Vol. 213, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 4, 1995 Pages 273-281

MOLECULAR CHARACTERIZATION OF THE MURINE *SYK* PROTEIN TYROSINE KINASE cDNA, TRANSCRIPTS AND PROTEIN

Martin Flück, Gisela Zürcher, Anne-Catherine Andres, and Andrew Ziemiecki*

Laboratory for Clinical and Experimental Research, Department of Clinical Research, University of Bern, Tiefenaustrasse 120, CH-3004 Berne, Switzerland

Received June 24, 1995

SUMMARY: Using a RT-PCR based cloning strategy and conventional cDNA library screening we have cloned the murine *syk* cDNA. Sequence analysis of the 5350bp full length cDNA revealed a 5' untranslated region (UTR) of 477bp, an open reading frame of 1884bp and an unusually long 3' UTR of 2989bp containing a polyadenylation signal. The cDNA encodes a putative protein of 628 amino acids with two SH2 domains located N-terminally of the protein tyrosine kinase domain. The highest overall homology, 98%, was observed to the rat *syk*. Northern blot analysis revealed that the murine *syk* protein is encoded by two transcripts of approximately 5.4 and 3.5kb, the difference in size being attributable to differences in the 3' UTR. Rabbit antisera raised against a pGEX-*syk* bacterial fusion protein recognized specifically a protein of approximately 67kd with intrinsic protein tyrosine kinase activity in lymphoid cell extracts. The size of the *syk* protein could be confirmed by in vitro transcription/translation of the full length clone. Expression of *syk* was found in a variety of mouse organs with the highest levels in spleen, heart, mammary gland and thymus and in several lymphoid cell lines. The majority of the expression observed in whole mammary glands originated from the lymph node. Upregulated expression of *syk* was observed in aggressive, metastasizing mammary gland tumours but not in well differentiated, non-metastasizing tumors.

Protein tyrosine kinases (PTKs) are involved in many signalling pathways and have been shown to play a fundamental role in the control of cell proliferation and differentiation. Members of the PTK family of enzymes can be grouped into receptor and non-receptor type according to their cellular topology. Ligand binding to the extracellular part of the membrane spanning receptor PTKs activates their intracellular tyrosine kinase domain and triggers specific signal transduction pathways. The intracellular non-receptor PTKs act as signal transducer elements in a variety of cellular functions including coupling to transmembrane receptors lacking intrinsic PTK domains (1,2).

The interaction of B- and T-cell antigen receptors with intracellular non-receptor PTKs of both the src- and syk/zap70 family members is essential for their activation by antigens. In the resting lymphocytes, the src-family kinases are associated via their unique amino termini with the so called "tyrosine based activation motifs" (TAMs) found in the cytoplasmic tails of

* Corresponding author. FAX **41.31.302.5933.

0006-291X/95 $12.00

the multimeric receptors. Following receptor triggering, the invariant tyrosines found in the TAMs become phosphorylated and act as specific SH2 directed recruitment sites for src- and syk/zap70-family PTKs, and potentially other signalling molecules. The thereby assembled signal transducing complex is thought to be responsible for signal propagation and lymphocyte activation (reviewed in 3). Recently, the *syk* kinase has been reported to be directly activated upon intergrin $\alpha_{IIb}\beta_3$ ligation in platelets (4), which points to a role for *syk* in cell-matrix dependent signalling.

We are interested in PTK involvement in mammary gland biology. The development of the mammary gland takes place mainly in adult life when the mammary gland is continuously subjected to periodic phases of growth, differentiation and regression controlled by a complex interplay of growth factors, hormones, cell-cell and cell-matrix interactions (5). The established role of PTKs in the control of cell proliferation and intracellular signalling makes them prime candidates for involvement in these processes and also in the development of mammary neoplasias (6-8). We have employed a RT-PCR based cloning strategy (9) to identify PTKs expressed at different stages of mammary gland development (10,11). This approach coupled with screening of a murine spleen cDNA library led to the isolation of a full length cDNA encoding the previously uncharacterized murine *syk* protein.

