28. P. H. Warren and G. K. Kallemeyn, *J. Geophys. Res.* **98**, 5445 (1993).
29. P. H. Warren, G. J. Taylor, K. Keil, *Proc. Lunar Planet. Sci. Conf. 13, J. Geophys. Res.* **88**, A615 (1983).
30. We thank K. Edwards, K. Becker, E. Lee, T. Becker, T. Sucharski, J. Mathews, D. Cook, D. McMacken, C. Bowers, and J. Torson [all with U.S. Geological Survey (USGS)]; E. Malaret and H. Taylor (Applied Coherent Technologies); and C. Acton (Jet Propulsion Laboratory) for contributions to the Clementine data management and processing. Special thanks go to I. Lewis and H.-S. Park (Lawrence Livermore National Laboratory) for sensor calibrations and to T. Sorenson (AlliedSignal) and R. Reisse (Science Applications) for sequence planning. We also thank L. Gaddis (USGS), G. Blount (Texas A&M University), and two others for constructive reviews. This paper is LPI Contribution No. 848.

3 August 1994; accepted 1 November 1994

An Alternative to SH2 Domains for Binding Tyrosine-Phosphorylated Proteins

W. Michael Kavanaugh and Lewis T. Williams*

Src homology 2 (SH2) domains bind specifically to tyrosine-phosphorylated proteins that participate in signaling by growth factors and oncogenes. A protein domain was identified that bound specifically to the tyrosine-phosphorylated form of its target protein but differs from known SH2 sequences. Phosphotyrosine-binding (PTB) domains were found in two proteins: SHC, a protein implicated in signaling through Ras; and SCK, encoded by a previously uncharacterized gene. The PTB domain of SHC specifically bound to a tyrosine-phosphorylated 145-kilodalton protein. PTB domains are an alternative to SH2 domains for specifically recruiting tyrosine-phosphorylated proteins into signaling complexes and are likely to take part in signaling by many growth factors.

SH2 domains are amino acid sequences that are similar to a 100-residue noncatalytic region of the Src tyrosine kinase and are present in various signaling molecules (*1*). SH2 domains are functional protein motifs that bind tyrosine-phosphorylated targets by recognizing phosphotyrosine and specific adjacent residues (*2*). Activation of tyrosine kinases by growth factors, cytokines, and oncogenic agents therefore serves as a switch for assembling SH2 domain–containing proteins with their tyrosine-phosphorylated targets in signaling complexes in which downstream effectors are activated.

We identified a domain in the signaling protein SHC that binds specifically to the tyrosine-phosphorylated form of its target protein. The amino acid sequence of the PTB domain is not similar to that of any member of the known SH2 domain family. The SHC gene encodes 46- and 52-kD transforming proteins that are tyrosine phosphorylated in response to a number of growth factors and have been implicated as mediators of signaling from growth factor receptor and nonreceptor tyrosine kinases to Ras (*3*). The SHC protein contains (i) an SH2 domain at its COOH-terminus that binds tyrosine-phosphorylated targets such as activated growth factor receptors, (ii) a region of similarity to human alpha 1 collagen in the middle of the molecule that contains a binding site for the GRB2 adapter protein, and (iii) a 232-residue NH_2-terminus with no known function.

We investigated proteins that coimmunoprecipitated with SHC in growth factor–stimulated cells. Several tyrosine-phosphorylated proteins of approximately 145 kD (collectively called pp145) were present in anti-SHC immunoprecipitates from cells treated with either platelet-derived growth factor (PDGF) or fibroblast growth factor (FGF) but not in anti-SHC immunoprecipitates from unstimulated cells (Fig. 1). A similar tyrosine-phosphorylated protein or proteins were associated with SHC in B cells stimulated with antibodies to immunoglobin M, in activated T cells, in HepG2 hepatoma cells stimulated with interleukin-6, and in CCE embryonic stem cells stimulated with leukemia inhibitory factor (*4*). The number and electrophoretic mobility of the pp145 proteins varied slightly among different cell types. These may represent different proteins or the same protein with different amounts of phosphorylation. All of these proteins appear to bind to SHC in a similar manner. The pp145 proteins in fibroblasts or in B cells were not recognized by immunoblotting with antibodies to SHC, phospholipase C gamma, Ras guanosine triphosphatase–activating protein, the guanine nucleotide exchange factor Son of Sevenless (SOS), insulin receptor substrate 1, the guanine nucleotide exchange factor C3G, the transforming protein Eps 15, or the PDGF or FGF receptors (*5*).

