low temperatures, two cases arise depending on whether the stress is compressive or tensile. In tension, the mobility is expected to decrease with decreasing temperature and the flow stress is expected to continue to rise along the thermal activation curves until fracture intervenes. In compression (including indentation hardness), shear-induced transformation to a metallic β-tin phase is encountered (*13*) and the indentation hardness becomes nearly independent of temperature as a result of the change in mechanism (*11*). (Because of its technical importance, silicon was chosen as the prototype for this discussion, but quite similar arguments apply to other covalent crystals, both pure and impure.)

Some secondary effects in the motion of dislocations in covalently bonded crystals include doping, polarity in compounds composed of elements from groups III and V, and photoplasticity. In the context of the present model, doping simply changes the HOMO-LUMO gap in the vicinity of the dopant atoms. The polarity effect (difference in mobility of dislocations with half-planes ending on anions versus those with half-planes ending on cations) is related to the piezoelectric effect in these noncentrosymmetric crystals. This creates local electric fields, which alter the HOMO and LUMO energy levels. In the photoplastic effect, electrons are excited across the HOMO-LUMO gap by incident photons, thereby obviating the need for thermal excitation.

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26 April 1993; accepted 24 June 1993

Convergent Regulation of Sodium Channels by Protein Kinase C and cAMP-Dependent Protein Kinase

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The function of voltage-gated sodium channels that are responsible for action potential generation in mammalian brain neurons is modulated by phosphorylation by adenosine 3′,5′-monophosphate (cAMP)–dependent protein kinase (cA-PK) and by protein kinase C (PKC). Reduction of peak sodium currents by cA-PK in intact cells required concurrent activation of PKC and was prevented by blocking phosphorylation of serine 1506, a site in the inactivation gate of the channel that is phosphorylated by PKC but not by cA-PK. Replacement of serine 1506 with negatively charged amino acids mimicked the effect of phosphorylation. Conversion of the consensus sequence surrounding serine 1506 to one more favorable for cA-PK enhanced modulation of sodium currents by cA-PK. Convergent modulation of sodium channels required phosphorylation of serine 1506 by PKC accompanied by phosphorylation of additional sites by cA-PK. This regulatory mechanism may serve to integrate neuronal signals mediated through these parallel signaling pathways.

Action potentials in neurons are initiated by the opening of voltage-gated Na^+ channels. Although the rat brain Na^+ channel is a heterotrimer that consists of an α subunit of 260 kD, a β_1 subunit of 36 kD, and a β_2 subunit of 33 kD (*1*), the α subunit alone is sufficient to form functional voltage-gated ion channels when expressed from cloned complementary DNA in *Xenopus* oocytes (*2*) or mammalian cells (*3*, *4*). Alpha subunits of rat brain Na^+ channels are phosphorylated by cA-PK (*5*) and PKC (*6*), and four α subunits have been cloned and sequenced from rat brain (*7*, *8*). The large intracellular loop between homologous domains I and II ($L_{I/II}$) in each of these Na^+ channels contains multiple consensus sites (*9*) for phosphorylation by cA-PK and PKC that are phosphorylated in vitro and in intact cells (*10*, *11*).

Phosphorylation of rat brain Na^+ channels by PKC at Ser^{1506} in the inactivation gate formed by the short intracellular loop between domains III and IV ($L_{III/IV}$) slows their inactivation (*12*, *13*), and subsequent phosphorylation by PKC of a site in $L_{I/II}$ reduces peak Na^+ currents (*14*). Phosphorylation of Na^+ channels by cA-PK in $L_{I/II}$ also reduces the peak Na^+ current in excised membrane patches from cultured rat brain neurons, transfected mammalian cells, and *Xenopus* oocytes that express brain Na^+ channels (*15*, *16*). The reduction in peak current is caused by failure of channel activation during depolarizing stimuli, but the time course of Na^+ channel inactivation is not noticeably affected (*15*). Here, we show that phosphorylation of Na^+ channels through these two signaling pathways interacts to cause convergent regulation of Na^+ channel function.