MATERIAL AND METHODS

Animals and cell culture. Organs and mammary glands at various stages of development were isolated from female C57B16XSJL mice. The Wap-*ras* and Wap-*myc* transgenic mice have been described in detail previously (12,13). Mammary glands were fractionated into lymph nodes and multicellular epithelial organoids according to Schönenberger et al., (14). Murine haematopoietic cell lines LBB3.4.16 (B-cell), EL4.3+ (T-cell), EL4.1.10 (T-cell), RG17 (T-cell), L1210 (lymphoma) and Ag8.653 (myeloma) were a kind gift from Dr Rob MacDonald, Ludwig Institute for Cancer Research, Lausanne, Switzerland. The human breast cancer cell lines MCF7, MDA-MB 453 were a generous gift from Dr Mark Crompton, The Institute of Cancer Research, Royal Cancer Hospital, Sutton, UK.

cDNA library screening. A murine oligo-dT and random primed spleen cDNA library (Clontech, Palo Alto, CA) was screened with a PCR fragment highly homologous to the rat *syk* gene and several clones were plaque purified. The full-length sequence was determined using progressive exonuclease III digestion (Erase-a-Base system, Promega, Madison, WI) and Sequenase version 2.0 (USB, Cleveland, OH). Sequence comparisons were made using the FASTA programme (Programme manual for the Wisconsin Package, version 8, September 1994; Genetics Computer Group, Madison, WI). In vitro transcription-translation was performed using the TNT coupled reticulocyte lysate system (Promega) according to the manufacturers instructions. The murine *syk* cDNA sequence has been deposited with GenBank under the accession number Z49877.

Northern blot analysis. Total RNA was prepared using the guanidinium thiocyanate extraction method (15). Poly A+ mRNA was selected using oligo-(dT) cellulose (16). Aliquots of 5μg mRNA were denatured with glyoxal and electrophoretically separated before blotting onto nitrocellulose (17). Filters were hybridized with [α-^{32}P]dCTP labeled random primed fragments and washed to a final stringency of 1XSSC, 0.5% SDS at 65°C.

Antiserum production and immunoprecipitation. Polyclonal antiserum was raised in rabbits against a bacterially produced pGEX-syk fusion protein (*syk* amino acids 207-509).

LBB3.4.16 cells were metabolically labeled with ^{35}S-methionine (DuPont NEN, Regensdorf, Switzerland) in methionine free medium (50μCi/ml, 12 hrs). Cells were washed with ice-cold PBS on ice and lysed in RIPA buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 20 mM NaF, 100 μM $NaVO_4$, 1% Triton X-100, 1% Na-deoxycholate, 0.1% SDS, 1mM PMSF, 1mM ε-amino caproic acid). Pre-cleared lysates were incubated with serum on ice and antigen-antibody complexes isolated using protein-A bearing S. aureus bacteria (Pansorbin, Calbiochem, San Diego, CA). Proteins were resolved by SDS-PAGE and visualized by fluorography.

Auto-kinase assay and phospho-amino acid analysis. LBB3.4.16 cells were lysed in ice-cold PBS containing 1% Triton-X100, 100 μM $NaVO_4$, the lysates pre-cleared by centrifugation and immunoprecipitated as above. The protein-A antigen:antibody complexes were resuspended in kinase buffer (20 mM Tris-HCl, pH 7.5, 20 mM $MnCl_2$ containing 5 μCi [γ-^{32}P]ATP) and incubated at 30°C for 10 min. Labeled proteins were resolved by SDS-PAGE and the dried gel exposed directly to film. Areas containing the labeled *syk* proteins were excised and subjected to phospho-amino acid analysis (18).

RESULTS AND DISCUSSION

Cloning of the murine homologue of syk.

Using an RT-PCR cloning strategy we have previously isolated an approximately 200bp fragment from mature resting mammary glands with high homology to the rat *syk* gene (19). We have used this fragment to screen a murine spleen cDNA library and isolated a 5350bp full length clone (Fig. 1). The *syk* cDNA has a 5' UTR of 477bp followed by an open reading frame of 1884bp encoding a putative protein of 628 amino acids. The protein possesses two SH2 domains, SH2(N) and SH2(C) and a kinase domain displaying all of the conserved features expected of a tyrosine kinase (20). The overall structural characteristics are consistent with membership in the *syk/zap70* family of non-receptor PTKs (Fig. 2). At the amino acid level the murine protein shares the highest total homology to the rat *syk* protein (98%) followed by human and porcine. The kinase domain, the two SH2 domains and the domain between SH2(N) and SH2(C) are well conserved between species whereas the very N-terminus and the sequence between the SH2(C) and the kinase domain are moderately conserved. The two SH2 domains between them share only 36% homology reflecting possible differences in binding specificities for phosphorylated tyrosine(s). Interestingly, the C-terminal 9 amino acids are absolutely conserved between species and contain three tyrosine residues which may have regulatory function. The unusually long 3' UTR of 2989bp contains two UA-rich motifs, one immediately adjacent to the polyadenylation signal, commonly found in the 3' UTRs of lymphokine and proto-oncogene mRNAs and thought to regulate stability and/or translation initiation efficiency (21). The 3' UTR terminates with a classical polyadenylation signal.