To characterize binding of SHC to pp145, we separated proteins from cell lysates by SDS–polyacrylamide gel electrophoresis (SDS-PAGE), transferred them to nitrocellulose, and incubated them with ^{32}P-labeled glutathione-S-transferase (GST)–SHC fusion protein. ^{32}P-labeled SHC bound specifically to three proteins of approximately 180, 145, and 120 kD from lysates of PDGF-stimulated fibroblasts but did not bind any proteins from lysates of unstimulated cells (Fig. 2A). The 180-kD band comigrated with the autophosphorylated PDGF receptor; the 145-kD band comigrated with the major pp145 protein identified in Fig. 1; the identity of the 120-kD protein is unknown. The ^{32}P-SHC probe also bound 145-kD proteins present in anti-SHC immunoprecipitates from PDGF-stimulated cells but did not bind any proteins in immunoprecipitates from unstimulated cells (Fig. 2A). Therefore, the ^{32}P-SHC probe apparently binds to the same 145-kD protein or proteins that associate with SHC in vivo. These experiments demonstrate that SHC binds pp145 directly and that interaction of SHC and p145 in vitro requires PDGF stimulation in vivo.

To map the region of SHC responsible for binding to pp145, ^{32}P-SHC probes were prepared that contained deletions of various domains. Deletion of the SH2 domain of SHC eliminated binding to the p180 and p120 proteins in lysates of PDGF-stimulated cells but did not affect binding to pp145 in either cell lysates or in anti-SHC immunoprecipitates (Fig. 2B). Further, the isolated SH2 domain from SHC bound to p180 and p120 but not to pp145 (Fig. 2B).

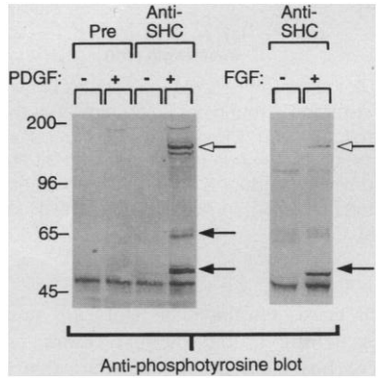

Fig. 1. Association of SHC with 145-kD tyrosine-phosphorylated proteins in vivo. Balb/3T3 fibroblasts were stimulated with PDGF (left); L6 myoblasts expressing the human FGF receptor 1 were stimulated with bFGF (right) (*12*). Cell lysates were immunoprecipitated with preimmune serum or antiserum to SHC and immunoblotted with antibody to phosphotyrosine. Black arrows indicate the 52-kD SHC protein and a 66-kD SHC-related protein seen in fibroblasts. The pp145 proteins are indicated by open arrows. Each lane contains equal amounts of SHC, as determined by immunoblotting with antiserum to SHC (*5*).

Cardiovascular Research Institute, Department of Medicine, University of California, San Francisco, CA 94143, USA.

*To whom correspondence should be addressed.

Therefore, binding of SHC to p120 and p180 was due to the SH2 domain, but binding of SHC to pp145 did not require the SH2 domain. By further deletional analysis, we identified a 186–amino acid fragment representing residues 46 to 232 of SHC that bound pp145 (Fig. 2C). This fragment bound specifically to pp145 and not to any other proteins in nitrocellulose-filter binding assays of lysates or of anti-SHC immunoprecipitates from cells stimulated with PDGF or FGF or from activated B cells. This region of SHC also specifically bound pp145 in solution-binding assays (Fig. 2D). The pp145-binding domain (designated the PTB domain) is located in the NH_2-terminal portion of SHC, for which no function had been previously assigned.

