



## Identification of *Xenopus* Cortactin: Two Isoforms of the Transcript and Multiple Forms of the Protein

Authors: Yamashita, Atsushi, Katsube, Takanori, Hashimoto, Naoko, Tomita, Kimiko, Takahisa, Manabu, et al.

Source: Zoological Science, 18(3) : 331-336

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.18.331>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Identification of *Xenopus* Cortactin: Two Isoforms of the Transcript and Multiple Forms of the Protein

Atsushi Yamashita<sup>†1</sup>, Takanori Katsube<sup>†2</sup>, Naoko Hashimoto, Kimiko Tomita, Manabu Takahisa, Ryu Ueda and Shin Togashi<sup>†3\*</sup>

Neurogenetics Research Project, Mitsubishi Kasei Institute of Life Sciences, Minamiooya 11, Machida-shi, Tokyo 194-8511, Japan

**ABSTRACT**—Cortactin was initially identified as a substrate for Src tyrosine kinase. It interacts with the filamentous actin in the cell cortex through the tandem repeats of 37-amino acid. In this report, we describe the identification of a *Xenopus* homolog of cortactin. The deduced amino acid sequence shares over 70% identity with human, mouse, and chicken cortactin. Northern and Western blot analyses revealed that *Xenopus* cortactin is widely expressed in *Xenopus* tissues. Analysis of the transcripts using polymerase chain reaction revealed two isoforms being different in the number of the tandem repeats. The major isoform has 6.5 tandem repeats but the minor one has 5.5 tandem repeats. As the sixth repeat, which is missed in the minor isoform, is encoded by a single exon flanked by introns on both sides, these two isoforms are likely to be generated by alternative splicing. We propose that cortactin regulates the construction of actin cytoskeleton by altering the number of its tandem repeats.

## INTRODUCTION

The cortical cytoskeleton is a dense network of actin filaments (F-actin) and associating actin-binding proteins. Cells undergo rearrangement of the cortical cytoskeleton during cell division, cell migration, oncogenic transformation, and in response to various extracellular stimuli (Schmidt and Hall, 1998). Src protein tyrosine kinase plays a pivotal role in some of these cellular processes (Thomas and Brugge, 1997). Its constitutive activation results in transformation of cells and in a gross alteration of cell morphology. Cortactin was originally identified as a highly phosphorylated protein in v-Src-transformed chicken embryo fibroblast cells (Wu *et al.*, 1991). Stimulation of its phosphorylation is also observed in response to various extracellular stimuli, which often induce rearrangement of the cortical cytoskeleton (Bhattacharya *et al.*, 1995; Dehio *et al.*, 1995; Huang *et al.*, 1997; Liu *et al.*, 1996; Maa *et al.*, 1992; Zhan *et al.*, 1993). Cortactin has an F-actin binding activity and primarily localizes within cortical structures, such as membrane ruffles, lamellipodia, and podosomes, which are enriched for cytoskeletal proteins (Wu and Parsons, 1993).

Cortactin also has an F-actin cross-linking activity, which is down-regulated by Src mediated tyrosine phosphorylation (Huang *et al.*, 1997). Thus, cortactin is thought to be involved in the Src signaling pathway regulating cytoskeleton reorganization. However, its precise function in the process is obscure.

*Xenopus laevis* is an attractive model animal because we can access the embryos easily to analyze gene function in development utilizing microinjection of cloned DNA, synthetic mRNA, or antibodies into the oocytes and embryos (Vize *et al.*, 1991). Previously, injection of a synthetic mRNA encoding a constitutive active form of Src into *Xenopus* oocyte was revealed to induce a gross alteration in the cortex (Unger and Steele, 1992). Several observations suggest the involvement of *Xenopus* cortactin (Xcortactin) in this process (Thorn *et al.*, 1999; Unger and Steele, 1992). Here, we demonstrate the molecular cloning and characterisation of Xcortactin. This study enables us to investigate the function of cortactin in developing organisms, which will provide further information on the function of cortactin on Src signaling pathways regulating cytoskeleton organization.

\* Corresponding author: Tel. +81-42-778-9481; FAX. +81-42-778-9481.