Expression of the syk gene and protein in murine tissues.

The murine *syk* gene encodes two transcripts of approximately 5.5 and 3.5kb found predominantly in spleen but also in heart, lung, thymus, mammary gland and intestine (Fig. 3a). *syk* expression was also detected in two of the haematopoietic cell lines analyzed; the B-cell line LBB3.4.16 and the lymphoma B/T-cell line L1210 (Fig. 3b). The observed expression

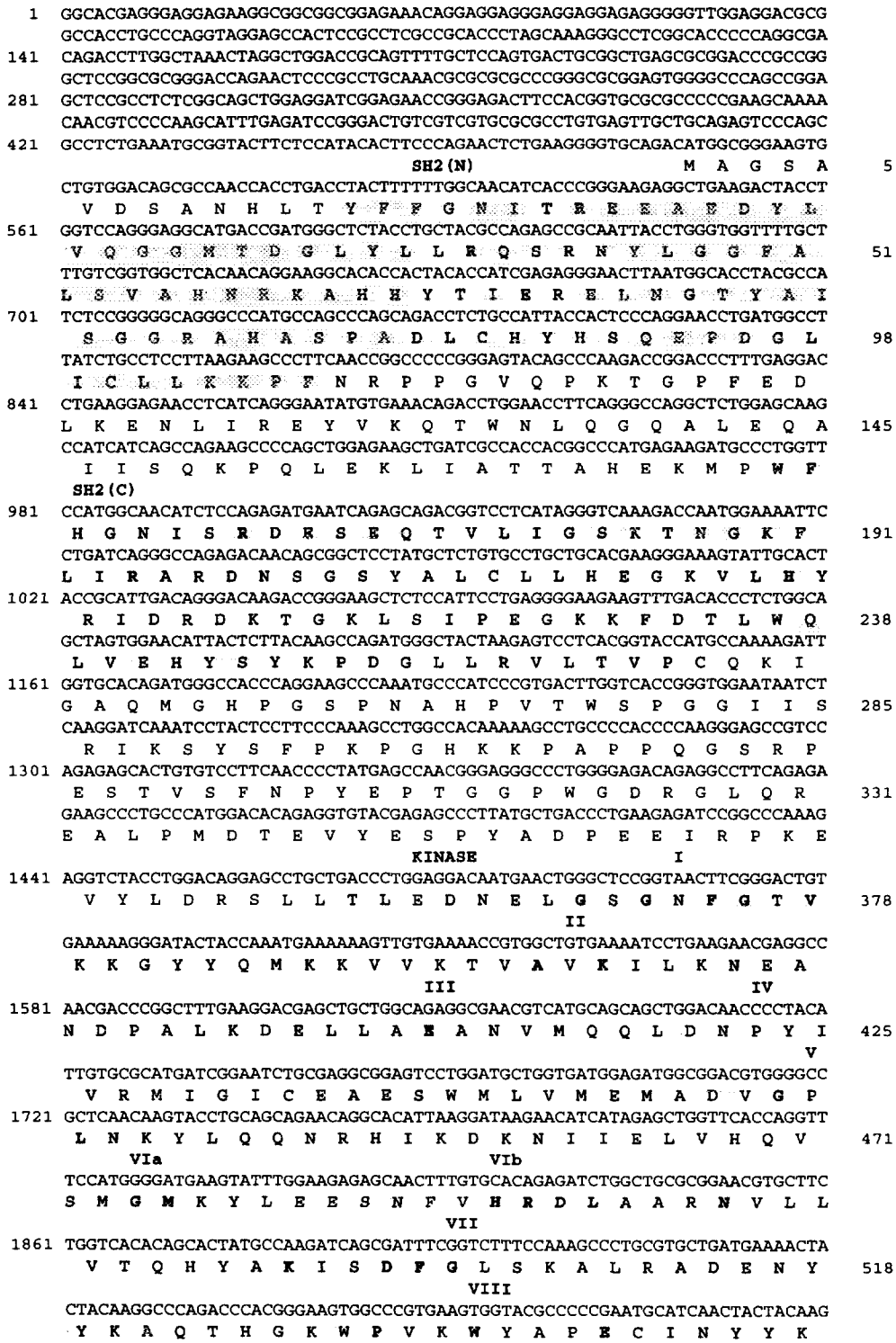


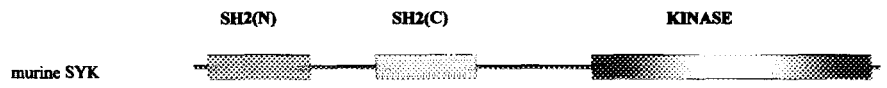
Figure 1. Nucleotide sequence and predicted amino acid sequence of murine *syk*. The SH2 and the kinase domains are shaded. Conserved amino acids within the SH2 domains and in domains I-XI of the kinase domain are in bold. Putative polyadenylation signals are in bold and underlined and UA-rich motifs are in italics and underlined.

```
                                                                IX
2001 TTCTCCAGTAAGAGTGACGTCTGGAGCTTCGGAGTCCTGATGTGGGAAGCGTTCTCCTATGGGCAGAAGC
     F  X  X  X  S  D  V  W  S  F  G  V  L  M  W  E  A  F  S  Y  G  Q  K  P   565
                                                                X
     CCTACAGAGGGATGAAAGGGAGCGAAGTGACCGCCATGCTGGAGAAAGGAGAGCGGATGGGGTGCCCTGC
       Y  X  G  M  K  G  S  E  V  T  A  M  L  E  K  G  E  R  M  G  C  P  A
                                                                XI
2141 AGGATGCCCGAGAGAGATGTACGACCTGATGAACCTGTGCTGGACTTACGATGTGGAGAACAGGCCAGGA
      G  C  P  R  E  M  Y  D  L  M  N  L  C  W  T  Y  D  V  E  N  R  P  G    611
     TTCACAGCTGTGGAACTGAGGCTTCGCAATTACTACTACGACGTGGTTAACTAACAGCTCCGGCGCCTGT
     F  T  A  V  E  L  R  L  R  N  Y  Y  Y  D  V  V  N  *                    628
