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16. From fiber diffraction data of Arnott *et al.* (*6*) for poly dT·dA·dT it was concluded that the triplex forms an A′-DNA type helix with C3′-endo sugar puckers. Our analysis of the sugar puckers of 31- and 32-base intramolecular triplexes from phase-sensitive correlated spectroscopy (COSY) coupling patterns indicates that almost all of the sugars except the cytosines in the third strand are predominantly S-type (C2′-endo). Thus, the intramolecular triplex may form a structure closer to an underwound B-DNA type helix than an A′-DNA helix (*27*).
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A Retroviral Oncogene, *akt*, Encoding a Serine-Threonine Kinase Containing an SH2-Like Region

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The v-*akt* oncogene codes for a 105-kilodalton fusion phosphoprotein containing Gag sequences at its amino terminus. Sequence analysis of v-*akt* and biochemical characterization of its product revealed that it codes for a protein kinase C–related serine-threonine kinase whose cellular homolog is expressed in most tissues, with the highest amount found in thymus. Although Akt is a serine-threonine kinase, part of its regulatory region is similar to the Src homology–2 domain, a structural motif characteristic of cytoplasmic tyrosine kinases that functions in protein-protein interactions. This suggests that Akt may form a functional link between tyrosine and serine-threonine phosphorylation pathways.

THE AKT8 VIRUS (*1*), THE ONLY acute transforming retrovirus isolated from a rodent T cell lymphoma (AKR) to date, transforms mink lung cells in culture. Virus rescued from nonproducer mink cells by two poorly leukemogenic amphotropic murine leukemia viruses was inoculated into newborn mice and shown to be tumorigenic (*2*). A defective clone of the AKT8 virus clone contained v-*akt*, a gene of cellular origin (*3*). The presumed human homolog of v-*akt* was cloned by screening a human genomic DNA library with a virus-derived probe under conditions of reduced stringency (*3*), and it was mapped to chromosome 14q32, proximal to the immunoglobulin heavy chain locus (*4*), a region frequently affected by translocations and inversions in human T cell leukemia or lymphoma, mixed lineage childhood leukemia, and clonal T cell proliferations in ataxia telangiectasia (*5*). The putative *AKT* gene was amplified in a human gastric carcinoma (*3*). The molecular characterization of a nondefective AKT8 virus clone presented here reveals that Akt is a protein kinase C (PKC)–related serine-threonine kinase whose noncatalytic domain contains a Src homology–2 (SH2)–like region. This structural feature suggests that Akt may form a functional link between tyrosine and serine-threonine phosphorylation pathways.

We cloned the AKT8 proviral DNA from AKT8-transformed mink lung cells using a Moloney murine leukemia virus (M-MuLV) long terminal repeat (LTR) probe. A restriction map of the integrated AKT8 provirus is shown in Fig. 1. Hybridization of restriction endonuclease–digested AKT8 DNA to a M-MuLV probe representing the entire viral genome identified a nonhybridizing region of possible cellular origin included in a 3.5-kb Bgl II–Sma I DNA fragment. Sequence analysis of this fragment, which we expected to contain the transduced cellular oncogene (Fig. 2), revealed that AKT8 has sequences from a mink cell focus-forming virus and the gene encoding Akt. The 5′ recombination breakpoint maps at nucleotide 785 from the *gag* ATG codon in the region coding for the capsid protein p30. The 3′ recombination breakpoint maps at nucleotide 298 from the *env* ATG codon in the region coding for the *env* gene product gp70. The v-*akt* gene codes for a 763–amino acid protein (86 kD) generated by the fu-

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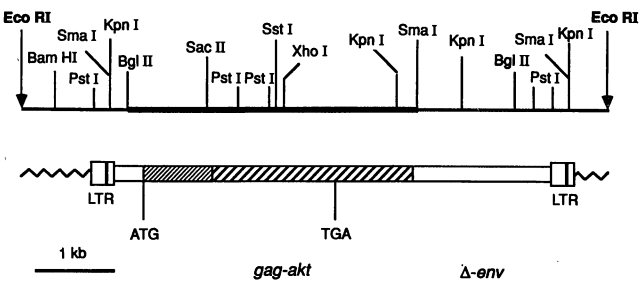