Because both SHC and p145 are tyrosine phosphorylated in growth factor–stimulated cells, we examined how the binding properties of these two molecules were influenced by tyrosine phosphorylation. Phosphotyrosine was not detected on immunoblots of a baculovirus-derived SHC protein probe when antibody to phosphotyrosine was used, and treatment of the SHC probe with tyrosine-specific phosphatases had no effect on binding to pp145 (5). Therefore, tyrosine phosphorylation of SHC is not required for binding. To investigate the relation of phosphorylation of p145 to binding with SHC, we transferred pp145 derived from anti-SHC immunoprecipitates from PDGF-stimulated cells to nitrocellulose and treated it with tyrosine-specific phosphatases. Dephosphorylation of immobilized pp145 completely eliminated binding to ^{32}P-SHC (Fig. 3A). This effect was prevented by inclusion of the tyrosine phosphatase inhibitor sodium orthovanadate. Conversely, when p145 in lysates of unstimulated cells, which does not bind SHC, was phosphorylated with recombinant PDGF receptor, the p145 then bound to the PTB domain of SHC (5). Therefore, binding of SHC to p145 requires p145 to be tyrosine phosphorylated.

To further investigate the mechanism of PTB binding to pp145, we separated proteins in cell lysates by SDS-PAGE, transferred them to nitrocellulose, and assayed binding of ^{32}P-SHC to pp145 in the presence of phosphotyrosine. Binding of SHC to pp145 was competitively inhibited by high concentrations of phosphotyrosine but not by similar concentrations of phosphoserine (Fig. 3B). Phosphotyrosine also inhibited binding of SHC to p180 and p120, which are SH2 domain–dependent interactions. These experiments suggested that binding of the PTB region to pp145 involves recognition of phosphotyrosine, as does SH2 domain binding.

Taken together, these results demonstrated that (i) the PTB domain of SHC specifically binds to pp145 and not to other proteins, (ii) that this interaction

