to and activates transcription from the adenovirus major late promoter USF E box site (E_{USF}). HeLa nuclear extract that contains USF binding activity was a gift from R. Metz.
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23. Rat embryo fibroblasts (Whittaker Bioproducts) were transfected by a modified calcium phosphate method (*29*). The c-*myc* and H-*ras* plasmids (5 μg of each) and 10 μg of the E6 or E6mutR plasmids were used for transfections with the pBluescript SK+ (Stratagene) vector used as carrier DNA to bring the total plasmid DNA to 20 μg in each transfection. The human c-Myc (LTR-H*myc*), adenovirus E1a (p1A), and mutant H-*ras* (pT22) expression vectors plasmid have been described (*19, 30*). The CMV E6 and CMV E6mutR vectors were generated by moving the Eco RI–Hind III inserts from pBS+ to pcDNAI (Invitrogen), which encodes a cytomegulovirus promoter immediately upstream of the insert coding regions. Cells were divided (1:3) 2 days after transfection and fed at 4-day intervals with Dulbecco's modified Eagle's medium that contained 5% fetal bovine serum. Foci appeared 8 to 12 days after transfection and were scored at 14 days by giemsa staining.
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25. Fragments that encoded the desired amino acid sequence were amplified by the polymerase chain reaction and cloned into the pBS+ vector (Stratagene). The parental expression vector E12/5′, from which E6, the E6mut clones, E6Δ, and $EM_{B/HZ}$ were derived, was produced from a fragment that encoded amino acid residues 112 to 336 from pE12R (*2*) with the 5′ primer, 5′-GAAAGCTTACCATGGCTGGCAGCTCCGGGGATGC-3′ (E12-E) and the 3′ primer, 5′-GAGTCGACGCTCCTTCTCCCGCTCG-3′ (E12-C). E6 was generated by subcloning into E12/5′, a pE12R-derived polymerase chain reaction (PCR) fragment produced by the following primers: 5′ primer, 5′-AGGTCGACGAACACACAACGTCTTGGAGCGCCAG CGCAGGAACAAAGTGCAGGAGGCCTTG-3′ (Mbasic) and 3′ primer, 5′-GAGAATTCTTAACAGGTTCCGCTCTCGCACTTG-3′ (E12-B). The E6mut constructs were generated by subcloning into E12/5′ pE12R-derived PCR fragments produced with the 3′ primer E12-B and the mutant E6 5′ primers listed below. Mutations were introduced individually at the underlined positions noted above in the primer Mbasic: E6mutN, CAG at the first position (Asp to Gln); E6mutB2Q, CAG at the second position (Arg to Gln); E6mutR, GTG at the third position (Arg to Val). E6Δ was derived from E6 by subcloning a PCR fragment generated with the primers E12-C and E12-F, AGAAGCTTACCATGGCGGCTGACCACTCGGAGGAG [the translation initiation site in E6Δ corresponds to amino acid 296 in E12 (*2*)]. $EM_{B/HZ}$ was constructed by subcloning into the E12/5′ vector a PCR fragment that encoded amino acids 347 to 439 (c-Myc basic-HLH-zipper) from pHL*myc* 1/0 (*25*); the primer sequences were 5′ primer, 5′-AGGTCGACGAACACACAACGTCTTGG-3′ and 3′ primer, 5′-GAGAATTCAGGACATTTCTGTTAGAAG-3′. The parental expression vector 5′Hmyc from which M_NE6 was derived was produced from a fragment that encoded amino acids 1 to 346 from pHL*myc* 1/0 (*25*) with the 5′ primer, 5′-GAAAGCTTCCCCGCCGCTGCCAGGAC-3′ and 3′ primer, 5′-CAGTCGACGGACATTCTCCTCGGTGTCCG3′. M_NE6 was constructed by subcloning the Sal I–Eco RI fragment from E6 into the 5′Hmyc vector. The structure of the basic-HLH region of all chimeric constructs was verified by DNA sequencing. The TFE-3 clone pBS-λ3 (*10*) was obtained from H. Beckmann and T. Kadesch.
26. Transcription and translation reactions including [^{35}S]methionine (40 μCi) were performed as described (*3*). An aliquot (~0.5 μl) of a standard 50-μl translation reaction was analyzed by SDS–polyacrylamide gel electrophoresis and fluorography.
27. Standard binding reactions were performed as described (*2*) with the addition of a highly degenerate nonspecific single-stranded oligonucleotide (~0.6 μg). Binding reactions contained reticulocyte extract (2 μl) programmed with E6 or TFE-3 RNA and double-stranded E_{USF} probe (~0.5 ng), which was produced by annealing complementary oligonucleotides as described (*3*) and end-labeling with γ-[^{32}P]ATP. For competition, annealed unlabeled E_{USF} (~100 ng) was added prior to the addition of the extract proteins. Protein-DNA complexes were fractionated on 5% polyacrylamide gels (37.5:1, acrylamide:bis-acrylamide) as described (*2*) and processed for autoradiography.
28. The proteins in the binding reactions were derived from HeLa nuclear extract, in the case of USF (~5 μg of nuclear extract protein per reaction was used), or from programmed reticulocyte extracts (*25*) in the case of TFE-3 and E6. For methylated E_{USF} oligonucleotides, 5-methyl cytosine was incorporated at the central CpG of the recognition site GGACACGTGACC on each strand (Genosys). Hemimethylated oligonucleotides were generated by annealing either methylated strand with the complementary unmethylated strand.
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Distinct Protein Targets for Signals Acting at the c-*fos* Serum Response Element

