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17. Generally labeled RNA transcripts 47 and 31 nucleotides in length were transcribed from a pSP64 plasmid containing the required sequence inserted between the Hind III and Eco RI sites; transcription was effected with SP6 RNA polymerase + [α-^{32}P]CTP (cytidine 5′-triphosphate) following plasmid linearization with Hha I or Bal I, respectively. The RNAs were purified by 10% polyacrylamide gel electrophoresis (PAGE; 7*M* urea, 90 m*M* tris-borate, *p*H 8.0, with 2 m*M* EDTA). The 5′ end–labeled transcripts were prepared by successive treatments of the unlabeled transcripts with calf intestinal phosphatase, and then T_4 polynucleotide kinase + [γ-^{32}P]ATP (adenosine triphosphate). Purification was effected by 20% PAGE.
18. The RNA transcripts were not processed by Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ba^{2+}, or Ca^{2+}. Incubation in the presence of Fe^{2+}, Zn^{2+}, or Sm^{2+} resulted in nonspecific degradation. Although initial experiments suggested that Mn^{2+}-dependent RNA strand scission was facilitated by spermidine, more recent experiments have indicated that no spermidine is required. As anticipated, 25 m*M* EDTA completely inhibited RNA processing.
19. That RNA strand scission was not the result of some protein contaminant having ribonuclease activity was shown by (i) demonstration that strand scission of the hairpin was insensitive to proteinase K and (ii) the finding that a chemically synthesized 31-nt RNA substrate identical in sequence with the RNA derived by transcription also underwent Mn^{2+}-dependent strand scission.
20. See also J. J. Butzow and G. Eichhorn, *Nature* **254**, 358 (1975).
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22. The chemical nature of Mn^{2+}-promoted strand scission was also studied by degradation of the derived products. The 31-nt RNA substrate prepared by transcription in the presence of [α-^{32}P]ATP was treated with Mn^{2+}, and the 13-nt product was isolated following PAGE separation. Digestion of the product with nuclease P_1 afforded a mixture of mononucleotides, which was analyzed by polyethyleneimine chromatography in comparison with authentic standards and shown to contain a strong band for [^{32}P]5′-AMP (adenosine 5′-monophosphate) and a weaker band corresponding to [^{32}P]pG>p, as predicted. The formation of radiolabeled pG>p reinforced the inference (Fig. 2) that the 3′-terminus of the 13-nt product contained a 2′,3′-cyclic phosphate. Furthermore, since the source of radiolabel in the 31-nt substrate was [α-^{32}P]ATP, the labeled phosphate in pG>p must have derived from an adjacent adenine nucleotide which, after hydrolytic cleavage from the labeled phosphate, formed the 5′ end of the 18-nt product (compare with Fig. 1). In a parallel experiment the 18-nt product, derived from a substrate that had not been radiolabeled, was treated successively with T_4 polynucleotide kinase + [γ-^{32}P]ATP and then with nuclease P_1. As anticipated, the sole radiolabeled product detectable by thin-layer chromatographic analysis was [^{32}P]5′-AMP, verifying that adenosine (containing a free 5′-OH group) was the nucleotide at the 5′ end of the 18-nt product.
23. In the context of this proposed mechanism, we note that Mn^{2+}-supported proton dissociation from a β-hydroxyethyliminodiacetic acid chelate at a pK_a value 2.2 units lower than that of the respective Mg^{2+} chelate [S. Chaberek, Jr., R. C. Courtney, A. E. Martell, *J. Am. Chem. Soc.* **74**, 5057 (1952)], which provides a further basis for the observed metal specificity of RNA strand scission.
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Expression of a Zinc Finger Gene in HTLV-I– and HTLV-II–Transformed Cells

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Gene products encoded by the human T cell leukemia virus (HTLV) types I and II mediate transformation by the transactivation of cellular genes necessary for proliferation, probably including transcriptional regulatory factors. By searching for factors that may control proliferation, a zinc finger gene (225) was identified that was constitutively expressed in all HTLV-I– or HTLV-II–transformed cell lines examined, whereas in normal T cells it was only transiently expressed after mitogenic stimulation. The 225 gene was also constitutively expressed in two HTLV-I–transformed helper T cell clones, but not in the parental cell lines. Thus this putative cellular transcriptional factor, which was abnormally expressed in retrovirus-infected cells, may have a role in transformation.