Fig. 1. Restriction endonuclease map and structural organization of the AKT8 provirus. (**Top**) Restriction map of the clone λAKT8-AB obtained by screening a partial genomic library of Eco RI–digested DNA from AKT8-transformed mink lung cells. The Bgl II–Sma I fragment shown in bold was sequenced. (**Bottom**) Structure of the AKT8 provirus. The wavy lines at both ends represent the cellular DNA sequences flanking the provirus. The hatched bar represents v-*akt*, which is subdivided into 5′ *gag*-derived and 3′ c-*akt*–derived segments. ATG and TGA define the beginning and the end of the v-*akt* open reading frame.

sion of Gag (p15, p12, and truncated p30) to the noncatalytic domain of the Akt kinase. The transduced gene contains an intact 3′ untranslated region and polyadenylate tail, suggesting that oncogene capture occurred during reverse transcription (*6*).

A search of the GenBank and National Biomedical Research Foundation databases revealed that the catalytic domain of the Akt kinase is most closely related to the rabbit PKCα (68% similarity, 47% identity) (7), the human PKCβ (68% similarity, 47% identity) (*8*), the rabbit PKCγ (68% similarity, 47% identity) (7), and the rat PKCδ (67% similarity, 46% identity) (*9*). The region of similarity is confined to the catalytic domain of these proteins (Fig. 3A). On the NH_2-terminal side from this domain, there is an amino acid sequence resembling the SH2 domain identified as a conserved motif of the cytoplasmic tyrosine kinases (*10*). The SH2-like region of Akt extends over a stretch of 77 amino acids (amino acids 285 to 361) corresponding mostly to box B and part of box C of the SH2 domain (Fig. 3) (*11*). The Akt SH2-like domain lacks about 20 amino acids found in the COOH-terminal region of other SH2 domains. Akt is also missing some highly conserved SH2 residues, such as the leucine at position 34, the arginine at position 51, and the histidine at position 78 (Fig. 3). The *gag-akt* fusion site is adjacent to the region encoding the SH2 domain, as are the fusion sites for *gag*-actin-*fgr* (*12*) and *gag-abl* (*13*). On the COOH-terminal side of the SH2-like region, v-Akt contains a domain rich in glutamic acid residues (12 out of 69 residues, 17.4%). A full-length cDNA clone of c-*akt* is identical to v-*akt* in the relative position of the catalytic and SH2-like domains (*14*). This confirms that these two regions represent linked components of one cellular gene and that the replicating virus did not transduce two separate oncogenes. The Akt SH2-like domain is highly conserved between mice and humans. Comparison of the v-Akt protein sequence between amino acids 301 and 341 to the SH2-like domain in the protein from the putative human genomic *AKT* clone mapped on chromosome 14q32 revealed 95% identity (*14*).

```
      ┌→ p15
  1   MGQTVTTPLSLTLEHWGDVQRIASNQSVDVKKRRWVTFCS   40
 41   AEWPTFGVGWPQDGTFNLDIILQVKSKVFSPGPHGHPDQV   80
 81   PYIVTWEAIAYEPPPWVKPFVSPKLSLSPTAPILPSGPST  120
           p15 ←┬→ p12
121   QPPPRSALYPALTPSIKPRPSKPQVLSDNGGPLIDLLTED  160
161   PPPYGEQGPSSSDGDGDREEATSTPEIPAPSPMVSRLRGK  200
              p12 ←┬→ Δp30
201   RDPPAAVSTTSRAFPLRLGGNGQLQYWPFSSSDLYNWKNN  240
                      Δp30.←┐
241   NPSFSEDPGKLTALIESVLTTHAREETLIIIPGLPLSLGA  280
281   TDTMNDVAIVKEGWLHKRGEYIKTWRPRYFLLKNDGTFIG  320
321   YKERPQDVDQRESPLNNFSVAQCQLMKTERPRPNTFIIRC  360
361   LQWTTVIERTFHVETPEEREEWATAIQTVADGLKRQEEET  400
                                  ┌→ Catalytic
401   MDFRSGSPSDNSGAEEMEVSLAKPKHRVTMNEFEYLKLLG  440
      domain
441   KGTFGKVILVKEKATGRYYAMKILKKEVIVAKDEVAHTLT  480
481   ENRVLQNSRHPFLTALKYSFQTHDRLCFVMEYANGGELFF  520
521   HLSRERVFSEDRARFYGAEIVSALDYLHSEKNVVYRDLKL  560
561   ENLMLDKDGHIKITDFGLCKEGIKDGATMKTFCGTPEYLA  600
601   PEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHEKL  640
641   FELILMEEIRFPRTLGPEAKSLLSGLLKKDPTQRLGGGSE  680
681   DAKEIMQHRFFANIVWQDVYEKKLSPPFKPQVTSETDTRY  720
721   FDEEFTAQMITITPPDQDDSMECVDSERRPHFPQFSYSAS  760
761   GTA   763
```