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The c-*fos* serum response element (SRE) is a primary nuclear target for intracellular signal transduction pathways triggered by growth factors. It is the target for both protein kinase C (PKC)–dependent and –independent signals. Function of the SRE requires binding of a cellular protein, termed serum response factor (SRF). A second protein, $p62^{TCF}$, recognizes the SRE-SRF complex to form a ternary complex. A mutated SRE that bound SRF but failed to form the ternary complex selectively lost response to PKC activators, but retained response to PKC-independent signals. Thus, two different signaling pathways act through discrete nuclear targets at the SRE. At least one of these pathways functions by recruitment of a pathway-specific accessory factor ($p62^{TCF}$). These results offer a molecular mechanism to account for the biological specificity of signals that appear to act through common DNA sequence elements.

REGULATION OF CELLULAR PROLIFeration and differentiation is coordinated by extracellular signals. The receptors that transduce these signals share common structures and enzymatic activities, and activate common intracellular signal transduction systems. Yet growth factors act with a remarkable degree of specificity—they trigger unique responses in a given cell and, sometimes, different responses in different cells. Ultimately, this specificity must reside in the genome, because these responses are driven by changes in cellular gene expression.

The c-*fos* proto-oncogene is a primary genomic target for extracellular signals (*1*). Many of these signals act through a small regulatory element that flanks the c-*fos* gene, the serum response element (SRE) (*2*). The SRE is the site of action for at least two distinct signal transduction pathways—one that involves the activation of protein kinase C (PKC) and another that transmits signals by a PKC-independent mechanism (*3–8*). For the SRE to function, binding of a cellular protein termed serum response factor (SRF) is required (*9–14*). Yet the precise function of SRF in signal transduction is not clear, because there is no change in the properties of SRF isolated from stimulated cells. Indeed, the SRE remains constitutively bound by protein in cells regardless of the transcriptional state of the gene (*15*).

Two additional proteins bind to the SRE in vitro. One protein, $p62^{DBF}$ (for direct binding factor) binds directly and asymmetrically to the 5′ side of the SRE (*16*). A second protein, $p62^{TCF}$ (for ternary com-

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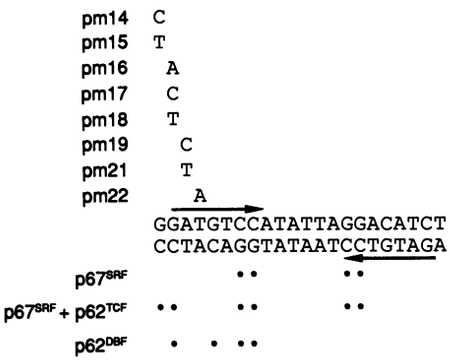