ACTIVATION OF RESTING PERIPHERAL T lymphocytes by antigen or mitogen initiates a cascade of sequential and interregulated transcriptional events that ultimately result in DNA synthesis, lymphocyte proliferation, and differentiated immunologic function (*1*). The regulation of this activation program and the identification of genetically responsive genes that are essential to the proliferative response has been approached in our laboratory by cloning inducible genes from a cDNA library generated during the early stages of T lymphocyte activation. More than 60 distinct gene transcripts representing genes activated during the transition of quiescent (G_0) cells through the early prereplicative (G_1) stage of the cell cycle were identified. These transcripts presumably include transcriptional regulatory molecules that control the initial stages of the response to cellular activating agents (*2*). Some of the early regulatory genes involved in mitogen or antigen-induced T cell activation and division may also participate in the initiation and maintenance of cell proliferation after transformation of T lymphocytes by HTLV-I and HTLV-II. These lymphotropic retroviruses are associated with specific T cell malignancy;

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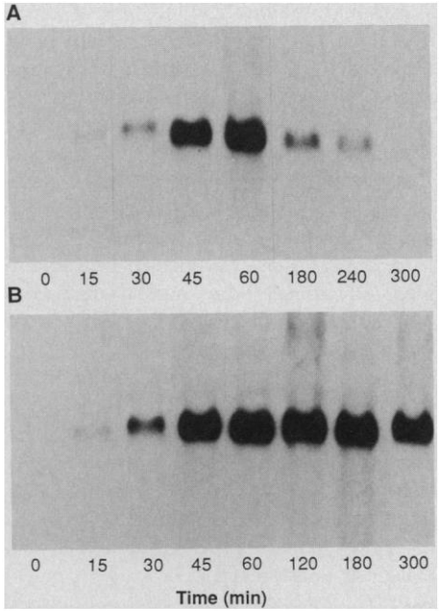

Fig. 1. Kinetics of 225 expression in activated human peripheral T cells. (**A**) Northern blot analysis of total cellular RNA obtained at the indicated times after PHA and PMA stimulation of peripheral T lymphocytes. (**B**) Analysis of 225 expression under same conditions as (A), except that cycloheximide was added at the time of activation with PHA and PMA. T lymphocytes, obtained from nylon wool column purification of peripheral mononuclear cells, were resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum at a concentration of 1×10^6 cells per milliliter. PHA (1 μg/ml), PMA (20 ng/ml), and cycloheximide (10 μg/ml) were added and portions were removed at the indicated times for extraction of total RNA with guanidine and phenol. Blots in both (A) and (B) contained 10 μg of total cell RNA per lane and were hybridized with probes from the 5′ (nucleotides 194 to 862) and 3′ (nucleotides 2779 to 3117) regions of the 225 cDNA.

HTLV-I is the etiological agent of adult T cell leukemia (ATL) (*3*), and HTLV-II has been isolated from several patients with variant T lineage hairy cell leukemia (*4, 5*). T cells transformed by these viruses resemble mitogen-activated lymphocytes. Initially after infection in vitro with HTLV-I or HTLV-II, T cells are polyclonally stimulated (*6*). In addition, tumor cells from ATL patients, as well as T lymphocytes transformed in vitro with either of these viruses, display an activated T cell phenotype (7) that is characterized by expression of CD25, the α chain of the interleukin-2 (IL-2) receptor. This cellular gene appears to be transactivated, at least in part, by the virally encoded Tax protein (*8*). Other lymphokines and growth factors that are expressed in HTLV-I–transformed cells may be transactivated by viral genes; some of these may contribute to the uncontrolled proliferation of transformed cells (*9*).