Fig. 2. Predicted amino acid sequence of v-Akt. We subcloned the Bgl II–Sma I DNA fragment of the AKT8 provirus into the plasmid pBluescript SK(−) and subjected the fragment to nested deletions in both directions using the Exonuclease III–Mung Bean Nuclease method (Stratagene). We sequenced subclones with overlapping deletions with the Sequenase version 2.0 system (United States Biochemical) using double-stranded DNA as template; we resolved GC-rich areas by sequencing single-stranded DNA or by using deoxyinosine triphosphate. The boundaries of p15, p12, Δp30 (truncated p30), and the v-Akt catalytic domain are identified by arrows. The SH2-like domain is boxed. The single-letter amino acid code is used (*26*). Three putative N-glycosylation sites are marked by asterisks. There is a glutamic acid–rich region COOH-terminal to the SH2-like domain. Tyr^{609} is a potential target of phosphorylation. The acidic context of Tyr^{609} (EVLEDNDYGRAVD) resembles the phosphorylation motif of v-Src Tyr^{416} (RLIEDNEYTARQGAK) (*27*). The sequence data have been submitted to GenBank under accession number M61767.

A

```
                       I                        II                   III                  IV                   V
              1   *  *  *                                                                                           86
Akt           NEFEYLKLLG KGTFGKVILV KEKATGRYYA MKILKKEVIV AKDEVAHTLT ENRVLQ.NSR HPFLTALKYS FQTHDRLCFV MEYANG
Rabbit PKCα   TDFNFLMVLG KGSFGKVMLS ERKGTDELYA VKILKKDVVI QDDDVECTMV EKRVLALPGK PPFLTQLHSC FQTMDRLYFV MEYVNG
Human PKCβ    TDFNFLMVLG KGSFGKVMLS ERKGTDELYA VKILKKDVVI QDDDVECTMV EKRVLALPGK PPFLTQLHSC FQTMDRLYFV MEYVNG
Rabbit PKCγ   TDFNFLMVLG KGSFGKVMLA DRKGTEELYA IKILKKDVVI QDDDVECTMV EKRVLALMDK PPFLTQLHSC FQTVDRLYFV MEYVNG
Rat PKCδ      ENFTFQKVLG KGSFGKVLLA ELKGKERYFA IKYLKKDVVL IDDDVECTMV EKRVLALAWE NPFLTHLICT FQTKDHLFFV MEFLNG
Consensus     tdFnflmvLG KGsFGKVmL. erKgt.elyA .KiLKKdVvi qdDdVecTmv EkRVLalpgk pPFLTqLhsc FQTmDrLyFV MEyvNG
                                           VI                                         VII                  VIII
              87                                                                                            172