Fig. 1. SRE mutants used in this report. The sequence shown is the 22-bp serum response element sequence, which is sufficient to restore serum responsiveness to a truncated c-*fos* promoter (*5, 11*). Below the sequence is a summary of methylation interference data for each of the protein-DNA complexes known to form at the serum response element (*9, 11, 16, 17*). The dots indicate guanosine residues (on either strand) at which methylation interferes with protein binding. Above the sequence are the eight randomly generated single nucleotide substitutions (sequence of the upper strand) used in the experiments in Fig. 2. The pm18 substitution was built into a wild-type c-*fos* promoter to generate the −356pm18 plasmid used in Fig. 3.

plex factor), does not bind directly to the SRE, but instead recognizes the SRF-SRE complex to form a ternary complex (*17*). This ternary complex contains additional protein-DNA contacts not observed for the SRF-SRE complex (*17*) (Fig. 1), and in vivo footprinting studies are consistent with the presence of this ternary complex at the SRE prior to and after serum stimulation (*15*).

To correlate binding of these proteins with SRE function in vivo, we generated a collection of mutants that carried nucleotide substitutions in the 5′ flanking region of the SRE (Fig. 1). Electrophoretic mobility shift assays were carried out with probes generated from each mutant (*18*) (Fig. 2A). The probes were labeled internally so that they had comparable specific activities. The wild-type probe formed both the SRF and the SRF-p62^{TCF} complexes (Fig. 2A, lane 1; labeled B and A, respectively). In contrast, each probe that carried a single substitution in the three outer base pairs of the SRE yielded an SRF complex that was similar in strength to wild type, but that formed little or no ternary complex. As a control, a probe with a substitution in the fourth position of the SRE was tested (pm22, Fig. 1). This probe bound SRF with lower affinity, but formed the ternary complex with high efficiency (Fig. 2A, lane 9). Similar behavior was observed for other mutants at this and more internal positions (*19*).

In order to test the function of these mutants in vivo, SRE-containing restriction fragments were transferred to a truncated c-*fos* promoter fused to the bacterial CAT gene. This promoter, which lacks an SRE, is not inducible by serum, but insertion of a wild-type SRE restores serum responsiveness (*5*). Each construct was transfected into BALB/c 3T3 fibroblasts and tested for serum inducibility (*20*). A construct that carried a wild-type SRE was strongly inducible by serum (Fig. 2B, lanes 1 and 2, T), whereas one that carried a previously characterized SRE mutant (pm12) (*5, 16*) was not, although endogenous c-*fos* was induced normally in these cultures (lanes 3 and 4, E). Each construct that carried a substitution that prevented ternary complex formation was induced by serum at least as well as wild type (lanes 5 to 18). Only the pm22 mutant, which bound SRF poorly but still formed the ternary complex, was significantly less inducible than wild type (lanes 19 and 20). These results suggest that ternary complex formation is not required for serum responsiveness in BALB/c 3T3 cells.

To determine whether such a mutation retained this phenotype when placed in its natural sequence context, we mutated an intact c-*fos* promoter to carry the pm18 (Fig. 1) mutation, a G to T transversion in the second position (Fig. 1). Probes that spanned the SRE (nucleotides at positions −356 to −275 in the c-*fos* regulatory region) were prepared from the wild-type and pm18 promoters and tested for their abilities to form the ternary complex in vitro by electrophoretic mobility shift assay. Both probes bound p62^{DBF} (complex C; Fig. 3A, lanes 3 and 4) and SRF (complex B; Fig. 3A, lanes 5 and 6) at equivalent levels, but the pm18 mutant failed to form the ternary complex (complex A; Fig. 3A, lanes 5 and 6). These promoters were then tested for in vivo function (Fig. 3B). Both the wild-type and mutant promoters retained responsiveness to serum (compare lanes 1 and 2 with 5 and 6), consistent with the conclusion that ternary complex formation is not required for serum stimulation. However, the pm18 mutant was dramatically impaired for response to the phorbol ester, phorbol myristate acetate (PMA) (lane 7) and responded at an intermediate level to purified, recombinant c-Sis protein, a form of platelet-derived growth factor (PDGF) (lane 8). This observation suggests that the ternary complex is required for response to PMA but not for other signals.