After mitogen induction, we most frequently isolated cDNAs representing a gene designated 225 from our subtractive T cell library (*2*) that was enriched for activated cDNAs. The induction kinetics of this gene were rapid. The 225 RNA transcripts were identified on Northern (RNA) blots within 15 to 30 min after exposure of peripheral T cells to phytohemagglutinin (PHA) and phorbol myristate acetate (PMA) (Fig. 1A). The RNA expression peaked by 45 to 60 min and decreased to undetectable levels by 5 hours. Addition of the protein synthesis inhibitor cycloheximide to the stimulated cells resulted in a two- to fourfold superinduction of 225 and a prolongation of elevated expression beyond 4 hours (Fig. 1B). In addition to PHA and PMA, mitogenic monoclonal antibodies directed to the CD3 or CD2 proteins (*2, 10*), antigen in the context of antigen-presenting cells, and PMA alone also stimulated 225 expression. A nearly full-length 225 cDNA sequence, based on its estimated size from Northern blots, is 3157 nucleotides in length. An open reading frame extending from nucleotides 249 to 1877 encodes a protein of 543 amino acids with an unmodified molecular size of 56 kD (Fig. 2). The sequence contains three tandem zinc finger regions. Zinc finger motifs have been identified in several eukaryotic proteins with transcriptional regulatory activity; the zinc finger domains usually function as the nucleic acid–binding regions of these regulatory molecules. Each of the zinc fingers in the 225 gene contains paired cysteine and histidine residues that are believed to tetrahedrally complex a Zn^{2+} ion and provide structural stability to the finger conformation (*11*). A nucleotide sequence homology search indicated that gene 225 showed a high degree of similarity (85% of the nucleotides and 91% of the amino acids) with a zinc finger–containing gene present in rat and mouse cells [designated *NGFI-A* in rat, and *egr-1*, *Krox-24*, *zif/268*, and *tis8* in mouse (*12*)]; therefore, 225 is likely to represent the human homolog of these genes. With a mouse *egr-1* probe, the gene has been localized to the long arm of human chromosome 5 (*13*). The 225 gene is also related to, but distinct from, the human gene *egr-2* (homolog of the mouse gene *Krox-20*) (*14*). The high expression in early activation and the sequence-specific DNA binding activity of the mouse homolog *zif/268* (*15*) suggested that the 225 gene may encode a transcription factor that participates in the early stages of T cell proliferation.

The DNA-binding proteins are also a likely class of host cell regulatory molecules that HTLV-I or HTLV-II may use to effect the malignant transformation of lymphocytes. Such cellular genes probably exist, since the viral genome does not include a known transforming gene (*16*). Transactivation of the 225 gene by these lymphotropic retroviruses would further support participation of this protein in the regulation of T lymphocyte growth. We examined 18 HTLV-I–infected cell lines that were from the peripheral blood of ATL patients or that were established after in vitro transformation of peripheral lymphocytes or umbilical cord blood cells, four T cell lines from acute lymphocytic leukemia (ALL) patients, and a Sezary syndrome cell line (Fig. 3, A and B). All HTLV-I–transformed cell lines that we tested showed constitutive expression, at varying levels, of gene 225, whereas only one T cell line (HSB) among those examined that were not transformed by HTLV-I expressed this gene. The ALL cells represent a more immature stage of T cell differentiation, whereas the HuT 78 cells have a similar post-thymic maturation phenotype as the ATL cell lines. Although 225 expression correlated with the presence of Tax,

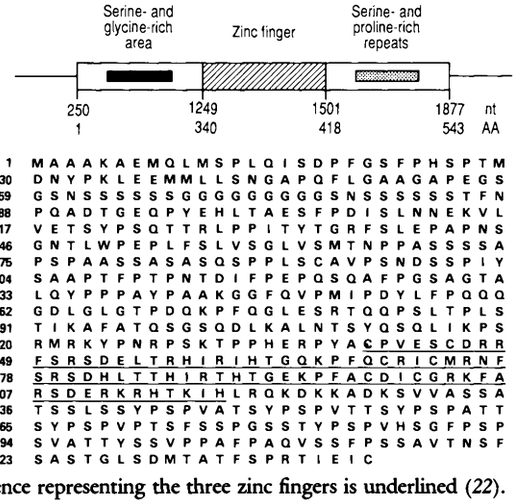

Fig. 2. Protein sequence for 225, deduced from the nucleotide sequence (*21*). The 225 cDNA was sequenced by the dideoxy chain-termination method with overlapping clones obtained from the subtractive library or from a λ zap cDNA library constructed with poly-adenylated mRNA obtained from peripheral T lymphocytes and Jurkat cells stimulated with PHA and PMA in the presence of cycloheximide for 2 and 4 hours. The full length of both strands was sequenced. Nucleotide (nt) and amino acid (AA) positions are indicated, as well as the zinc finger region, a serine- and glycine-rich region, and the eight amino acid serine- and proline-rich repeats near the COOH-terminus. The amino acid sequence representing the three zinc fingers is underlined (*22*).

alternative pathways for 225 activation must exist, because this gene was expressed constitutively in at least one HTLV-I–negative T cell line (HSB). Two HTLV-II–transformed cell lines, Mo-T (established from a variant hairy cell leukemia) and C-344 (a cord blood cell line transformed in vitro with HTLV-II) showed constitutive expression of gene 225 (Fig. 3C). The Mo-B cell line, derived from the same patient as Mo-T, contains both Epstein-Barr virus (EBV) and HTLV-II genomes and also expressed 225 (Fig. 3C) (*5*).