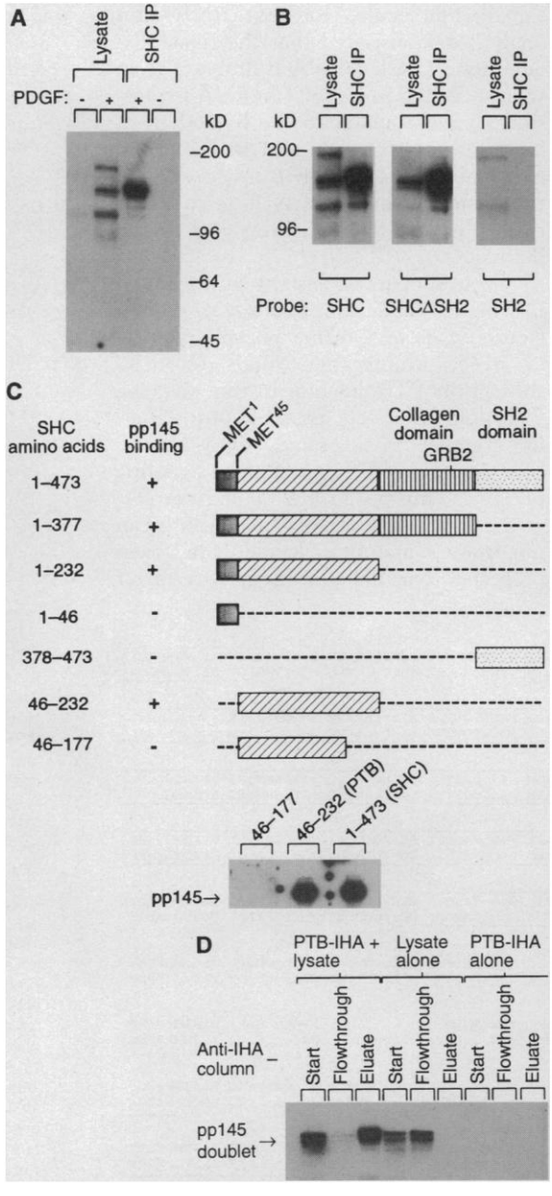

Fig. 2. Binding of SHC to pp145. (**A**) Binding of full-length SHC to proteins in cell lysates or in anti-SHC immunoprecipitates (SHC IP) from quiescent (−) or PDGF-stimulated (+) Balb/3T3 cells. Proteins were analyzed by SDS-PAGE and transferred to nitrocellulose. The filters were incubated with ^{32}P–GST-SHC fusion proteins as probes (*13*). (**B**) The pp145 protein in lysates or anti-SHC immunoprecipitates from PDGF-stimulated fibroblasts were blotted with ^{32}P-SHC (left), SHC in which the SH2 domain had been deleted (SHCΔSH2, residues 1 to 377, middle), or the isolated SH2 domain from SHC (residues 378 to 473, right). (**C**) Mapping of the pp145-binding domain on SHC. Fragments of SHC corresponding to the indicated residues were prepared as ^{32}P-GST fusion proteins and as probes as described above. The organization of the SHC protein is shown, including the two translation start sites (MET^1 and MET^{45}); the GRB2 binding site; and the PTB, collagen, and SH2 domains. Shown are blots of anti-SHC immunoprecipitates from PDGF-stimulated fibroblast lysates with the use of three representative probes. (**D**) Binding of the PTB domain to pp145 in solution. GST-SHCΔSH2 protein containing the IHA epitope was incubated with lysate of activated B cells and then purified by immunoaffinity chromatography with monoclonal antibody to IHA (*14*). The starting material, column flowthrough, and SDS eluates were analyzed by blotting with ^{32}P-labeled PTB domain probe as described above.

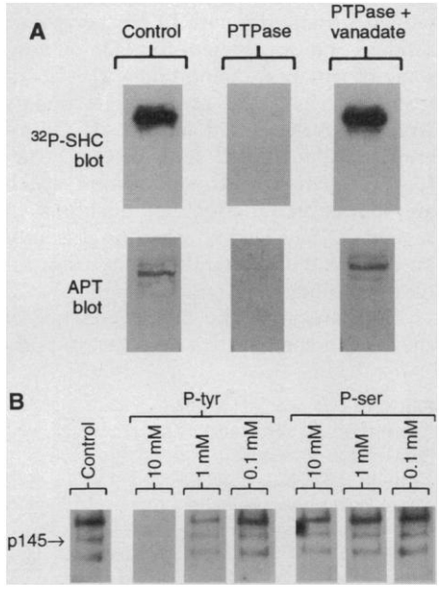

Fig. 3. Involvement of phosphotyrosine in binding of SHC to pp145. (**A**) Proteins in anti-SHC immunoprecipitates from PDGF-stimulated fibroblasts were immobilized on nitrocellulose and treated with tyrosine-specific phosphatases in the presence or absence of the protein tyrosine phosphatase (PTPase) inhibitor sodium orthovanadate (*15*). The filters were then blotted with ^{32}P–GST-SHC (top) or immunoblotted with antibody to phosphotyrosine (bottom). (**B**) Lysates from PDGF-stimulated cells were blotted with ^{32}P–GST-SHC in the presence of the indicated concentrations of phosphotyrosine (P-tyr) or phosphoserine (P-ser).