PMA is an activator of PKC, an important mediator of growth factor signals (*21*). That the ternary complex mutant responded poorly to PMA treatment suggests that this mutant failed to respond to PKC activation. The residual response of the mutant of c-Sis and serum suggests that the mutant retained response to PKC-independent signals. To test this idea, we removed PKC from cells by chronic phorbol ester treatment (*22*) and examined the response of the promoters to PKC-independent signals. Under these conditions, the wild-type and mutant promoters behaved similarly, responding with the following hierarchies: serum > c-Sis > PMA (Fig. 3C). Thus, both promoters remain responsive to PKC-independent signals. These results support the idea that ternary complex formation is required for PKC-dependent activation of c-*fos*, but not for

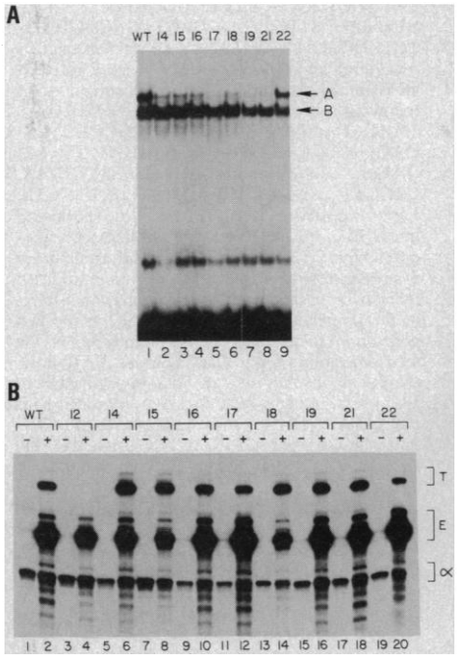

Fig. 2. Binding properties in vitro and serum responsiveness in vivo of the serum response element mutants. (**A**) Electrophoretic mobility shift assay. Uniformly labeled DNA probes of comparable specific activity were prepared from each of the mutants (Fig. 1). Electrophoretic mobility shift assays were performed with heparin-agarose–purified p62^{TCF} and SRF. A, the position of the ternary complex that contained both proteins; B, the complex that contained SRF alone. (**B**) Transient expression assay. Each SRE derivative, including wild type (WT) and a previously characterized mutant (pm12) (*5, 16*) that binds none of the proteins, was transferred to a truncated mouse c-*fos* promoter that carried sequences from −151 to +109. The plasmids were transiently transfected into duplicate cultures of BALB/c 3T3 cells together with a human α-globin internal control plasmid. After a 48-hour incubation in medium that contained 0.5% calf serum, one dish from each pair of duplicates was fed fresh medium that contained 10% calf serum (lanes marked +). The remaining dish in each pair was untreated (lanes marked −). All dishes were harvested 45 min later. Cytoplasmic RNA was isolated and assayed by RNase protection. T, probe fragment protected by transcripts of the transfected c-*fos* plasmid; E, probe fragment protected by endogenous c-*fos* mRNA; α, probe protected by transcripts of the α-globin internal control plasmid.

PKC-independent activation. Thus, SRF, alone or with unidentified accessory proteins, is sufficient to respond to PKC-independent signals, while $p62^{TCF}$ confers PKC responsiveness to SRF.

An intact SRE is required for the response of the c-*fos* promoter to both PKC-dependent and PKC-independent signals (*5*). Here we show that these signals act on overlapping targets. That is, a mutation in the 5′ flanking region of the SRE abolished response to PKC-dependent signals only. This phenotype correlated with loss of ternary complex formation at the SRE. A previous study (*17*) showed that mutant SRE's that fail to form the ternary complex lose response to serum. In our experiments, serum response was retained. One explanation for this discrepancy is differences in second-messenger production in the cells used. Thus, in our experiments serum-stimulated c-*fos* transcription is activated largely by PKC-independent pathways. Loss of response to PKC had little effect on serum induction. In other systems, however, serum may act predominantly through the PKC pathway. Loss of response to PKC would then lead to significant loss of serum response.