Because peak Na^+ currents in excised membrane patches are reduced by treatment with cA-PK (*15*), we were surprised to observe that treatment of intact Chinese hamster ovary (CHO) cells that stably express rat brain type IIA Na^+ channel α subunits (CNaIIA-1 cells) (*4*) with the membrane-permeant analog 8-bromo–adenosine 3′,5′-monophosphate (8-Br-cAMP) either had no effect on peak Na^+ currents or increased peak Na^+ currents (Fig. 1A) recorded in cell-attached membrane patches (*17*). Because both cA-PK and PKC reduce peak Na^+ current in excised patches, we examined the interaction between these two kinases in modulating rat brain Na^+ channel function in intact cells. Partial activation of PKC by treatment with an intermediate concentration (25 μM) of oleoylacetylglycerol (OAG) slowed the inactivation of the Na^+ current in cell-attached patches but did not reduce the peak Na^+ current (Fig. 1, B and C) (*14*). Subsequent addition of 8-Br-cAMP in the presence of OAG reduced peak Na^+ current, which indicates that PKC phosphorylation was required to observe an effect of phosphorylation by cA-PK (Fig. 1, B and C).

To examine the mechanism of this regulatory interaction, we studied the effects of cA-PK on Na^+ currents in excised inside-out patches (*17*) from CHO cells that expressed either wild-type or mutant Na^+ channels in which Ser^{1506}, the site of phos-

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phorylation by PKC in the inactivation gate domain, had been converted to alanine (S1506A) (*18*). Normally, Na^+ current is reduced by 40 to 50% when the catalytic subunit (1 μM) of cA-PK is applied to the cytoplasmic surface of excised patches (*15*) (Fig. 1D). In six cells in this series, the peak Na^+ current was reduced to 61.3 ± 4.2% of the control (SEM). When the same experiment was done with CHO cells that expressed the S1506A mutant, cA-PK had no effect (Fig. 1E); 96.6 ± 4.7% (SEM) (n = 16) of the peak Na^+ current remained after cA-PK treatment. This demonstrates that the reduction of the Na^+ current by cA-PK phosphorylation in $L_{I/II}$ requires the presence of Ser^{1506} in $L_{III/IV}$, presumably because this site must be phosphorylated.

Serine 1506 is contained within a PKC consensus sequence (KKLGSKK) (*9*, *18*), and peptides corresponding to this region of the Na^+ channel are good substrates for phosphorylation by PKC but not by cA-PK (*13*). All seven phosphopeptides from the Na^+ channel α subunit that are phosphorylated by cA-PK in intact cells or in vitro are derived from phosphorylation of four sites located in $L_{I/II}$ (*10*). A phosphopeptide (designated C) derived from phosphorylation of purified Na^+ channels by PKC contains Ser^{1506} (*11*). To confirm that this site is not phosphorylated by cA-PK under the conditions of our experiments, we phosphorylated purified Na^+ channels with cA-PK (1 μM) and ATP (1 mM), and the resulting phosphopeptides were identified by two-dimensional mapping. We observed the four phosphopeptides that represent the major peptides derived from the four sites normally phosphorylated by cA-PK (peptides 1, 2, 6, and 7), but phosphopeptide C was not detectably phosphorylated (Fig. 2). These results indicate that Ser^{1506} must be phosphorylated by PKC in order for cA-PK to cause the observed reduction in peak Na^+ current.

To examine whether the negative charge added by phosphorylation of Ser^{1506} is the critical factor in permitting the action of cA-PK, we constructed mutants in which Ser^{1506} was replaced by the negatively charged amino acids aspartate or glutamate (*19*). When these mutant Na^+ channels that carried a permanent negative charge at position 1506 were expressed in CHO cells (*20*), the resulting Na^+ current was reduced in the normal fashion by exposure to 1 μM cA-PK (Fig. 3, A and B). The voltage dependence of Na^+ channel activation was shifted by approximately +20 mV in both of these mutants, but phosphorylation by cA-PK did not alter the current-voltage relation for either S1506E (Fig. 3C) or S1506D (*18*). Peak current was reduced to 52.7 ± 7.2% (SEM, n = 3) of the control in S1506D and to 43.5 ± 13.8% (SEM, n = 4) of the control in S1506E. Thus, adding a fixed negative charge at position 1506 substitutes for phosphorylation in permitting the reduction of peak Na^+ current by phosphorylation of sites in $L_{I/II}$ by cA-PK.

