We have observed, however, a translocation of the *bcl*-1 locus to a variable-region gene in diffuse B-cell lymphoma carrying the t(11;14) chromosome translocation (*10*). As the t(14;18) chromosome translocations seem to occur during VDJ joining, we can infer that these translocations occur during the pre–B-cell stage of differentiation of the involved B cells. While leukemia 380 cells display the phenotype of pre–B cells (*11*), the neoplastic B cells of follicular lymphomas are more mature, as shown by the analysis of surface markers and by the fact that they do not express the enzyme terminal transferase (*15*). The expression of the neoplastic phenotype appears to be switched on at later stages of B-cell differentiation. The observation that, in FL 966 and FL 1144, the *bcl*-2 locus has recombined with a γ chain gene is consistent with this interpretation. In these two follicular lymphomas, the *bcl*-2 gene at first recombined with the J_H region 5′ of the C_μ gene, and then, because of heavy-chain switching, the *bcl*-2 J_H DNA segment translocated to a γ chain gene.

The observation that the expression of the neoplastic phenotype occurs after B-cell differentiation has several important implications. For instance, it may be a mistake to conclude that a B-cell tumor is polyclonal on the basis of immunoglobulin gene rearrangement (*16*). As the productive immunoglobulin gene rearrangements might occur after the t(14;18) chromosome translocation and the involved heavy genes might undergo switching, a truly monoclonal tumor (unicellular in origin) might appear to be biclonal or oligoclonal on the basis of immunoglobulin gene rearrangements.

Furthermore, as the expression of the neoplastic behavior in the B cells carrying the translocation often occurs after B-cell differentiation, it seems likely that the translocation of *bcl*-2 to the heavy-chain locus is not sufficient to induce a clinical neoplasm of pre-B cells. It must, however, induce some proliferative advantage, as the t(14;18) translocation has been observed in pre–B-cell leukemias carrying both the t(14;18) and the t(8;14) chromosome translocation (*11*). In these cases, the expansion of the clone carrying the t(14;18) translocation may increase the probability of the occurrence of a t(8;14) chromosome translocation. This translocation, in turn, leads to a highly malignant B-cell neoplasm (*11*).

The failure of the activated *bcl*-2 gene to induce clinical neoplastic behavior in a pre–B cell could be due to the ability of pre–B cells to respond to regulators in their environment. Such ability could be lost at later stages of B-cell differentiation. Alternatively, additional genetic changes may be required for development of a clinical tumor. It is not clear, however, why such changes should usually occur in more differentiated B cells and not in pre–B cells.

Another possibility is that the differentiated stage of the B cells harboring the activated *bcl*-2 gene is critical for the expression of neoplastic behavior. Different cell types might respond differently to the product of the same activated oncogene. For example, in patients with chronic myelogenous leukemia (CML), the entire bone marrow may be taken

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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20. Supported in part by grants from the American Cancer Society, March of Dimes, and National Cancer Institute, CA16685. We thank P. C. Nowell for helpful suggestions and K. A. Reinersmann for preparation of the manuscript.

31 May 1985; accepted 11 July 1985

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-

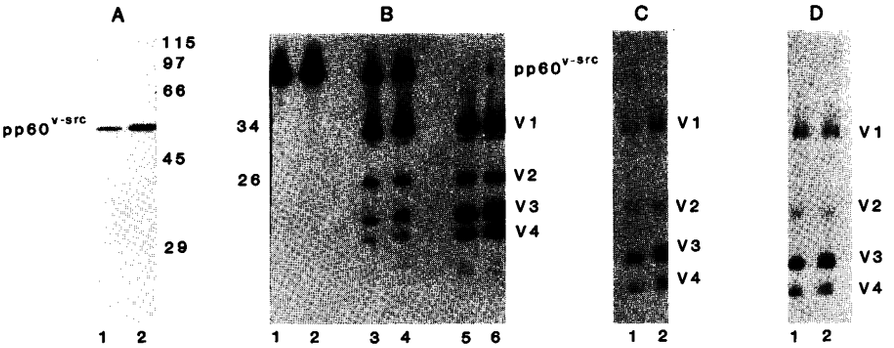

Fig. 1. Partial protease analysis of pp60$^{v\text{-}src}$ from TPA- and DMSO-treated cells. (A) The cells were European field vole (*Microtus agrestis*) cells transformed by the Schmidt-Ruppin strain (subgroup D) of RSV (clone I–T; originally provided by A. J. Faras, University of Minnesota Medical 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.