Our results raise a paradox. The recognition specificity of $p62^{TCF}$ for the c-*fos* SRE is quite stringent, because each of the seven nucleotide substitutions in the outer three positions of the SRE substantially reduced ternary complex formation. Yet SRE's in other growth factor–regulated genes share little sequence similarity in this region; only the central core, which binds SRF, is conserved (*23*). One possible explanation for this paradox comes from studies of the yeast MCM1 protein, which is related to SRF in primary amino acid sequence and DNA recognition specificity (*24*). MCM1 adopts a different conformation depending on the site to which it is bound (*25*). If this is true for SRF, then SRF bound to different SRE's will display distinct surfaces to $p62^{TCF}$. Because $p62^{TCF}$ appears to require contacts with both SRF and the SRE, changes in SRF contacts are likely to be balanced by changes in the DNA contacts required for optimum $p62^{TCF}$ binding. Therefore, it may not be valid simply to compare flanking sequence of SRE's in which the central sequences differ. Some of these SRE's may not form the ternary complex and thus would be responsive to a different spectrum of signals. The existence of distinct classes of SRE's with different patterns of signal responsiveness could account in part for the unique biological activities of different growth factors. This is a striking example of how flanking sequences can influence the function of a transcription factor.

$p62^{TCF}$ may be viewed as an adapter that allows SRF to respond to a new signal. Such an adapter could function between SRF and upstream signals, providing a physical target for the PKC pathway, and, therefore, coupling SRF to that pathway. $p62^{TCF}$ could be a direct target for a covalent modification, or, together with SRF, it could present a surface for interaction of another protein factor. Alternatively, $p62^{TCF}$ could function as a downstream adapter that bridges SRF to the transcriptional machinery.

These data strengthen the parallels between nuclear signaling pathways in mammalian cells and in yeast (*26*). Several genes from *Saccharomyces cerevisiae* that are induced by mating pheromones carry a binding site for MCM1 (*24*). For the α-specific *STE3* gene, pheromone induction requires a ternary complex of MCM1 and the MATα1 protein (*27*). This MCM1-MATα1 complex is similar to the SRF-$p62^{TCF}$ complex in its structural and functional organization; MATα1 flanks an MCM dimer on one side only and provides a signal-responsive activation function to MCM1. In contrast, for the α-specific *STE2* gene, pheromone induction requires interaction of MCM1 with a different protein, STE12, bound to a nearby site (*28*). These two target architectures in yeast may be analogous to the two distinct signal-responsive targets at the c-*fos* SRE. We suggest, therefore, that $p62^{TCF}$ may be functionally related to MATα1.

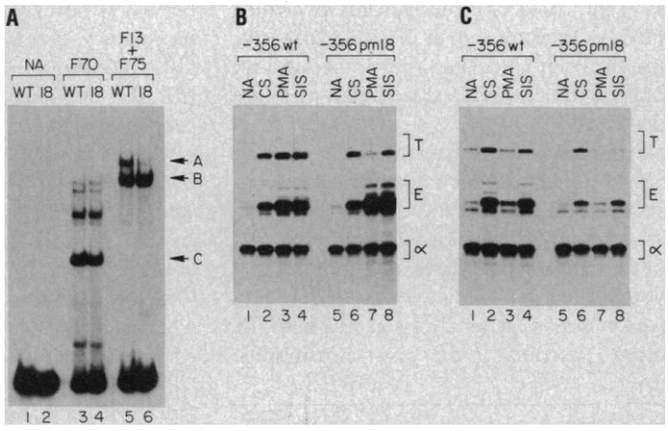

Fig. 3. Binding properties in vitro and inducibility in vivo of a c-*fos* promoter carrying a single nucleotide substitution (pm18). (**A**) Electrophoretic mobility shift assay. Uniformly labeled DNA probes contained c-*fos* promoter sequences from −356 to −275 and were either wild type in sequence (lanes marked WT) or carried a single nucleotide substitution (pm18) (Fig. 1) (lanes marked 18). Assays were performed with no added protein (lanes 1 and 2), with a heparin-agarose fraction (fraction 70) (*16*) that contained $p62^{DBF}$ (lanes 3 and 4), or with heparin-agarose fractions that contained $p62^{TCF}$ and SRF (fractions 13 and 75, respectively) (lanes 5 and 6). A, ternary complex; B, SRF complex; C, $p62^{DBF}$ complex. (**B**) Transient expression assay of c-*fos* promoters treated with various inducers. BALB/c 3T3 cells were transfected with wild-type (lanes 1 to 4) or pm18 (lanes 5 to 8) promoter plasmids and left untreated (lanes 1 and 5), or treated with fresh medium that contained 10% calf serum (lanes 2 and 6), phorbol myristate acetate (Sigma) (50 ng/ml) (lanes 3 and 7), recombinant c-Sis protein (Amgen Biologicals) (40 ng/ml) (lanes 4 and 8). Treatment was for 45 min. RNase protection assays were performed as in Fig. 2. (**C**) Transient expression assays of wild-type and pm18 promoters in cells treated with phorbol dibutyrate. BALB/c 3T3 cells were transfected and induced as described in (B), except that after transfection cells were treated for 48 hours with phorbol dibutyrate (Sigma) (200 ng/ml).