Akt           GELFFHLSRE RVFSEDRARF YGAEIVSALD YLHSEKNVVY RDLKLENLML DKDGHIKITD FGLCKEGIKD GATMKTFCGT PEYLAP
Rabbit PKCα   GDLMYHIQQV GRFKEPHAVF YAAEIAIGLF FLQS.KGIIY RDLKLDNVML DSEGHIKIAD FGMCKENIWD GVTTKTFCGT PDYIAP
Human PKCβ    GDLMYHIQQV GRFKEPHAVF YAAEIAIGLF FLQS.KGIIY RDLKLDNVML DSEGHIKIAD FGMCKENIWD GVTTKTFCGT PDYIAP
Rabbit PKCγ   GDLMYHIQQV GKFKEPQAVF YAAEISIGLF FLHK.RGIIY RDLKLDNVML DSEGHIKIAD FGMCKEHMMD GVTTRTFCGT PDYIAP
Rat PKCδ      GDLMFHIQDK GRFELYRATF YAAEIICGLQ FLHG.KGIIY RDLKLDNVML DKDGHIKIAD FGMCKENIFG ENRASTFCGT PDYIAP
Consensus     GdLmyHiqqv grFkep.AvF YaAEIaigLf fLhs.kgiiY RDLKLdNvML DseGHIKIaD FGmCKEniwd gvttkTFCGT PdYiAP
                            IX                              X                                XI
              173                                                                                           258
Akt           EVLEDNDYGR AVDWWGLGVV MYEMMCGRLP FYNQDHEKLF ELILMEEIRF PRTLGPEAKS LLSGLLKKDP TQRLGGGSED AKEIMQ
Rabbit PKCα   EIIAYQPYGK SVDWWAFGVL LYEMLAGQAP FEGEDEDELF QSIMEHNVAY PKSMSKEAVA ICKGLMTKHP GKRLGCGPEG ERDIKD
Human PKCβ    EIIAYQPYGK SVDWWAFGVL LYEMLAGQAP FEGEDEDELF QSIMEHNVAY PKSMSKEAVA ICKCLMTKHP GKRLGCGPEG ERDIKE
Rabbit PKCγ   EIIAYQPYGK SVDWWAYGVL LYEMLAGQPP FDGEDEDELF QSIMEHNVSY PKSLSKEAVS ICKGLMTKHP AKRLGCGPEG ERDVRE
Rat PKCδ      EILQGLKYSF SVDWWSFGVL LYEMLIGQSP FHGDDEDELF ESIRVDTPHY PRWITKESKD IMEKLFERDP AKRLGVTG.. ..NIRL
Consensus     EiiayqpYgk sVDWWafGVl lYEMlaGqaP FegeDedeLF qsImehnvay Pks.skEav. ickgLmtkhP .kRLGcgpeg erdi.e

              259                                                                                    342
Akt           HRFFANIVWQ DVYEKKLSPP FKPQVTSETD TRYFDEEFTA QMITITPPDQ DDSMECVDSE RRPHFPQFSY SASGTA
Rabbit PKCα   HAFFRYIDWE KLERKEIQPP YKPK.ACGRN AENFDRFFTR HPPVLTPPDQ EVIRNIDQSE ....FEGFSF VNSEFLKPEV KS
Human PKCβ    HAFFRYIDWE KLERKEIQPP YKPK.ACGRN AENFDRFFTR HPPVLTPPDQ EVIRNIDQSE ....FEGFSF VNSEFLKPEV KS
Rabbit PKCγ   HAFFRRIDWE KLENREIQPP FKPKV.CGKG AENFDKFFTR GQPVVTPPDQ LVIANIDQSD ....FEGFSY VNPQFVHPIL QSSV
Rat PKCδ      HPFFKTINWN LLEKRKVEPP FKPKVKSPSD YSNFDPEFLN EKPQLSFSDK NL...ID.SM DQTAFKGFSF VNPKYEQFLE
Consensus     HaFFryIdWe klerkeiqPP fKPkvacgr. aenFDrfFtr hppvltppDq evirnidqSe ....FegFSf vnseflkpev kSSV
```