These results indicate that phosphorylation of Ser^{1506} is the limiting factor that prevents cA-PK from reducing peak Na^+ currents in intact cells. To verify this idea, we converted the consensus sequence surrounding Ser^{1506} to a site more favorable for phosphorylation by cA-PK (*9*) by converting Lys^{1507} and Lys^{1508} to glutamine by oligonucleotide-directed mutagenesis (K1507Q; K1508Q) (*18*, *19*). Whereas Na^+ currents in excised inside-out patches from cells that expressed wild-type Na^+ channels (Fig. 4, A and C) were reduced only 9% after exposure to 100 nM cA-PK, Na^+ currents from cells that expressed mutant K1507Q; K1508Q were reduced 43% (Fig. 4, B and C) when exposed to this low cA-PK concentration. Thus, the mutant channels are approximately 10-fold more sensitive than the wild-type channels to regulation by cA-PK (*15*). This result confirms that in excised patches containing wild-type Na^+ channels, the level of phosphorylation of Ser^{1506} is a limiting factor in

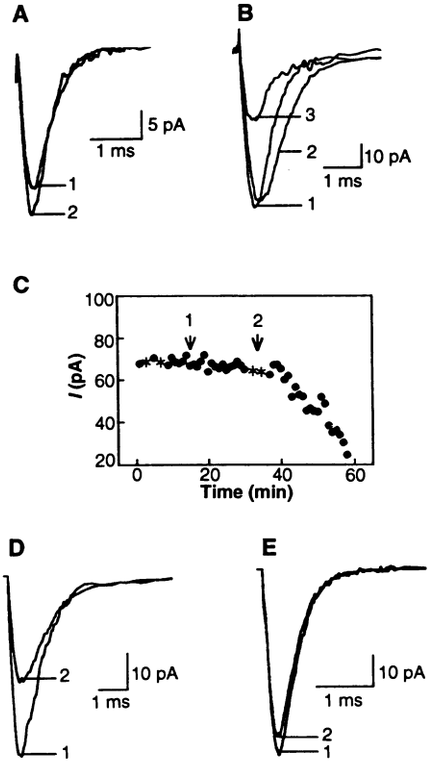

Fig. 1. Interaction between phosphorylation of Ser^{1506} by PKC and phosphorylation by cA-PK in the regulation of peak Na^+ current. (**A**) Na^+ currents during a test pulse to 0 mV from a holding potential of −110 mV before (trace 1) and after (trace 2) addition of 8-Br-cAMP (2 mM) to the bathing medium of a CNaIIA-1 cell during cell-attached patch recording. The pipette solution contained 140 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 10 mM Hepes (pH 7.3); the bath solution contained 150 mM KCl, 1.5 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM Hepes (pH 7.4). (**B**) A similar experiment in which Na^+ currents were recorded under control conditions (trace 1), after treatment with OAG (25 μM) (trace 2), and after treatment with OAG (25 μM) plus 8-Br-cAMP (2 mM) (trace 3). (**C**) Time course of the change in peak Na^+ current (I) during treatment with OAG and 8-Br-cAMP. The Na^+ currents were recorded as in (B) as a function of time with test pulses to −10 mV. Asterisks indicate times when complete current-voltage relations were determined. No change in the voltage dependence of activation of the Na^+ current was observed. Arrows indicate the time of addition of OAG (25 μM) (1) and 8-Br-cAMP (2 mM) (2). The results shown in (A) through (C) are typical of four similar experiments. (**D**) Macroscopic Na^+ current recorded in an excised inside-out patch from CNaIIA-1 cells that express wild-type Na^+ channels before (trace 1) and after (trace 2) exposure of the cytoplasmic surface of the patch to cA-PK (1 μM) and adenosine triphosphate (ATP) (1 mM). Currents were recorded during voltage steps to a test potential of −20 mV from a holding potential of −120 mV. (**E**) A similar experiment to that in (D) with S1506A Na^+ channels.