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18. The starting plasmid for mutagenesis was pUC119, which carried an SRE oligonucleotide in the Hinc II site of the polylinker (*5*). Single-stranded phage DNA was prepared from transformed *Escherichia coli* TG-2 infected with M13K07 helper phage. The oligonucleotide used for mutagenesis had the following sequence: 5′-CTGCAGGTCGTCGGATGTCCATATT-3′. Underlined positions were synthesized from a preparation of precursors with ~80% of the wild-type nucleotide and 20% of an equimolar mixture of four precursors. The oligonucleotide mixture was isolated by gel electrophoresis and purified by chromatography on a Sep-Pak column (Waters). Mutagenesis was performed with the Amersham kit. The mutagenesis reaction was transformed into *E. coli* TG-2, and ampicillin-resistant colonies were picked randomly for phage DNA preparation. Phage DNA was sequenced with a universal sequencing primer and Sequenase enzyme (United States Biochemical). Of ~100 phages se-

quenced, about 35% carried mutations in the SRE. Of these mutants, we obtained 13 of the 18 possible single substitutions, as well as five mutants with multiple substitutions. Mobility shift probes of similar specific activities were prepared by primer extension of phagemid DNA in the presence of α-^{32}P-dCTP. DNA was annealed to universal sequencing primer, and the primer was extended with Klenow polymerase for 60 min at room temperature. The Klenow was heat-inactivated and the reaction was treated with Hind III and Eco RI, which excises a 79-bp internally labeled fragment that carried the SRE derivative. The labeled fragments were gel purified, eluted from the gel slice by diffusion, and used directly without further purification. DNA-binding assays were performed as described, with heparin-agarose–purified protein fractions (*16*). SRF was eluted from the heparin-agarose column at ~450 mM KCl, p62^{DBF} at 350 mM KCl, and p62^{TCF} flowed through the column.

19. R. Graham and M. Gilman, unpublished data.
20. To assay the activity of the mutant SRE's in vivo, we transferred them to a c-*fos*–CAT fusion plasmid that carried mouse c-*fos* sequences from −151 to +109 (*4, 8*). Phage DNA inserts were amplified by the polymerase chain reaction (PCR) in 50-μl reactions that contained phage DNA (~1 ng), forward and reverse sequencing primers (50 pmol of each), deoxynucleoside triphosphates (250 μM), and Taq polymerase (Perkin-Elmer Cetus) (1.25 units). The amplification cycle was 30 s at 94°C, 30 s at 55°C, 60 s at 72°C, and was repeated for 25 cycles. The PCR products were digested with Hind III and Sal I, and the fragments that carried the SRE were isolated from a polyacrylamide gel and ligated into the c-*fos*–CAT plasmid that had been cleaved with the same enzymes. This construction placed the SRE oligonucleotide sequence immediately upstream of position −151, separated by 7 bp. All clones were sequenced to determine that the correct SRE oligonucleotide had been subcloned. Transfection and serum stimulation of BALB/c 3T3 cells and ribonuclease (RNase) protection assays were performed as described (*5*) [L. A. Berkowitz, K. T. Riabowol, M. Z. Gilman, *Mol. Cell. Biol.* **9**, 4272 (1989)] with one modification. A total of 10 μg of cytoplasmic RNA was subject to RNase protection analysis. Half of this material (representing 5 μg of RNA from each culture) was analyzed by gel electrophoresis to compare signals from endogenous c-*fos* to ensure that all cultures were equally treated. The remaining samples were refractionated in amounts adjusted to equalize the signals from the α-globin internal control; this controls for variations in transfection efficiency and RNA recovery and facilitates comparison of signals from the transfected c-*fos*–containing plasmids. These are the gels shown in the figures. Therefore, signals from the transfected c-*fos* plasmids may be directly compared, but signals from the endogenous c-*fos* gene may not, because they represent signals from different amounts of cellular RNA.
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Association of B Cell Antigen Receptor with Protein Tyrosine Kinase Lyn