requires stimulation of cells by various growth factors, and (iii) that the PTB domain specifically recognizes the tyrosine-phosphorylated form of p145. This combination of specificities is functionally similar to those of SH2 domains. We also observed that pp145 could not be effectively dephosphorylated while it was complexed to SHC in immunoprecipitates. This suggested that the PTB domain protected the phosphotyrosine of pp145 from phosphatase action, as do SH2 domains (6). However, PTB domains are clearly different in sequence from SH2 domains. The amino acid sequence of the PTB region of SHC is different from that of all members of the known SH2 domain family. The only recognizable sequence similarity is a short motif present in the extreme NH_2-terminal end of the PTB domain—GVSYLVR (7)—which is somewhat similar to the consensus sequence for phosphotyrosine binding in SH2 domains: G(S or T)FLVRES (Fig. 4) (7, 8). However, mutation of the arginine in this motif (Arg^{55} of SHC) to leucine did not affect binding of the PTB domain to pp145 (5); the analogous mutation in SH2 domains abolishes binding to tyrosine-phosphoryl- ated targets (9). The remainder of the PTB domain has little or no sequence similarity with highly conserved residues or motifs present in SH2 domains, some of which are important for SH2 domain function. The predicted secondary structure of the PTB domain is also different from that of SH2 domains (5). Structural comparison of these domains, which are similar in function but dissimilar in sequence, may lead to new insights into protein-protein interactions in tyrosine kinase signaling.

Comparison of the DNA sequence of the PTB domain with sequences in computer databases revealed a gene that contained a putative PTB domain. A partial clone of this gene, EST03775, was originally obtained by sequencing expressed sequence tags from human brain complementary DNA (cDNA) clones (10) but had not been recognized as a putative signaling molecule. By screening human placental and HepG2 cDNA libraries with EST03775, we identified a larger partial clone encoding a protein with a PTB domain and an SH2 domain that were similar to those of SHC, which we call SCK (for SHC-like) (Fig. 4). The conserved region of the PTB domain correlated with the minimal region necessary for binding to pp145. The identification of a PTB domain in SCK establishes that PTB domains are present in more than one gene. Although the biological function of SCK is unknown, the presence of both SH2 and PTB domains strongly suggests that it is a signaling molecule. Northern (RNA) blot analysis demonstrated that the tissue distribution of SCK mRNA is different from that of SHC mRNA. SCK mRNA expression is much greater in the liver than in other tissues and is present in the brain. SHC mRNA expression is approximately the same in the liver as it is in other tissues, except that it is very low in the brain.

These data are consistent with a model in which stimulation of cells with growth factors leads to tyrosine phosphorylation of p145, which then binds to SHC through its PTB domain. In this way, the PTB domain directs assembly of a signaling complex in the same way as do the SH2 domains of SHC or of other signaling proteins. Thus pp145, the target or targets of the SHC PTB domain, may also be an important signaling molecule. It has been proposed that SHC links growth factor receptor and nonreceptor tyrosine kinases to activation of Ras; one mechanism for this link is formation of a complex of SHC with GRB2, an adapter protein, and with SOS, a guanine nucleotide exchange factor for Ras (11). We speculate that p145 may also participate in the regulation of Ras signaling by virtue of its association with SHC.

Fig. 4. Comparison of the amino acid sequences of SHC and SCK (7). Shown is a comparison of residues 11 to 432 of SHC with a SCK partial clone. Bold uppercase letters represent identical residues. The PTB domains of SHC (Fig. 2C) and of SCK are enclosed in boxes; the SH2 domains are underlined. Asterisks mark the FLVRES-like sequence in the PTB domain.