2281 CCGTGCACACCACGGATTCCCAAGCGATCACAGGAAATTCATTCAGATGAACTGGCTCTCAGAGTTTCAT
     CTCCCTCTGCCCGGAGTGAGAGCTAAGCTAACATAGGACTCACCCTCACAACAGGTCTGGTGCCCAGAGA
     CAGACAAGCAGCAAGACCTTGGGGCCTGTGGAGTCGTCTCTCTGGTCTTTGTTTCCATCTGTGTGGTCTT
     CGCTATCGGTCACGTTTGGGAGCCATTTCCAAATTCTCTTGACATCATTCCGTTCCTCTGGGTCCAGGAT
2561 TTCAGTGTCCCCTGAAGATCAGAAAAGAAAAGTGCTTTGCCACTCCAGAAAGACATAAAGAGAAACCTCG
     CTGGTGCAGTCAGCAAGCTGGAAGGTGGTTAAGGACAATGACAAGCCTGGACACTGGTTTTACTCTCAGC
     GCTGAGACAGAGATACTGTGTGGATGACATGCTGAGATGTGCTGGCTTGGCTGACACGCCCCTGGAGTCC
     ACCTCCCTGCAGTCGTGTGTGTTTGCTGGAGGAGGTGTGAATAACGTTCTGTGTGGAGGCTGCCGGGGGA
2841 GGTGAGCTCCCATGATCCTCTGCGACTGAGCAGAAGTGTGTCAGGCAAGGGTCAGACTCCCCTCTCTGCA
     GAGGGAATGCATCCAGGACTCCTTGTTGCTGCCACTAAAAAATTGCCAACCAGCAACTTAAGGGAGGAAT
     GGCTCATTTGAGCTCACAGTTGGAGGGGATAGGCCATCATGGTGGAAAAGACAACAGGCAGGAAAGGCTT
     GGTGGCATGAAAAGGAAGCTGGAAGGTCACATGGCATCCACATTCAGGAAACAAAGAATGAACAGGAAGT
3121 GGGGCCAGGCTATAAGGCCCTAAAGGCCACCTCCGGTGACCCAGTTCCTTCAGATCCATCTATCAAAGAT
     TCCACAGGCCTCTCAGACACTGAATGCCAACAGCTGGGGACCAAATGTTCAAGCACACAAGGTGCAGTGC
     CAGGCAGGGGCCTTCCTGAATGATTACACAGTGTGGTATCCTGACAGAGCGGGTGAGCCATCTCAGGTCT
     GTCCCCTTATGAGCCCCGTTTACCATCCCAGATTCCCCCTTGGGACCCCCCTCATCTGGATCATTCCCCT
3401 CCCCCCAGGCCCTATCTCCAAACACCTAGGTACTAAGTGAACCTAGGTACTAAGTCTTCAACACATGAAT
     TCTGAGGGGACACATTCATACTGGAGCCGATGGCAGCCTGGTGACCAAGACCATCTCAGGATGAGTTTCT
     GAATGGAATCTTCATTCGCCATAAAACCAGAGATGTGACCTTCCCCACAAGGAAGCCTTGCTAAGTGCGA
     CATTGAGCTGAGGACACACAAGCTGAGAATTCCCCCTCTGTCCTCCTAGCCTTGGCTAACTACTGACCAT
3681 ACACCGAATGTCACCCATTCACGGCACTTTTGCTGAGGACCTAAAAGCTACAAAGGCTGCATAGCCTTCC