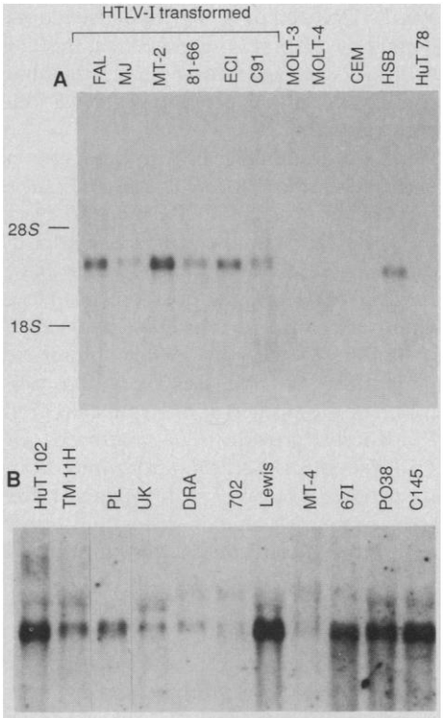

Fig. 3. Constitutive expression of zinc finger gene 225 by HTLV-I– or HTLV-II–transformed cell lines. (**A**) Northern blot of HTLV-I–transformed cell lines and HTLV-I–negative T lymphocyte lines. FAL and MJ were established from peripheral blood of ATL patients, whereas the remaining HTLV-I–transformed cell lines were established after in vitro transformation of peripheral lymphocytes (MT-2, 81-66, and ECI) or cord blood cells (C91) with HTLV-I. HTLV-negative T cell lines include T-ALL cell lines MOLT-3, MOLT-4, CEM, and HSB and the Sezary syndrome cell line HuT 78. (**B**) Northern blot analysis of additional HTLV-I–transformed cell lines including ATL cells (PL, UK, and Lewis) or cell lines established after in vitro transformation with HTLV-I of peripheral lymphocytes (DRA, 702, MT-4, and 671) or cord blood cells (PO38 and C145). (**C**) Northern blot showing constitutive expression of 225 by HTLV-II cell lines Mo-T, Mo-B, and C-344. The Mo-B lane contained less RNA than the other lanes. All HTLV-I and HTLV-II cell lines in (A), (B), and (C) except MT-2, HuT 102, C91, and Mo-B were cultured in media supplemented with recombinant IL-2 (10 to 100 U/ml).

To explore further the relation between HTLV-I transformation and 225 expression, we assessed two paired T cell clones that included two parental nontransformed clones and their HTLV-I–transformed counterparts (Fig. 4). The parental clones were $CD4^+$ helper T cells (TM5 and TM11) with specificity for tetanus toxoid. These clones were established from a normal donor and required periodic antigen stimulation for growth. Cultivation of TM5 and TM11 with irradiated HTLV-I–producing cells generated transformed cell lines that carried HTLV-I proviral genomes and proliferated independently of antigen exposure (TM5H and TM11H) (*17*). The parental uninfected cell lines transiently expressed 225 after mitogenic stimulation of resting cells, but otherwise did not constitutively express the 225 gene. In contrast, the HTLV-I–transformed companion cell lines constitutively expressed high levels of the 225 gene. In additional experiments, the 225 gene expression was activated after transformation of human cord blood lymphocytes by a *Herpesvirus saimiri* vector carrying the HTLV-I Tax and Rex transactivating proteins, and 225 was concomitantly activated in permanently transfected cell lines stimulated to make the Tax protein (*18*).