REFERENCES AND NOTES

1. I. Sadowski, J. C. Stone, T. Pawson, *Mol. Cell. Biol.* **6**, 4396 (1986).
2. J. A. Escobedo, D. R. Kaplan, W. M. Kavanaugh, C. W. Turck, L. T. Williams, *ibid.* **11**, 1125 (1991); L. C. Cantley *et al.*, *Cell* **64**, 281 (1991); T. Pawson and G. D. Gish, *ibid.* **71**, 359 (1992); S. Zhou *et al.*, *ibid.* **72**, 767 (1993); G. Waksman, S. E. Shoelson, N. Pant, D. Cowburn, J. Kuriyan, *ibid.*, p. 779.
3. G. Pelicci *et al.*, *Cell* **70**, 93 (1992); M. Rozakis-Adcock *et al.*, *Nature* **360**, 689 (1992).
4. W. M. Kavanaugh, S. Robbins, L. T. Williams, unpublished data.
5. W. M. Kavanaugh and L. T. Williams, unpublished data.
6. R. B. Birge, J. E. Fajardo, B. J. Mayer, H. Hanafusa, *J. Biol. Chem.* **267**, 10588 (1992).
7. Single-letter abbreviations for the amino acid residues are as follows: 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.
8. B. J. Mayer, P. K. Jackson, R. A. Van Etten, D. Baltimore, *Mol. Cell. Biol.* **12**, 609 (1992).
9. L. E. Marengere and T. Pawson, *J. Biol. Chem.* **267**, 22779 (1992); K. B. Bibbins, H. Boeuf, H. E. Varmus, *Mol. Cell. Biol.* **13**, 7278 (1993); S. Katzav, *Oncogene* **8**, 1757 (1993).
10. M. D. Adams, A. R. Kerlavage, C. Fields, J. G. Venter, *Nat. Genet.* **4**, 256 (1993).
11. S. E. Egan *et al.*, *Nature* **363**, 45 (1993).
12. Balb/3T3 cells or L6 myoblasts expressing the human FGF receptor 1 [D. E. Johnson, P. L. Lee, J. Lu, L. T. Williams, *Mol. Cell. Biol.* **10**, 4728 (1990)] were grown to confluence in Dulbecco's modified Eagle's medium containing bovine calf serum (10%), antibiotics, and (for L6 cells) G418 (800 μg/ml). Cells were stimulated at 37°C for 10 min with either BB PDGF (2 nM) or basic FGF (bFGF) (25 ng/ml) and lysed at 4°C in lysis buffer (1 ml/10^6 cells) [20 mM tris-HCL (pH 8.0), 137 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM sodium orthovanadate, 100 mM NaF, 10 mM sodium pyrophosphate, 1 mM EGTA, 1.5 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, aprotinin (0.15 units/ml), and 20 μM leupeptin]. Lysates were cleared by centrifugation at 13,000*g* for 10 min at 4°C. Immunoprecipitations were done as described [W. M. Kavanaugh, A. Klippel, J. A. Escobedo, L. T. Williams, *Mol. Cell. Biol.* **12**, 3415 (1992)]. SHC antibodies were raised against bacterially expressed GST–SH2 domain fusion protein from SHC essentially as described [G. Pelicci *et al.*, *Cell* **70**, 93 (1992)].
13. A full-length human cDNA clone of SHC was obtained from polymerase chain reaction (PCR) of K562 or SK cell cDNA on the basis of the published sequence. Sequencing revealed two differences from the published sequence: a silent T to C transition at position 1276 and an insertion of three bases (GCA), which resulted in an in-frame alanine insertion corresponding to amino acid 308. Because all clones contained the same changes, this probably represents a polymorphism. The cDNA was cloned into a baculovirus expression vector (pV-IKS) that contained, from 5′ to 3′, the gene encoding glutathione-S-transferase, the influenza virus hemagglutinin (IHA) epitope tag, a recognition sequence for cyclic adenosine monophosphate (cAMP)–dependent protein kinase, and the gene encoding SHC. Proteins were expressed by infection of Sf9 insect cells with recombinant baculovirus as described [D. K. Morrison *et al.*, *Cell* **58**, 649