     ATCTAGGTCTAGGGACATGTACAGATGGGAGCATCTCACCTTCACCTCTGAGACACTGGGACATTCACAG
     CCAGGGATGGTACAGGAGTCCCTGCTGGTGGTGGGCTCAATGAACAGAGACGATGGTGAAATGGGTGCCT
     CTCTTTAGACCAGTGGTTCTCAACCTTCCTAATGCTGAGATCTTTCAATACAGGTCCTTGCGTTGTGGCG
3961 ACTCCCAACCATGAAGTTATTTTGTTGCAGATTGTCACTGAATTTTGCTACTATGATGAATCGTAGTGTA
     GATATATGATATGCAGGATATCGGATATGTAAATATCTGGTATGCAGGATATGTGAAATGAGGGCACATC
     CCACAGGTTGGGAACCACTGCCTTTGATAGGGAGGCACTATGCTTAGAGCATGGTTAGGAAGTATTTAGA
     AGCTATTGGTGTGTCTTGGAATTGTGTTAGTAGGTCAACCGTCCTGTGGAGGGAAGGAAGGGGGTGGAGG
4241 AAATCCCTGTATGTTGAATTCATTGAGCAGACCTGCAGAACACAGCCTTGGTGAGGAAGTCCCATGGGTC
     AGACCACCTGTAGATTTAGTCAGGAGAGGAAGGGCCACTGTATAGTTATGGAGACAGGACTGTCTGCTCT
     GAGCAAGAAGTTCTCTGCTGAGCTCCTAGCCTACCTTCCCTTGTCCCCAGGGCTATAGAAAGGCCACCTC
     GAAGACCAGGGAGCATGTGTGAGAAGTCTGTGGAGGCCTCTGCCTTCTTCCTGGCCTCTCTAGCAGTGCC
4521 TCTCCTTCCCAGGGATTTGGGACTGACCGTTATCTGGCTATGACTGAGCAGAGTGGAGGGATGGGCTTTG
     CCCCTTCAGAAAGCCGAAACCGGTTCCCATGTGCAGAGTCACATGTGGCCATCGACCAGGGCTCTCATCT
     GGTGAAAACTGTGTCTTACTGTCAAGATTAATTCTCTCCCCTGGGCCACTGAGGAGGGCACATGGCAAGA
     GGATCCTCCCTAGAGGATTTAGACTATGAATGCCCACTAAATTTGCAAGGTCAGAAACTAGCCAAGGTCC
4801 TTCTCAGGCATCTATCCTTAACTTGGTCTCTCCACAAGCTGCCTCTGATGCTCCGAGCCCCTTCTGCCTG
     GCGACTGGGCTCCCTGTGTGCTTTTACCTATGACCCTTGGTCAGCAGAGTACAAGGAGCACCTAAGCTGC
     CTCGCCACCTCCCACTGACTGCAAACACCACATACCTCATGCGGAACCGAATGTCTCTCCAGTAAACACC
     CCAGCCAGCCATTCATAAAAACCTGTCTCTGTGTGCGCTGGGACATGTCCTCCCTGTACCCCGGGCCTGC
5181 TTTGTGTGCCAGACAGTGACTCCACAGGGATGCCAGGTCTGTGATTGCATTGCTCCTTGCAGAGGGAAGC
     CCTCGGTTGTCCTTCTCTGGGAATGCTGCCACCCAGAGCAGCACACAGTGATTGCCCTGTTATTTCAGAA
     ATTATTTTAAATAAAGATCTACCTCGTGCC
```