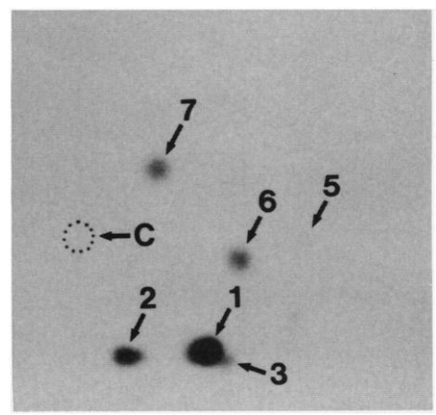

Fig. 2. Analysis of tryptic phosphopeptides (1 through 7) from purified Na^+ channels phosphorylated by cA-PK. Purified Na^+ channels were phosphorylated for 2 min in the presence of 1 μM cA-PK and 1 mM [γ-^{32}P]ATP (1250 Ci/mmol), and ^{32}P-labeled α subunits were separated by SDS–polyacrylamide gel electrophoresis, located by autoradiography, and subjected to tryptic phosphopeptide analysis as described (*11*). Tryptic phosphopeptides were separated by high-voltage electrophoresis in the horizontal direction at pH 1.9 (cathode to the right), followed by thin-layer chromatography in the vertical direction. The dotted circle marks the expected migration position for phosphopeptide C containing Ser^{1506} from samples phosphorylated by PKC.

the action of cA-PK. In contrast, in the mutant channels Ser^{1506} is readily phosphorylated by cA-PK, and Na^+ current reduction is observed at lower kinase concentrations.

These results in excised patches suggested that it might also be possible to reduce peak Na^+ currents mediated by mutant K1507Q; K1508Q Na^+ channels in intact cells by increasing the intracellular concentration of cAMP. When Na^+ currents were recorded in cell-attached patches on intact cells that expressed wild-type channels, exposure to 8-Br-cAMP caused no reduction in peak Na^+ current (Fig. 4D). In contrast, when mutant K1507Q; K1508Q Na^+ channels were studied in cell-attached patches on intact cells, the same 8-Br-cAMP concentration reduced the peak current by approximately 50% (Fig. 4E). Furthermore, cA-PK slowed Na^+ channel inactivation in this mutant as PKC does in wild-type channels (Fig. 4E), which is consistent with phosphorylation of Ser^{1506} by cA-PK in the mutant but not in the wild-type channels. Thus, Na^+ currents in intact cells are readily reduced by cAMP-dependent phosphorylation, but concomitant phosphorylation of Ser^{1506} is required.

Our experiments demonstrate a requirement for phosphorylation of Na^+ channels by PKC to allow phosphorylation by cA-PK to reduce peak Na^+ currents in intact cells that express wild-type or mutant Na^+ channels. Consistent with our studies of transfected cells, Na^+ current is reduced in rat striatonigral neurons after activation of dopamine D1 receptors, which raise intracellular cAMP levels, and increased after activation of dopamine D2 receptors, which reduce intracellular cAMP levels (*21*). Thus, cA-PK–mediated Na^+ current regulation occurs in rat brain neurons in response to the activation of neurotransmitter receptors, which presumably reflects concurrent basal phosphorylation of Ser^{1506} by PKC in those cells. Although phosphorylation by cA-PK has a direct inhibitory effect on Na^+ channel activity, as observed in these experiments, Na^+ currents due to rat brain type IIA Na^+ channels expressed in *Xenopus* oocytes can be increased by 8-Br-cAMP (*22*), and expression of functional Na^+ channels in PC-12 cells requires cA-PK activity (*23*). At present, the molecular basis for these other effects of cA-PK on brain Na^+ channels is unknown.

Under physiological conditions, PKC, but not cA-PK, phosphorylates Ser^{1506} (*10–12*), which slows Na^+ channel inactivation directly (*12, 13*). Further activation of PKC causes phosphorylation of one or more consensus phosphorylation sites in $L_{I/II}$ and results in a reduction of peak Na^+ current (*14*). Likewise, reduction of peak Na^+ current by cA-PK results from phosphorylation at one or more of the four distinct consensus sites for that kinase in $L_{I/II}$ that are known to be phosphorylated in vitro and in intact cells (*10*) but only when Ser^{1506} is phosphorylated or substituted with a negatively charged amino acid. Thus, the effect of cA-PK on peak Na^+ current is conditional, dependent on phosphorylation of Ser^{1506} by PKC. This conditional regulation has the potential to serve as an integrative mechanism because two inputs linked to different second messenger systems impinging on the same neuron would cause a much greater response than activation of either input alone. Multiple consensus phosphorylation sites for cA-PK and PKC are a common feature of many ion channels (*1, 24*). Interactions between these two regulatory pathways such as those described here may play a general role in ion channel regulation.