B

```
                    1                                                                      60
Akt         285     NDVA....IV KEGWLHK... ..RG.....E Y..IKTWR.. ..PRY..FLL KNDGTFIG.Y
PLC-γ1-N    550                   WFHGKLG AGRDGRHIAE R.LLTEYCIE TGAPDGSFLV RESETFVGDY
PLC-γ1-C    664             ES KE.WYHASLT ..RAQ...AE H.MLMRV... ..PRDGAFLV RKRN.EPNSY
v-Fps       810       EVQKPLCQQ A..WYHGAIP ..RSEV..QE ..LLKYS... ....GDFLV RESQ.GKQEY
v-Crk       248                   WYWGRLS ..RGD...AV S.LL.Q.G.. ..QRHGTFLV RDSGSIPGDF
Lck         114     NFVAKANSLE PEPWFFKNLS ..RKD...AE RQLLA.PG.. .NT.HGSFLI RESESTAGSF
v-Abl       235     LYITPVNSLE KHSWYHGPVS ..RNA...AE Y.LLS.SGI. .N..GSFLV RESESSPG.Q
v-Src       135     NYVAPSDSIQ AEEWYFGKIT ..RRE...SE R.LLLNP... ENPR.GTFLV RESETTKGAY
v-Yes       419     NYVAPADSIQ AEEWYFGKMG ..RKD...AE R.LLLNPG.. .NQR.GIFLV RESETTKGAY
GAP-N       178                   WYHGKLD ..RT...IAE E.RL...RQA ..GKSGSYLI RESDRRPGSF
GAP-C       348                   WFHGKIS ..KQE...AY N.LLMTVGQA .....CSFLV RPSDNTPGDY

                    61                                                                     117
Akt         322     KERPQDV.DQ RESPLNNFSV AQCQLMKTER PRPNTF.... .......... ..IIRCL
PLC-γ1-N    596     TLSF..WR.N ..G.KVQHCR IHSRQDAGTP KFFLTDNL.. .......... ..VFDSL
PLC-γ1-C    703     AISF..RAE. ..G.KIKHCR V...QQEGQT VMLGNSE... .......... ...FDSL
v-Fps       872     VLSV.LW.D. ..G.QPRHFI IQ.AADN..L YRLEDDG... .......... ...LPTI
v-Crk       283     VLSVSE.S.. S...RVSHYI VNSLGPAGGR RAGGEGPGAP GLNPTRFLIG DNVFDSL
Lck         164     SLSVRDF.DQ NQGEVVKHYK IR.NLDNGGF YISPRIT... .......... ...FPGL
v-Abl       282     R.SISLRYE. ..G.RVYHYR IN.TASDGKL YVSSESR... .......... ...FNTL
v-Src       185     CLSVSDF.DN AKGLNVKHYK IR.KLDSGGF YITSRTQ... .......... ...FSSL
v-Yes       469     SLSIRDW.DE VRGDNVKHYK IR.KLDNGGY YITTRAQ... .......... ...FESL
GAP-N       214     VLSFLSQT.N V....VNHFR I..IAMCGDY YIGGRR.... .......... ...FSSL
GAP-C       384     SLYFRT.SEN IQ..R...FK I.CPTPNNQF MMGGRY.... .......... ...YNSI
```

Fig. 3. (**A**) Comparison of the amino acid sequences (*26*) of the catalytic domains of v-Akt, rabbit PKCα, human PKCβ, rabbit PKCγ, and rat PKCδ. The 11 subdomains characteristic of the catalytic domain of members of the protein kinase family are indicated (*28*). The three asterisks and the arrow mark the characteristic motif of the adenosine triphosphate (ATP) binding site. Amino acid residues diagnostic for the serine-threonine kinase specificity are boxed (*28*). Dots indicate gaps. In the consensus sequence, lowercase letters indicate the amino acid found in the majority of the sequences, and dots represent unconserved amino acids. (**B**) Amino acid sequence alignment of the SH2-like domain of v-Akt and the corresponding regions (*11*, *29*) of PLC-γ1 GAP (N and C, NH_2- and COOH-terminal SH2 domains, respectively), v-Fps, Crk, Tck/Lck, v-Abl, v-Src, and v-Yes. Identical amino acids between Akt and one or more of the other listed proteins are boxed and in bold letters; similar amino acids are in bold letters only. Dots indicate gaps. The groups of similar amino acids were defined as follows: small R groups with near neutral polarity (A, G, P, S, T); nonpolar chain R groups (I, L, M, V); acidic and uncharged polar R groups (D, E, N, Q); basic polar R groups (H, K, R); and aromatic nonpolar or uncharged R groups (F, W, Y). The databases were searched with the programs Wordsearch and FastA. Sequence alignments were done with the output files from the program Bestfit and edited with the program Lineup. Wordsearch, FastA, Bestfit, and Lineup are part of the Genetics Computer Group software package (*30*).