Figure 1 – *Continued*

of *syk* in spleen, thymus, heart and lung as well as in haematopoietic cell lines with B-cell characteristics agrees with the known function of the *syk* protein in B-cells and platelets.

In order to characterize the difference between the two *syk*-specific transcripts, Northern blots of spleen, thymus and mammary gland RNA were hybridized with probes derived from two different parts of the *syk* cDNA. The probe spanning the coding region of



length (aa)	13	93	60	92	105	256	9	total
rat SYK	-	98%	100%	98%	94%	98%	100%	98%
human SYK	64%	93%	98%	94%	75%	98%	100%	92%
porcine SYK	62%	88%	100%	91%	75%	96%	100%	90%
murine ZAP-70	39%	58%	65%	50%	27%	63%	56%	54%
human ZAP-70	39%	57%	65%	50%	26%	63%	33%	53%

Figure 2. Total and domain amino acid sequence homologies of murine *syk* to rat (19), human (24) and porcine (25) *syk* and murine (26) and human (27) *zap70*.

syk detected both transcripts whereas the probe containing nucleotide 4040 to 5350 of the 3' UTR recognized exclusively the large transcript of 5.5 kb in all organs (Fig. 3c). This indicates that the size difference between the two *syk*-specific mRNAs is due to different lengths of the 3' UTRs and that we have cloned the cDNA corresponding to the large transcript. Interestingly, two putative polyadenylation signals are found at position 3661 and 3959 which could be involved in the polyadenylation of the 3.5 kb transcript. The 3' UTRs of mRNAs are

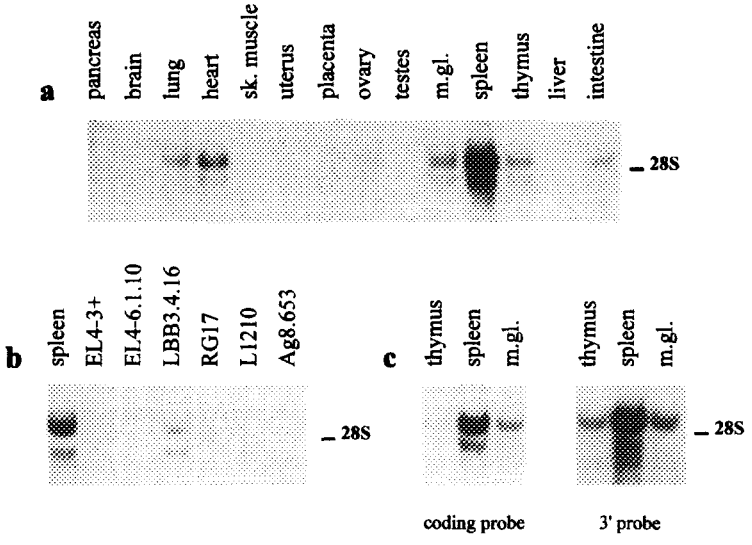


Figure 3. Expression of *syk* in mouse organs and haematopoietic cell lines. Poly(A) selected RNA prepared from the organs and cell lines indicated was analyzed by Northern blotting. Equal loading was verified by acridine orange staining of the gels. The probe used for hybridization corresponded to the entire *syk* cDNA. The position of 28S rRNA is indicated.

thought to be important inter alia for the stability of the mRNA (21). Half-life determination by actinomycin D inhibition of mRNA synthesis in LBB3.4.16 cells indicated that both RNA populations have the same half-life of approximately 6 hrs (data not shown).

The characterization of the murine *syk* protein is summarized in figure 4. In vitro transcription-translation of the full length *syk* cDNA yielded a protein of approximately 67 kd molecular weight. Antibodies generated against a bacterially produced GST-*syk* fusion protein specifically recognized an approximately 67 kd protein in ^{35}S-methionine metabolically labelled LBB3.4.16 cells. Immunoprecipitation of LBB3.4.16 cell extracts with syk specific antiserum followed by an in vitro kinase assay resulted in phosphorylation of the 67 kd protein and phospho-amino acid analysis revealed that the phosphorylation was exclusively on the amino acid tyrosine. Thus, the murine *syk* gene encodes an approximately 67 kd protein with intrinsic tyrosine kinase activity.