(1989); M. D. Summers and G. E. Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures* (Texas Agricultural Station, College Station, TX, 1987)]. Deletions of SHC indicated in Fig. 2C were obtained by PCR and cloned into the same vector. GST-SHC fusion proteins were purified by binding to glutathi- one-agarose [K. Guan and J. E. Dixon, *Anal. Biochem.* **192**, 262 (1991)]. The bound proteins were then incubated in 20 mM tris-HCL (pH 7.5), 1 mM dithiothreitol (DTT), 100 mM NaCl, 12 mM $MgCl_2$, 0.5 mCi of [γ-^{32}P]ATP (6000 Ci/mmol), and 250 units of cAMP-dependent protein kinase catalytic subunit from bovine heart tissue for 1 hour at room temperature. The beads were then washed extensively and eluted with 10 mM glutathione. The specific activity of all preparations was typically $>1 \times 10^7$ cpm/μg. SDS-PAGE analysis showed a single band at the predicted sizes for the GST-SHC fusion proteins with either Coomassie staining or autoradiography. Immunoprecipitates or portions of cell lysates containing equal amounts of total protein were separated by SDS-PAGE and transferred to nitrocellulose. The filters were blocked for 2 hours at 4°C in nonfat dry milk (5%) in hybridization buffer [20 mM Hepes (pH 7.7), 75 mM KCl, 0.1 mM EDTA, 2.5 mM $MgCl_2$, 1 mM DTT, and 0.05% Triton X-100]. The filters were then incubated overnight at 4°C in hybridization buffer containing milk (1%) and 2.5×10^5 cpm/ml of ^{32}P–GST-SHC fusion protein as a probe. The filters were then washed three times in hybridization buffer with milk (1%), dried, and exposed to x-ray film with an intensifying screen for 6 to 36 hours at −70°C.

14. Lysate was prepared in hybridization buffer from 2.5×10^7 BAL17 B cells stimulated by cross-linking the B cell antigen receptor as described [T. M. Saxton *et al.*, *J. Immunol.* **153**, 623 (1994)]. The lysate was incubated with approximately 250 ng of GST-SHCΔSH2 protein containing the IHA epitope tag for 1 hour at 4°C. The mixture was then subjected to immunoaffinity chromatography with the use of a monoclonal antibody to IHA covalently linked to agarose beads. The column was washed with 50 column volumes of hybridization buffer and eluted with 2% SDS. Proteins in equal fractions of the starting mixture, column flowthrough, and SDS eluate were separated by SDS-PAGE, transferred to nitrocellulose, and blotted with ^{32}P-labeled PTB domain protein probe. In B cells, pp145 was seen as a doublet.
15. Anti-SHC immunoprecipitates from PDGF-stimulated fibroblasts immobilized on nitrocellulose filters were incubated in 25 mM imidazole (pH 7.0), 50 mM NaCl, 2.5 mM EDTA, 5 mM DTT, acetylated bovine serum albumin (100 μg/ml), and 5 units each of LAR and T cell tyrosine-specific phosphatases for 60 min at 30°C. An equivalent sample was treated identically except that 5 mM sodium orthovanadate was included. The filters were then washed extensively and blotted with ^{32}P–GST-SHC as above, except that the hybridization buffer included 1 mM sodium orthovanadate.
16. We thank P. P. Di Fiore and B. Knudsen for the Eps 15 and C3G antibodies, respectively, and W. J. Fantl, J. A. Escobedo, D. Schneider, and T. Quinn for reviewing the manuscript. Supported by NIH grants K11 HL02714 and R01 HL32898 and by the Daiichi Research Center.

3 August 1994; accepted 26 October 1994

Identification of Herpesvirus-Like DNA Sequences in AIDS-Associated Kaposi's Sarcoma

Yuan Chang,* Ethel Cesarman,† Melissa S. Pessin, Frank Lee, Janice Culpepper, Daniel M. Knowles,† Patrick S. Moore

Representational difference analysis was used to isolate unique sequences present in more than 90 percent of Kaposi's sarcoma (KS) tissues obtained from patients with acquired immunodeficiency syndrome (AIDS). These sequences were not present in tissue DNA from non-AIDS patients, but were present in 15 percent of non-KS tissue DNA samples from AIDS patients. The sequences are homologous to, but distinct from, capsid and tegument protein genes of the Gammaherpesvirinae, herpesvirus saimiri and Epstein-Barr virus. These KS-associated herpesvirus-like (KSHV) sequences appear to define a new human herpesvirus.