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Antigen is thought to cross-link membrane-bound immunoglobulins (Igs) of B cells, causing proliferation and differentiation or the inhibition of growth. Protein tyrosine kinases are probably involved in signal transduction for cell proliferation and differentiation. The Src-like protein tyrosine kinase Lyn is expressed preferentially in B cells. The Lyn protein and its kinase activity could be coimmunoprecipitated with IgM from detergent lysates. Cross-linking of membrane-bound IgM induced a rapid increase in tyrosine phosphorylation of at least ten distinct proteins of B cells. Thus, Lyn is physically associated with membrane-bound IgM, and is suggested to participate in antigen-mediated signal transduction.

B CELLS HAVE MEMBRANE-BOUND immunoglobulins (mIgs) on their surface, which are receptors for specific antigens. Cross-linking of the mIgs of resting B cells by antigens or antibodies to Ig generally activates B cells to enter the G_1 phase of the cell cycle, in which they become susceptible to proliferative signals provided by helper T cells (*1, 2*). Cross-linking can also cause B cell tolerance, by inhibiting growth of immature and mature B cells (*3*). These responses are preceded by phosphatidylinositol (PI) turnover, activation of protein kinase C, and Ca^{2+} mobilization (*2, 4, 5*), which are apparently dependent on the function of GTP-binding proteins (G proteins) (*6*). Because B cell antigen receptors have few cytoplasmic amino acids (*7*), mIgs might be associated with other signal transducing molecules. mIgM and mIgD associate with at least three sets of polypeptide chains at the plasma membrane (*8*). However, the molecular mechanisms by which the mIgs transmit signals intracellularly have not yet been elucidated, partly because no catalytic molecules associated with mIgs are identified.

Protein tyrosine kinases probably participate in signal transduction systems that control cell proliferation and differentiation (*9, 10*). Protein tyrosine kinases can be divided into receptor-type kinases and nonreceptor-type kinases (or Src-like kinases). The Src-like kinases are generally associated with the internal portion of the plasma membrane and have no extracellular or transmembrane sequences (*10*). Thus, Src-like kinases may act as signal transducers in association with surface receptors that lack an intracellular catalytic domain. The T lymphocyte–specific Src-like kinase p56^{lck} is physically and functionally associated with the T cell surface antigens CD4 and CD8 (*11*). Possible involvement of protein tyrosine kinases in B cell activation is suggested by the observation that antibody to CD45, a membrane-bound phosphotyrosine phosphatase, inhibits anti-IgM–induced proliferation of B cells (*12, 13*). The *lyn* gene product is an Lck-like protein tyrosine kinase that is preferentially expressed in B cells, but not T cells (*14, 15*). Our present study with antibodies to Lyn suggests that the Lyn protein is physically associated with mIgM in B cells and functions in mIgM-mediated signaling.

WEHI-231 cells are murine B cell line that carry mIgM (*5, 16*). These B cells behave as normal B cells in terms of initial biochemical events such as PI turnover and Ca^{2+} mobilization after mIgM cross-linking (*5*). This suggests that these cells possess a proper signal transduction system for early responses to mIgM cross-linking. To determine if the Lyn protein is physically associated with mIgM, we examined immunoprecipitates of IgM from detergent (digitonin) lysates of WEHI-231 cells by immunoblotting with antibodies to Lyn (anti-Lyn), which were affinity-purified from antiserum (*15*) (Fig. 1A). Both forms of Lyn, p56^{lyn} and p53^{lyn} (*17*), were coimmunoprecipitated with IgM, but not with the class I nor class II major histocompatibility complex (MHC) molecules. No Lyn protein was detected in anti-IgM immunoprecipitates from digitonin lysates of A-20 cells, which do not express mIgM. Faint and broad bands detected in each precipitate were from the antibody solutions used for the immunoprecipitation (*18*). These data support the conclusion that the Lyn protein is physically associated with mIgM in B cells.

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