E-mail: stogashi@jet.sci.kitasato-u.ac.jp

<sup>†</sup> Present addresses: <sup>†1</sup>School of Science, Kitasato University, 1-15-1, Kitasato, Sagami-hara-shi, Kanagawa 228-8555, Japan; <sup>†2</sup>National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage-ku, Chiba-shi, Chiba 263-8555, Japan; <sup>†3</sup>Laboratory of Molecular Embryology, Department of Bioscience, Kitasato University, 1-15-1, Kitasato, Sagami-hara-shi, Kanagawa 228-8555, Japan

## MATERIALS AND METHODS

### Isolation of *Xenopus* cortactin cDNA

A 1.4-kb mouse cortactin cDNA fragment was amplified by reverse transcription and polymerase chain reaction (RT-PCR) using a set of degenerated primers as follows: 5'-TT(C/T)GT(T/C/A/G)AA(C/T)GA(C/T)GT(T/C/A/G)AG(C/T)GA-3' and 5'-TC(A/G)AA(T/C/A/G)GA(T/A/G)AT(C/T)TC(A/G)TC(A/G)TC-3'. The mouse cortactin cDNA fragment was labeled with <sup>32</sup>P and employed as a probe to

screen the *Xenopus* embryo cDNA library constructed in a lambda ZAPII vector (Stratagene, La Jolla, CA, USA). Phagemid DNA was isolated by *in vivo* excision from a positive cDNA clone and subjected to nucleotide sequencing on an ABI Prism 377 DNA Sequencer (Perkin Elmer, Foster City, CA, USA).

#### Preparation of nucleic acid and protein samples

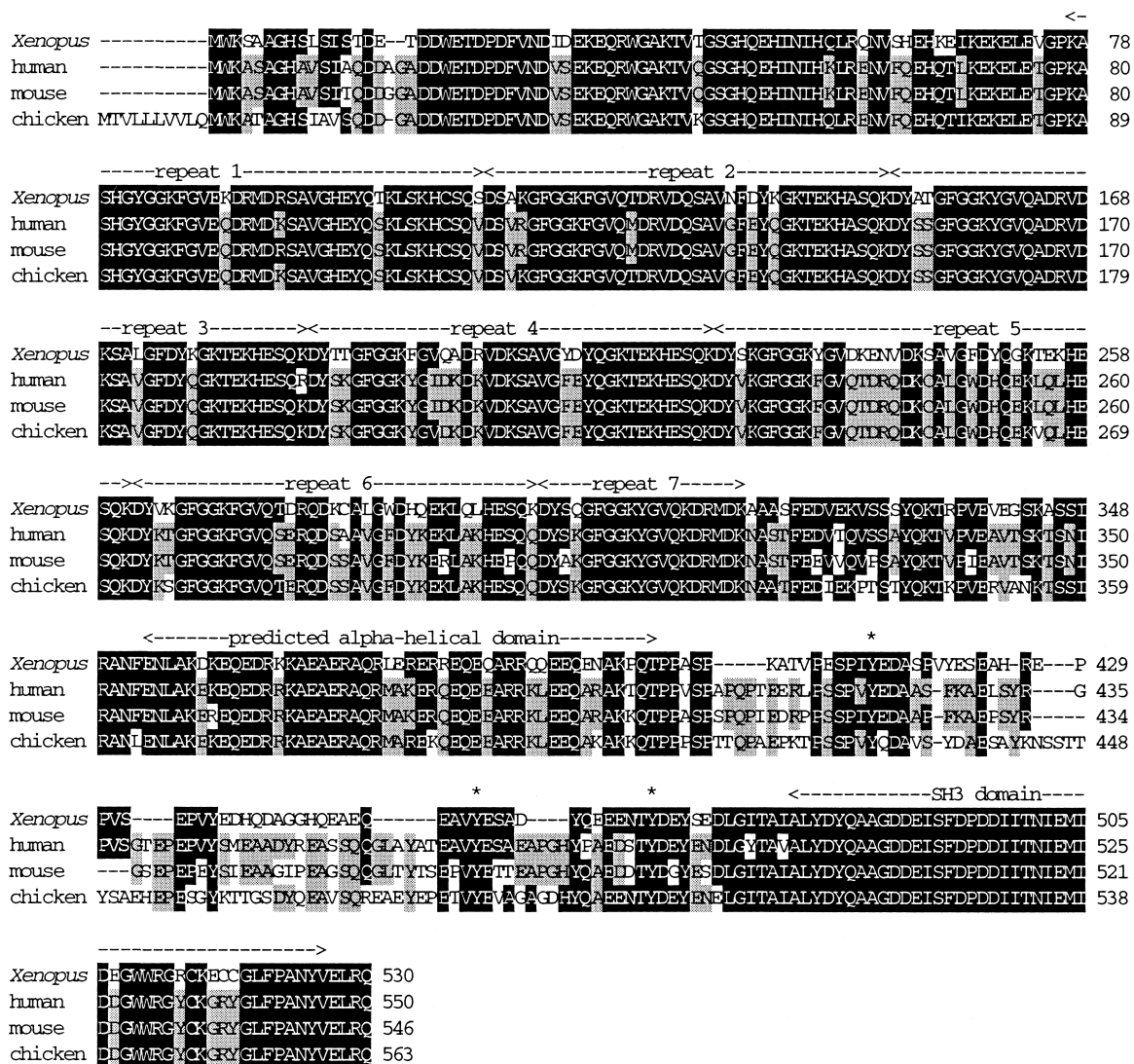
Tissue samples of brain, lung, heart, liver, skeletal muscle, kidney, ovary with immature oocytes, and testis were removed from adult *X. laevis* females or males. Ovarian tissues with mature oocytes were removed from adult *X. laevis* females pretreated with gonadotropin. Genomic DNA and total RNA samples were purified from these tissue samples using a TRIZOL Reagent (GIBCO BRL, Rockville, MD, USA). Tissue samples were lysed in 2 x SDS gel loading buffer (100 mM Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, and 2% 2-mercaptoethanol). The protein concentrations were quantified by the Bradford protein assay (Bio-Rad, Hercules, CA, USA).