Kaposi's sarcoma is the most common neoplasm occurring in persons with AIDS; approximately 15 to 20% of AIDS patients develop this neoplasm, which rarely occurs in immunocompetent individuals (*1*). Epidemiologic evidence indicates that AIDS-associated KS (AIDS-KS) may have an infectious etiology. Gay and bisexual male AIDS patients are approximately 20 times more likely than hemophiliac AIDS patients to develop KS, and KS may be associated with specific sexual practices among gay men with AIDS (*2*). KS is uncommon among adult AIDS patients infected through heterosexual or parenteral human immunodeficiency virus (HIV) transmission, or among pediatric AIDS patients infected through vertical HIV transmission (*3*). Agents suspected of causing KS include cytomegalovirus (CMV), hepatitis B virus, human herpesvirus 6 (HHV6), HIV, and *Mycoplasma penetrans* (*4*). Extensive investigations, however, have not demonstrated an etiologic association between any of these agents and AIDS-KS (*5*). Noninfectious environmental agents, such as nitrite inhalants, also have been proposed to play a role in KS tumorigenesis (*6*).

To search for foreign DNA sequences belonging to an infectious agent in AIDS-KS, we used representational difference analysis (RDA) to identify and characterize unique DNA sequences in KS tissue that are either absent or present in low copy number in nondiseased tissue obtained from the same patient (*7*). This method can detect adenovirus genome added in single copy to human DNA, but has not been used to identify previously uncultured infectious agents. RDA is performed by making simplified "representations" of genomes from diseased and normal tissues obtained from the same individual through polymerase chain reaction (PCR) amplification of short restriction fragments. The DNA representation from the diseased tissue is then ligated to a priming sequence and hybridized to an excess of unligated, normal-tissue DNA representation (*8*). Only unique sequences found in the diseased tissue that have priming sequences on both DNA strands are preferentially amplified during subsequent rounds of PCR amplification. This process can be repeated with different ligated priming sequences to enrich the sample for unique DNA sequences that are found only in the tissue of interest.

The initial round of amplification-hybridization from KS and excess normal-tissue DNA resulted in a diffuse banding pattern (Fig. 1, lane 2), but four bands at approximately 380, 450, 540, and 680 base pairs (bp) were identifiable after the second amplification-hybridization (Fig. 1, lane 3). These bands became discrete after a third round of amplification-hybridization (Fig. 1, lane 4). Control RDA, performed by hybridizing DNA extracted from AIDS-KS tissue against itself, produced a single band at ~540 bp (Fig. 1, lane 5). The four KS-associated bands (designated KS330Bam, KS390Bam, KS480Bam, and KS631Bam after digestion of the two flanking 28-bp ligated priming sequences with Bam HI) were gel purified.

KS390Bam and KS480Bam Southern (DNA) hybridized nonspecifically to both KS and non-KS human tissues and were not further characterized. The remaining two RDA bands, KS330Bam and KS631Bam, were cloned and sequenced (*9*). KS330Bam

Y. Chang and M. S. Pessin, Department of Pathology, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, NY 10032, USA.
E. Cesarman and D. M. Knowles, Department of Pathology, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, NY 10032, USA.
F. Lee and J. Culpepper, DNAX Research Institute, Department of Molecular Biology, 901 California Avenue, Palo Alto, CA 94304–1104, USA.
P. S. Moore, Disease Intervention Commission, New York City Department of Health, 125 Worth Street, New York, NY 10013 and Division of Epidemiology, School of Public Health, Columbia University, New York, NY 10013, USA.

*To whom correspondence should be addressed.
†Present address: Department of Pathology, New York Hospital–Cornell Medical Center, New York, NY 10021, USA.