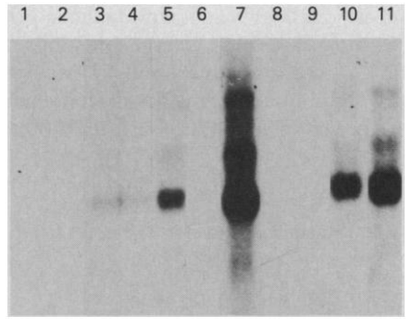

Fig. 4. Activation of 225 gene expression after HTLV-I transformation of antigen-dependent T cell helper clones TM5 and TM11. Quiescent TM11 cells (lane 1); TM11 cells 4 days after exposure to syngeneic antigen-presenting cells (APC) and two limiting flocculation units of soluble tetanus toxoid (TT) in the presence of IL-2 (lane 2); TM11 cells 1 hour (lane 3) and 4 hours (lane 4) after antigenic stimulation; TM11 cells 1, 4, or 18 hours after mitogenic activation with PHA and PMA (lanes 7, 8, and 9, respectively); TM11 cells and cycloheximide (CHX) in the presence of TT and APC for 4 hours (lane 5) or with CHX only (lane 6); HTLV-I–transformed TM5 and TM11 (TM5H and TM11H) cells (lanes 10 and 11, respectively).

Although the biologic function of 225 is not known, its sequence-specific DNA binding activity (*15*), its potential transactivating regions (*19*), and its expression pattern suggest a regulatory role in gene expression after a proliferative signal to the cell. The 225 gene represents the single known example among transcription factors whose mRNA expression is activated in all HTLV-I– and HTLV-II–transformed cells examined. Of course, other nuclear regulatory factors may be affected. It has been shown that the promoter for the transcription factor c-*fos* can be transactivated by Tax in transient transfection experiments and that the preexisting NF-κB factor is released from a cytoplasmic inhibitor in Tax-expressing cells and in some HTLV-I–transformed lines (*20*). Our data suggest that the deregulated expression of 225 may contribute to retroviral-mediated malignant transformation.

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19. The COOH-terminal region of the protein contains eight–amino acid–long repeats of proline, glutamic acid, serine, and threonine residues. Similar but not identical repeats exist in the β subunit of RNA polymerase II [L. Allison, J. Wong, V. Fitzpatrick, M. Moyle, C. Ingles, *Mol. Cell. Biol.* **8**, 321 (1988)]. This region of the 225 protein displays a high proline content (25%) and may function like the proline-rich activation region of CTF/NF-1 [N. Mermod, E. O'Neill, T. Kelly, R. Tjian, *Cell* **58**, 741 (1989)].
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21. Single letter abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
22. The nucleotide sequence of 225 has been submitted to GenBank and assigned the accession number M33672.
23. We thank A. S. Fauci for encouragement and support and for his critical reading of the manuscript. E. Tsachler and R. C. Gallo also generously provided RNA samples from several HTLV-I–transformed cell lines.

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In Vivo Receptor-Mediated Phosphorylation of a G Protein in *Dictyostelium*

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Extracellular adenosine 3′,5′-monophosphate (cAMP) serves multiple roles in *Dictyostelium* development, acting as a chemoattractant, a cell-cell signaling molecule, and an inducer of differentiation. The *Dictyostelium* G-protein α subunit Gα2 appears to be the major transducer linking the surface cAMP receptor to these intracellular responses. On stimulation of cells with cAMP, Gα2 is phosphorylated on one or more serine residues, resulting in an alteration of its electrophoretic mobility. Phosphorylation of Gα2 is triggered by increased occupancy of the surface cAMP receptor and is rapid and transient, coinciding with the time course of activation of physiological responses.

TRANSDUCTION OF A CAMP SIGNAL in *Dictyostelium* through the cell-surface cAMP receptor triggers multiple responses in the cells (*1*). The cAMP receptor contains seven transmembrane domains, a characteristic of G protein–linked receptors, such as rhodopsin and the β-adrenergic receptor (*2, 3*). A G-protein α subunit from *Dictyostelium*, Gα2, was determined to be the major transducer in this pathway by examination of the *Frigid A* complementation group (*4, 5*). These variants, which fail to carry out chemotaxis, increase intracellular cAMP or guanosine 3′,5′-monophosphate (cGMP) concentrations, or differentiate (*6*), contain point mutations (*7*) or deletions in the Gα2 gene (*5*). Because Gα2 is an essential component in the pathway, we investigated whether it undergoes covalent modification during cAMP stimulation.