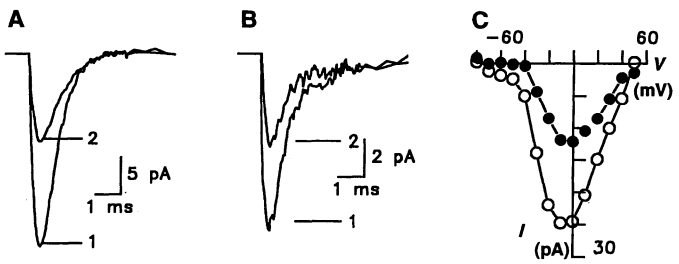

Fig. 3. Reduction of peak Na^+ currents in mutants with a fixed negative charge at position 1506 by treatment with cA-PK. (**A**) Macroscopic Na^+ currents recorded from excised inside-out patches from mutant S1506E before (trace 1) and after (trace 2) exposure of the cytoplasmic surface of the patch to the catalytic subunit of cA-PK (1 μM) and ATP (1 mM). Currents were recorded during voltage steps to 0 mV (*20*) from a holding potential of −120 mV. (**B**) A similar experiment with mutant S1506D. (**C**) Current-voltage relations for Na^+ currents mediated by mutant S1506E were determined before (○) and after (●) exposure to the catalytic subunit of cA-PK and ATP for test pulses to the indicated potentials from a holding potential of −120 mV.

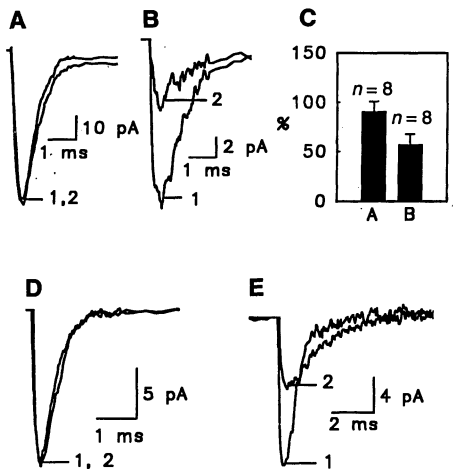

Fig. 4. Reduction of peak Na^+ currents by cA-PK for mutant K1507Q;K1508Q Na^+ channels in excised and cell-attached patches. (**A**) Wild-type Na^+ channels were co-expressed with plasmid HL-Rev_{ab} in CHO-K1 cells. HL-Rev_{ab} encodes a dominant negative mutant regulatory subunit of cA-PK, and its expression results in cells with reduced cA-PK activity (*15*). Macroscopic Na^+ currents were recorded from wild-type Na^+ channels in an excised patch before (trace 1) and after (trace 2) exposure to 100 nM cA-PK. The test pulse was to −20 mV from a holding potential of −120 mV. (**B**) A similar experiment to that in (A) was done with mutant K1507Q;K1508Q Na^+ channels. (**C**) Mean Na^+ current as percent of control in wild-type (A) and mutant (B) cells in response to 100 nM cA-PK. Wild-type and mutant recordings were obtained during a single series of experiments. (**D**) Wild-type Na^+ channels were expressed in CHO-K1 cells without HL-Rev_{ab}, and Na^+ currents in a cell-attached patch were recorded in response to a test pulse to 0 mV from a holding potential of −110 mV before (trace 1) and after (trace 2) exposure to 8-Br-cAMP (1 mM). The pipette solution was the same as that for the excised patch recording (*17*); the bath solution was also the same, except that 2.4 mM $CaCl_2$ was added. (**E**) A similar experiment to that in (D) was carried out for mutant K1507Q;K1508Q.

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18. Amino acid residue numbers refer to the rat brain type IIA sequence reported in (*8*). Mutant Na^+ channel constructs are referred to by the natural amino acid followed by the residue number (or numbers) and the mutant amino acid. For example, S1506A refers to mutation of the wild-type serine at position 1506 to an alanine. Abbreviations for the amino acid residues are: A, Ala; D, Asp; E, Glu; G, Gly; K, Lys; L, Leu; Q, Gln; and S, Ser.
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25 January 1993; accepted 9 July 1993

NF-κB Activation by Ultraviolet Light Not Dependent on a Nuclear Signal

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Exposure of mammalian cells to radiation triggers the ultraviolet (UV) response, which includes activation of activator protein–1 (AP-1) and nuclear factor kappa B (NF-κB). This was postulated to occur by induction of a nuclear signaling cascade by damaged DNA. Recently, induction of AP-1 by UV was shown to be mediated by a pathway involving Src tyrosine kinases and the Ha-Ras small guanosine triphosphate–binding protein, proteins located at the plasma membrane. It is demonstrated here that the same pathway mediates induction of NF-κB by UV. Because inactive NF-κB is stored in the cytosol, analysis of its activation directly tests the involvement of a nuclear-initiated signaling cascade. Enucleated cells are fully responsive to UV both in NF-κB induction and in activation of another key signaling event. Therefore, the UV response does not require a signal generated in the nucleus and is likely to be initiated at or near the plasma membrane.