Expression of the syk gene in normal and malignant mammary tissue.

We are interested in the role of PTKs in the normal and neoplastic development of the mammary gland. The relatively strong expression of *syk* in the mammary gland (Fig. 3a) prompted us to examine its expression in more detail, in particular, to determine how much of the expression could be accounted for by the lymph node found in each gland. Fractionation of mammary gland tissue into lymph nodes and epithelial organoids clearly revealed that the majority of the expression could be assigned to the lymph nodes (Fig. 5a).

In order to be able to study mammary gland carcinogenesis in vivo we have established Wap-*ras* and Wap-*myc* transgenic mice. These animals harbor either the Harvey-*ras* or *c-myc*

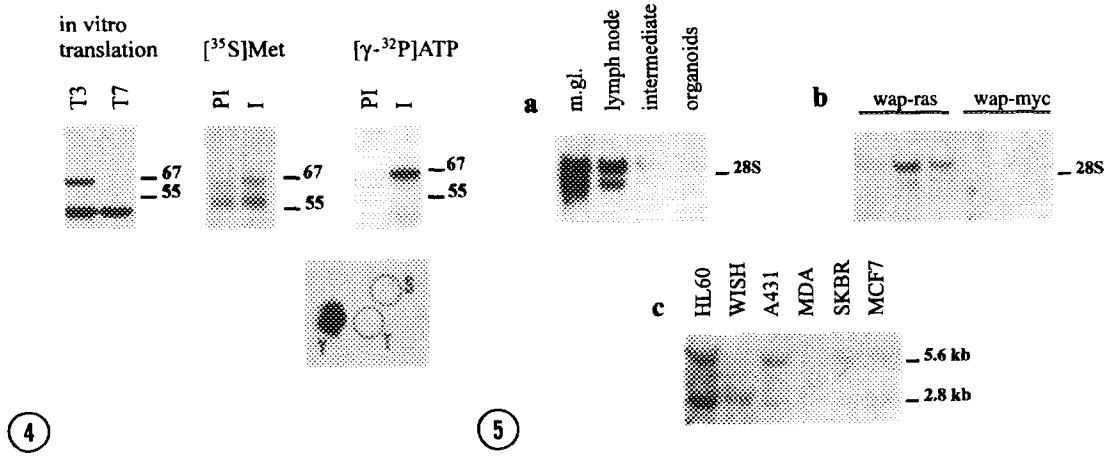


Figure 4. The murine *syk* protein. In vitro coupled transcription/translation was driven from the T3 or T7 promoter. PI: pre-immune serum, I: immune serum. S: phosphoserine, T: phosphothreonine, Y: phosphotyrosine.

Figure 5. Expression of *syk* in mouse mammary gland, mouse mammary tumours and human cell lines. Poly(A) selected RNA was analyzed by Northern blotting using full length *syk* as a probe.

oncogene under the control of the whey acidic protein (Wap) milk protein gene promoter resulting in targeted expression of the oncogene to the mammary epithelium. 80% of the female of either line develop adenocarcinomas. The Wap-*ras* females develop undifferentiated, invasive mammary tumours which often metastasize whereas the Wap-*myc* females develop highly differentiated mammary carcinomas which continue to secrete milk proteins, even in the absence of lactogenic hormones, and do not metastasize. We have used this mouse model to analyze *syk* expression during invasive and non-invasive carcinogenesis. Variable expression was consistently found in the aggressively growing Wap-*ras* induced tumors, whereas, no expression was found in the more benign mammary tumors of Wap-*myc* transgenic females (Fig. 5b). The source of the *syk* expression observed in the Wap-*ras* tumours is unknown, however, these tumours often show extensive lymphocyte invasion which could account for this expression (22). Alternatively, expression may be derived from the epithelial tumour cells. Interestingly, we have detected *syk* expression in several human tumor cell lines of epithelial origin such as A431, WISH and the breast carcinoma cell lines SKBR and MCF-7 (Fig. 5c). It is conceivable that the *syk* gene product is functioning in signalling pathways other than the classical B-cell activation pathway. Indeed, the recent observation of rapid activation of the *syk* kinase by integrin ligation in platelets (4) would support such a notion.