#### Northern and Western blot analyses

10 µg of total RNA samples were separated on 1.2% formaldehyde-agarose gel and transferred to a Hybond-N<sup>+</sup> nylon membrane. Probes were labeled with digoxigenin (PCR DIG Probe Synthesis kit, Roche molecular biochemicals, Mannheim, Germany). The hybridised probes were detected using a DIG Luminescent Detection kit (Roche molecular biochemicals). Western blot analysis was done as described previously (Katsube *et al.*, 1998). Anti-chicken cortactin (p80/85) monoclonal antibody was purchased from Upstate Biotechnology (Lake Placid, NY, USA). Anti-*Drosophila* cortactin polyclonal antibody was described previously (Katsube *et al.*, 1998).

#### RT-PCR

1 µg of total RNA samples were treated with DNase I (GIBCO BRL) and reverse transcribed by SuperScript II reverse transcriptase (GIBCO BRL) using the oligonucleotide primer RT. One-twentieth part of the cDNA products was subjected to a PCR using the oligonucle-



**Fig. 1.** Alignment of Xcortactin with the human, mouse, and chicken homologs. Identities with Xcortactin are shaded in black, and identities among the homologs are shaded in gray. Cortactin repeats (repeat 1-7), a predicted alpha-helical domain, and an SH3 domain are shown with broken lines above the aligned sequences. Asterisks represent conserved tyrosine residues targeted by Src tyrosine kinase (Huang *et al.*, 1998). The fourth, fifth, and sixth cortactin repeats of Xcortactin are aligned with the repeats at the corresponding positions of the homologs, but are most related to the third, fourth, and fifth repeats of them, respectively. The region between the predicted alpha-helical domain and the SH3 domain is rich in proline residues in all proteins, although the sequence homology within the region is quite limited.

otide primers Xcort-F and Xcort-R. The reaction was processed for 30 cycles at 95°C for 30 s, 67°C for 30 s, and 72°C for 2 min. The sequences of the oligonucleotide primers were as follows: RT, 5'-CGCTTGTAGCCAAAGCAGCCAAA-3'; Xcort-F, 5'-GGGTCTG-GACATCAAGAACACATC-3'; Xcort-R, 5'-CTGGTAGGACGAAG-AGACTTTCTC-3'.

#### Analysis of exon-intron structure of the *Xcortactin* gene

Two sets of oligonucleotide primers (G1, G2) and (G3, G4) were designed to amplify putative intron sequences. Each set of the primers and 0.2 µg of the genomic DNA was subjected to a 30 cycles of PCR at 95°C for 30 s and 68°C for 5 min. Nucleotide sequences of the oligonucleotide primers were as follows: G1, 5'-AGCGCTG-TTGGGTTTGACTACCAA-3'; G2, 5'-ACTCGTGCAGCTGAAGCTT-TTCCT-3'; G3, 5'-GTTTGGTGTGCAGACAGACAGACA-3'; G4, 5'-TC-TTTCTGCACACCGTACTTTCCG-3'.