To confirm that a *gag-akt* fusion protein was present in AKT8-transformed mink cells, we immunoprecipitated the fusion protein from lysates of uninfected (clone TR27173) and AKT8-transformed (clone 95785) mink lung cells (*3*) labeled with [^{35}S]methionine. The antibody used was a goat polyclonal antibody to the Rauscher virus Gag protein (*15*). SDS–polyacrylamide gel electrophoresis (SDS-PAGE) of the immune precipitates revealed a 105-kD protein that was present only in the transformed cells (Fig. 4A). Immunoprecipitation analysis after labeling in vivo with [^{32}P]orthophosphoric acid revealed that the 105-kD v-Akt protein was phosphorylated (Fig. 4B). The difference between the apparent molecular size and that inferred from the sequence of the v-Akt protein (105 and 86 kD, respectively) might be explained by the phosphorylation (Fig. 4B) and perhaps glycosylation of the protein (Fig. 2).

To confirm that the v-Akt protein product is a serine-threonine kinase, as predicted from its amino acid sequence, we immunoprecipitated it from AKT8-transformed mink cells with the antibody to Gag and tested its in vitro kinase activity. In control experiments, we assayed the in vitro kinase activity of proteins from nontransformed mink lung cells precipitated by the same antibody. The products of the in vitro kinase assays were analyzed by SDS-PAGE. These experiments confirmed that v-Akt is a kinase that phosphorylates itself, the immunoglobulin heavy and light chains, and three other proteins (35, 24, and 16 kD) (Fig. 4C). The in vitro–labeled v-Akt protein was hydrolyzed with HCl subsequent to elution from SDS-polyacrylamide gels. The released phosphoamino acids were identified as phosphoserine and phosphothreonine (Fig. 4D).

Expression of c-*akt* in normal mouse tissues was examined by Northern (RNA) blot analysis with a probe derived from the 3′ untranslated region of v-*akt* (nucleotides 2680 to 3264). The *akt* message was expressed in all tissues tested (brain, kidney, liver, spleen, testis, and thymus). The amount of expression was highest in thymus.