Exposure of mammalian cells to UV and other DNA-damaging agents activates the UV response (*1*). Two transcription factors that execute part of this induction response are AP-1 (*2*, *3*) and NF-κB (*2*). The latter may be responsible for stimulation of human immunodeficiency virus–type 1 (HIV-1) transcription (*2*, *4*) and thereby cause its activation in skin cells (*5*). However, it was also proposed that UV-mediated DNA damage activates HIV-1 by nonspecific decondensation of chromatin (*6*), an unlikely mechanism given the established role of NF-κB in HIV-1 induction (*7*). An unanswered problem is the mechanism by which UV activates NF-κB. Because NF-κB is stored as an inactive cytoplasmic complex by way of interaction with IκB (*8*), its activation requires the transfer of a UV-generated signal to that subcellular compartment. The UV response was proposed to be mediated by an intranuclear signaling cascade, elicited by damage to DNA (*1*). If this mechanism is involved in the activation of NF-κB by UV, then a nuclear signal should be transferred to the cytoplasm to induce dissociation of the NF-κB:IκB complex.

We demonstrated that exposure of HeLa cells to UV rapidly activates Src-family tyrosine kinases, followed by activation of the small guanosine triphosphate (GTP)–binding protein (G protein) Ha-Ras and the cytoplasmic serine-threonine kinase Raf-1 (*9*). This cascade increases the phosphorylation of c-Jun, an important component of AP-1 (*10*), on sites that stimulate its transcriptional activity (*9*). Because c-Src and Ha-Ras are primarily associated with the plasma membrane (*11*), we proposed that the signaling cascade leading to activation of AP-1 by UV is initiated at the plasma membrane (*9*). However, a very rapid transfer of a nuclear-generated signal to the plasma membrane could not be excluded. We now demonstrate that tyrosine kinases and Ha-Ras are also involved in activation of NF-κB by UV. With the use of enucleated cells, we show that activation of NF-κB and an early signaling event caused by UV do not require a nuclear signal. Therefore, the UV signaling cascade activating both NF-κB and AP-1 is initiated at or near the plasma membrane and is not elicited by DNA damage in the nucleus.

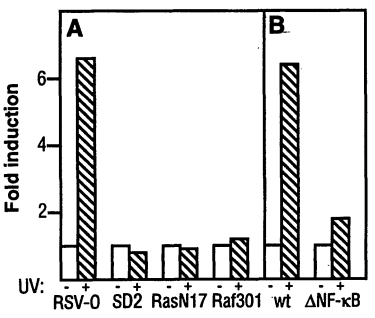

Fig. 1. Inhibition of HIV-1 promoter activation by dominant negative alleles of v-*src*, Ha-*ras*, and *raf-1*. (**A**) HeLa S3 cells were cotransfected with 3 μg of (−453/+80)HIV-LUC per plate, together with either the expression vector alone (RSV-O; 10 μg) or expression vectors encoding dominant interfering v-*srcSD2* (7 μg), Ha-*rasN17* (0.34 μg), and *raf301* (10 μg) alleles. The total amount of expression vector was kept constant at 10 μg with RSV-O. One-half of the cultures were exposed to UVC (40 J/m²) 2 hours after transfection. Luciferase activity was determined (*9*) 22 hours later. The results shown are averages of two separate experiments and are presented as fold induction above the basal (−453/+80)HIV-LUC expression, which averaged 70 U per microgram of cell protein. (**B**) HeLa S3 cells were cotransfected with 3 μg of (−453/+80)HIV-LUC or ΔNF-κB(−453/+80)HIV-LUC (*7*) per plate. One-half of the cultures were exposed to UVC (40 J/m²) 2 hours after the transfection, and LUC activity was determined 22 hours later. Shown are the averages of two separate experiments. Basal expression in these experiments averaged 21 U per microgram of protein.

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