ACKNOWLEDGMENTS

This work was supported by the Swiss National Science Foundation (31-30015-90), the Bernese Cancer League and the Foundation for Clinical and Experimental Cancer Research.

REFERENCES

1. Fantl, W.J., Johnson, D.E. and Williams, L.T. (1993) Annu. Rev. Biochem. 62, 453-481.
2. Bolen, J.B. (1993) Oncogene 8, 2025-2031.
3. Sefton, B.M. and Taddie. J.A. (1994) Curr. Op. in Immunology 6, 372-379.
4. Clark, E.A., Shattil, S. J., Ginsberg, M.H., Bolen, J. and Brugge, J.S. (1994) J. of Biol. Chem. 269, 28859-28864.
5. Howlett, A.R. and Bissell, M.J. (1993) Epith. Cell Biol. 2, 79-89.
6. Lehtola, L., Partanen, J., Sistonen, L., Korhonan, J., Warri, A., Harkonen, P., Clarke, R. and Alitalo, K. (1992) Int. J. Cancer 50, 598-603.
7. Sainsbury, J.R.C., Farndon, J.R., Needham, G.K., Malcom, H.A. and Harris, A.L. (1987) Lancet I, 1398-1402.
8. Berns, L.M.J.J., Klijn, J.G.M., van Staveren, I.L., Portengen, H. and Foekens, J.A. (1992) Cancer Res. 52, 1036-1039.
9. Wilks, A.F. (1989) Proc. Natl. Acad. Sci. USA 86, 1603-1607.
10. Blaschke, R.J., Andres, A.-C., Reid, H.H., Zürcher, G., Friis, R.R. and Ziemiecki, A. (1991) Behring Inst. Res. Comm. 89, 81-92.
11. Andres, A.-C., Reid, H.H., Zürcher, G., Blaschke, R.J., Albrecht, D. and Ziemiecki A. (1994) Oncogene 9, 1461-1467.
12. Andres, A.-C., Schönenberger, C.-A., Groner, B., Henninghausen, L., LeMeur, M. and Gerlinger, P. (1987) Proc. Natl. Acad. Sci. USA 84, 1299-1303.
13. Schönenberger, C.-A., Andres, A.-C., Groner, B., van der Valk, M., LeMeur, M. and Gerlinger, P. (1988) EMBO J. 7, 169-175.

14. Schönenberger, C.-A., Zuk, A., Groner, B., Jones, W. and Andres, A.-C. (1990) Dev. Biol. 139, 327-337.
15. Harpur, A.G., Andres, A.-C., Ziemiecki, A., Aston, R.R. and Wilks, A.F. (1992) Oncogene 7, 1347-1353.
16. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, Cold Spring Harbour Laboratory.
17. Thomas, P. (1980) Proc. Natl. Acad. Sci. USA 77, 5201-5205.
18. Hunter, T. and Sefton, B.M. (1980) Proc. Natl. Acad. Sci. 77, 1311-1315.
19. Benhamou M., Ryba, N.J.P., Kihara, H., Nishikata, H. and Siraganian, R.P (1993) J. Biol. Chem. 268, 23318-23324.
20. Hanks, S.K. and Quinn, A.M. (1991) Meth. Enzymol. 200, 38-62.
21. Jackson, R.J. (1993) Cell 74, 9-14.
22. Andres, A.-C., Bchini, O., Schubaur, B., Dolder, B., LeMeur, M. and Gerlinger, P., (1991) Oncogene 6, 771-779.
23. Koch, C.A. (1991) Science, 252, 668-674.
24. Law, C.-L., Sidorenko, S.P., Chandran, K.A., Draves, K.E., Chan, A.C., Weiss, A., Edelhoff, S., Disteche, C.M. and Clark, E.A. (1994) J. of Biol. Chem. 269, 12310-12319.
25. Taniguchi, T, Kobayashi, T., Kondo, J., Takahashi, K., Nakamura, H., Suzuki, J., Nagai, K., Yamada, T., Nakamura, S. and Yamamura, H. (1991) J. of Biol. Chem. 266, 15790-15796.
26. Genbank Accession no U04379.
27. Chan, A.C., Iwashima, M., Turck, C.W. and Weiss, A., (1992) Cell 71, 649-662.