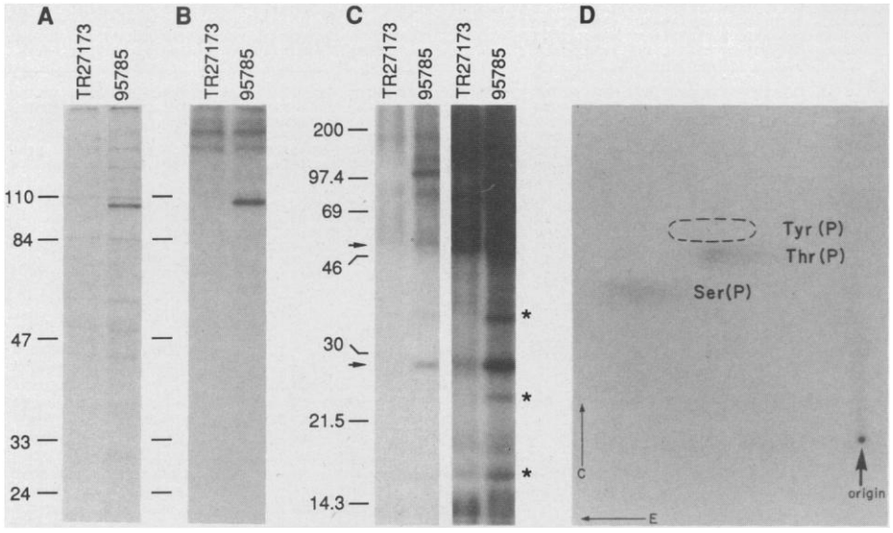

Fig. 4. Biochemical characterization of the *gag-akt* fusion protein. Uninfected TR27173 and AKT8-transformed 95785 mink lung cells (CCL64, American Type Culture Collection) were maintained in Dulbecco's modified Eagle's medium supplemented with penicillin, streptomycin, and kanamycin (50 units/ml, 50 μg/ml, and 100 μg/ml, respectively) and 10% calf serum. (**A**) Uninfected and AKT8-transformed cells were labeled in vivo for 2 hours with [^{35}S]methionine (Tran ^{35}S; ICN Biomedicals) (150 μCi per milliliter of media). Cells were then lysed and immunoprecipitated according to standard procedures (*31*) with a goat polyclonal antibody to the Gag p12 of Rauscher leukemia virus. Immunoprecipitates were analyzed by SDS-PAGE (10% gel). Molecular mass markers are shown in kilodaltons. (**B**) Uninfected and AKT8-transformed cells were labeled in vivo for 2 hours with [^{32}P]orthophosphoric acid (250 μCi/ml) (Du Pont Biotechnology Systems). Cells were then lysed, immunoprecipitated, and analyzed as above. (**C**) In vitro kinase assay. Unlabeled cell lysates from AKT8-transformed or control cells were immunoprecipitated with the antibody to Gag p12 and then incubated in a buffer containing 20 mM Pipes (pH 7), 20 mM $MnCl_2$, 20 μCi of [γ-^{32}P]ATP (7000 Ci/mmol) (ICN Biomedicals), and 5 μM ATP (*32*). Reaction products were analyzed by SDS-PAGE (12.5% gel). Two different exposures are shown. The labeled immunoglobulin heavy and light chains are indicated by arrows. The coprecipitated and labeled 35-, 24-, and 16-kD proteins are indicated by asterisks. (**D**) Phosphoamino acid analysis of the in vitro autophosphorylated v-Akt. The 105-kD *gag-akt* protein in (C) was eluted from the gel and digested in HCl (*32*). The hydrolysates were mixed with unlabeled phosphoserine, phosphothreonine, and phosphotyrosine standards and analyzed by thin layer electrophoresis, followed by thin layer chromatography (*33*). The heavy arrow indicates the origin. The arrows C and E indicate the orientation of the chromatographic and electrophoretic separations, respectively. The positions of the phosphoamino acid standards are indicated by Ser(P), Thr(P), and Tyr(P). Phosphoamino acid analysis of ^{32}P-labeled v-Akt immunoprecipitated from lysates of AKT8-transformed mink lung cells revealed that v-Akt was phosphorylated only on serine and threonine residues.

The genes v-*akt* and its cellular homolog c-*akt* appear to encode a new serine-threonine kinase related to PKC. The high amount of expression of c-*akt* in actively proliferating thymocytes and its involvement in the induction of T cell lymphomas suggest that it may have a role in the transduction of proliferative signals in T cells. An intriguing feature of the Akt kinase is an SH2-like domain detected in the region NH_2-terminal to the kinase domain. First identified as a conserved region present in all cytoplasmic tyrosine kinases (*10*), the SH2 domain was detected in the the protein encoded by the oncogene v-*crk* (*16*) and in two additional proteins involved in signal transduction, the phosphatidylinositol-specific phospholipase C-γ1 (PLC-γ1) (*11*) and the Ras guanosine triphosphatase–activating protein GAP (*17*). Mutational analysis of the SH2 domains of the tyrosine kinases encoded by the oncogenes v-*fps*, v-*src*, and c-*src* revealed that the SH2 domains modulate kinase and transforming activities of those proteins (*18–20*). Moreover, the phenotype of v-Src SH2 mutations may be host cell–specific (*21*). These findings suggest that SH2 is involved in protein-protein interactions between tyrosine kinases and their ligands and substrates. The finding that v-Crk, which lacks a kinase domain, induces protein phosphorylation provides additional support for the involvement of SH2 domains in protein-protein interactions (*16*). The SH2-mediated protein-protein interactions appear to be caused by the binding of SH2 domains to phosphotyrosine residues in the catalytic domain of tyrosine kinases (*18, 22*).