To examine the Gα2 protein during the course of cAMP stimulation, we analyzed samples on immunoblots with a specific peptide antibody (*5*). Gα2 normally migrates as a 40-kD band during SDS–polyacrylamide gel electrophoresis (SDS-PAGE). However, Gα2 underwent a time-dependent transition in electrophoretic mobility after application of the cAMP stimulus (Fig. 1). The reduced mobility form of Gα2, with an apparent size of 43 kD, appeared after 20 s and peaked after 1 to 2 min. The transition in mobility was transient; the reduced mobility form returned almost completely to the original form within 15 min (Fig. 1A).

The following observations suggest that the cAMP-induced change in Gα2 mobility is induced by an increased occupancy of the surface cAMP receptor. (i) The mobility transition of Gα2 is a response that adapts. Many cAMP-induced responses, such as the activation of adenylyl and guanylyl cyclases, adapt after several minutes of constant exposure to cAMP (*8*). Removal of cAMP results in resensitization of the system (*9*). After 15 min in cAMP, Gα2 had returned predominantly to the prestimulus form (Fig. 1A). A second addition of the same amount of cAMP did not induce a second change in Gα2 mobility (Fig. 1B, lane 1), unless the cAMP stimulus was first removed and a recovery period allowed (Fig. 1B, lanes 2 and 3). (ii) The cAMP dose-response of the transition in Gα2 mobility is similar to other responses coupled to the cAMP receptor; alteration in Gα2 mobility is induced by nanomolar cAMP and saturated at 200 to 500 n*M* cAMP (*10, 11*). (iii) In cell lines transformed with cAMP receptor antisense sequences, which do not express cAMP receptors during development (*3*) but do express Gα2, there is no cAMP-induced alteration of Gα2 (Fig. 2).

The receptor-mediated transition in Gα2 electrophoretic mobility suggested that the protein undergoes a reversible, covalent modification, such as phosphorylation. We

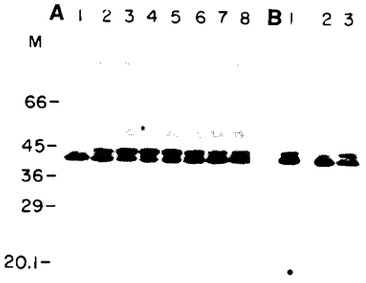

Fig. 1. Transient, receptor-mediated Gα2 modification. (**A**) Time course of Gα2 mobility shift in intact cells. Lanes 1 to 8 correspond to sampling times of 0, 20 s, 40 s, 1 min, 2 min, 5 min, 10 min, and 15 min after the addition of 1 μ*M* cAMP to basal state cells. (**B**) The effect of a second addition of cAMP. (Lane 1) Cells were exposed to 1 μ*M* cAMP for 15 min, after which a second addition of 1 μ*M* cAMP was made. The cells were sampled after a further 1 min. (Lanes 2 and 3) Cells were exposed to 1 μ*M* cAMP for 5 min then washed free of cAMP, placed into fresh medium, and incubated for 10 min without cAMP (lane 2) and then restimulated with 1 μ*M* cAMP for 1 min (lane 3). In both (A) and (B) cultures of 2×10^7 cells per milliliter were allowed to differentiate in DB (5 m*M* Na_2HPO_4, 5 m*M* KH_2PO_4, 2 m*M* $MgCl_2$, and 0.2 m*M* $CaCl_2$) as described (*18*). After 5 to 6 hours, cells were resuspended at a density of 5×10^7 cells per milliliter in DB containing 2 m*M* caffeine, which blocks the natural cAMP oscillations (*19*). Cells were shaken for 20 to 30 min to allow complete removal of endogenous cAMP and to allow the system to reach a basal state. Before the addition of cAMP, 10 m*M* dithiothreitol was added to inhibit extracellular phosphodiesterases (*20*). At the indicated times, cells were added to boiling SDS–sample buffer and subjected to SDS-PAGE as described (*21*), except that gels contained 0.033% SDS to improve separation of the Gα2 bands. Immunoblots were performed as follows: Gels were transferred to nitrocellulose (*22*), and after blockage of nonspecific sites with 3% bovine serum albumin (BSA), were incubated with antiserum to a peptide corresponding to the NH_2-terminal of Gα2 (*5*). Bands were visualized with ^{125}I-labeled protein A.

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