The detection of an SH2-like domain in Akt suggests that this oncogene product might interact with tyrosine kinases. Such interactions may lead to tyrosine phosphorylation of Akt, which contains a tyrosine residue in a sequence similar to a site autophosphorylated by v-Src (Fig. 2). However, the v-Akt protein was phosphorylated in vivo and in vitro only on serine and threonine residues (Fig. 4). The residues phosphorylated in c-Akt remain undetermined. Another potential outcome of such interactions is the phosphorylation of tyrosine kinases and other phosphotyrosine-containing proteins on serine and threonine residues. Crk, GAP, and the GAP-associated proteins p62 and p190 are phosphorylated not only on tyrosine but also on serine and threonine (*23, 24*). Furthermore, v-Crk is associated in

vivo with proteins that exhibit not only tyrosine but also serine and threonine kinase activities (*23*). Thus, Akt and other related proteins may represent a functional link between tyrosine and serine-threonine phosphorylation pathways.

The oncogenic activation of the retrovirally transduced *Akt* may be due to its fusion to *gag*. Because of the presumed myristylation of its NH_2-terminal Gly[1] residue (*25*), Akt is expected to be anchored to the cell membrane where its SH2-like domain might interact with tyrosine kinases and other phosphotyrosine-containing targets.

Note added in proof: A gene, *rac*, has been cloned that encodes a protein kinase related to PKC and the cyclic adenosine monophosphate–dependent protein kinase. The gene appears to be the human homolog of c-*akt* (*25a*).

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26. Abbreviations for the amino acid residues are as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine.
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Defining Protective Responses to Pathogens: Cytokine Profiles in Leprosy Lesions

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The immunological mechanisms required to engender resistance have been defined in few infectious diseases of man, and the role of specific cytokines is unclear. Leprosy presents clinically as a spectrum in which resistance correlates with cell-mediated immunity to the pathogen. To assess in situ cytokine patterns, messenger RNA extracted from leprosy skin biopsy specimens was amplified by the polymerase chain reaction with 14 cytokine-specific primers. In lesions of the resistant form of the disease, messenger RNAs coding for interleukin-2 and interferon-γ were most evident. In contrast, messenger RNAs for interleukin-4, interleukin-5, and interleukin-10 predominated in the multibacillary form. Thus, resistance and susceptibility were correlated with distinct patterns of cytokine production.

ONE OF THE KEY FUNCTIONAL PArameters determining the outcome of immune responses to infectious agents is the nature of the cytokines produced locally by immune cells, yet the patterns of cytokine production are unknown for most infectious diseases of man. Leprosy offers an attractive model for investigating the role of cytokines in resistance or susceptibility to infection for several reasons. Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* that primarily affects skin, the lesions of which are readily accessible to cellular and molecular analysis. Because leprosy is generally not life-threatening, the dynamics of infiltrating cells can be investigated over time. It is a disease that presents as a clinical and immunologic spectrum (*1*). $CD4^+$ T cells predominate in tuberculoid (resistant) lesions; in contrast $CD8^+$ T phenotypes predominate in lepromatous (susceptible) lesions (*2*). $CD4^+$ cells from tuberculoid lesions, but not lepromatous ones, proliferate in culture in response to *M. leprae* (*3*). On the other hand, $CD8^+$ cells from lepromatous lesions, but not tuberculoid ones, can be activated specifically by *M. leprae* to suppress CD4 T cell proliferation in vitro (*4*).

We examined the patterns of cytokine expression in lesions from 16 individuals (eight lepromatous and eight tuberculoid) who were at the poles of the spectrum of leprosy, to identify correlates of resistance and susceptibility to the pathogen (Fig. 1). Because several cytokine mRNAs exist at low levels in biopsy samples, we extracted total RNA from biopsy specimens, reverse-transcribed the polyadenylated mRNAs to obtain lymphokine cDNAs, and detected those cDNAs with high sensitivity by the polymerase chain reaction (PCR) with specific cytokine primers (*5*). PCR is a semiquantitative technique at best; to provide meaningful comparisons between different individuals and forms of the disease, we normalized the cDNAs to the β-actin PCR product, a marker for all cells, and to the

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