## RESULTS

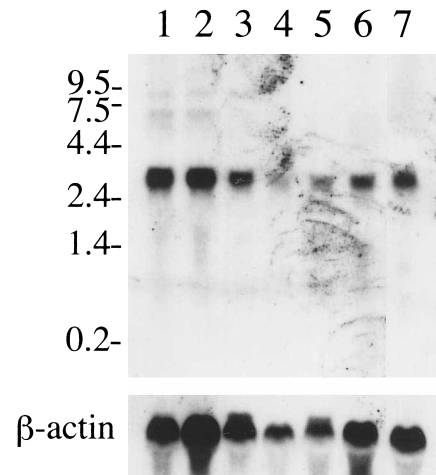
### Isolation of a *Xenopus* cortactin cDNA

To isolate a *Xenopus* cDNA encoding a cortactin homolog, a *Xenopus* embryo cDNA library was screened using a 1.4-kilobase (kb) mouse cortactin cDNA fragment as a probe. Several positive clones were isolated and the clone with the longest insert was sequenced. The clone showed significant homology to the previously reported cortactin cDNAs. It contained a 3578-basepair (bp) cDNA sequence including a 39-bp 5'-noncoding region, a 1593-bp open reading frame (ORF), and a 1946-bp 3'-noncoding region. Translation of the ORF revealed that the cDNA encodes a 530-amino acid protein with a calculated molecular mass of 59.8 kilodalton (kDa). This predicted *Xenopus* protein shares over 70% identity with the previously reported vertebrate cortactin and represents unique structures characteristic for cortactin, which include 6.5 tandem repeats of 37 amino acid (we will refer to these as cortactin repeats in this manuscript) at the N-terminal region, a predicted alpha-helical domain, a proline-rich domain, and a C-terminal src homology 3 (SH3) domain (Fig. 1). Thus, we concluded that the cDNA clone encodes a *Xcortactin*. It should be noted that careful examination of each repeating unit revealed the unique organization of the cortactin repeats of *Xcortactin*. Among the human, mouse, and chicken homologs, each repeating unit of a homolog represents highest homology to the one at the corresponding position of the others. The first, second, and third cortactin repeats of *Xcortactin* also represent highest homology to the ones at the corresponding position of the other vertebrate homologs. However, the fourth, fifth, and sixth cortactin repeats of *Xcortactin* represent highest homology to the third, fourth, and fifth ones, respectively, of the other vertebrate homologs. The nucleotide sequence of the *Xcortactin* cDNA has been submitted to the DDBJ/EMBL/GenBank with accession number AB027611.

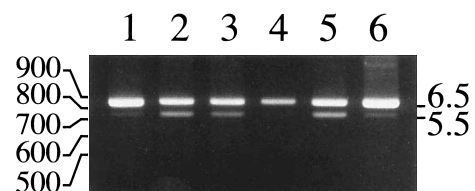
### Two isoforms of the *Xcortactin* transcript.

To examine the expression of the *Xcortactin* transcript in *Xenopus* tissues, Northern blot analysis was conducted. A transcript of about 3 kb was observed in the brain, lung, heart, liver, skeletal muscle, kidney, and testis (Fig. 2). This is consistent with the result of the mouse cortactin transcript, which

is expressed in most tissues other than B lymphocytes or plasma cells (Migliarese *et al.*, 1994). We further analyzed the *Xcortactin* transcript by RT-PCR. A set of primers designed to amplify the region encoding the cortactin repeats domain amplified two PCR products (Fig. 3). The larger product was dominantly amplified from all tissues examined. A moderate amount of the smaller product was amplified from lung, heart, and skeletal muscle, but in brain, liver, and ovary, the smaller product was hardly or not detected. Another set of primers, which also cover the cortactin repeats coding region, gave a consistent result (data not shown). Molecular cloning and nucleotide sequencing of the products revealed that the larger product (870-bp) matched the above-described *Xcortactin* cDNA. On the other hand, the smaller product missed the 111-nucleotide stretch corresponding to the sixth cortactin repeat, while the other portion matched the cDNA. The smaller product demonstrated an isoform of *Xcortactin* with 5.5 cortactin repeats.



**Fig. 2.** The *Xcortactin* transcript is widely expressed in *Xenopus* tissues. 10 µg total RNA samples from brain (lane 1), lung (lane 2), heart (lane 3), liver (lane 4), skeletal muscle (lane 5), kidney (lane 6), and testis (lane 7) of *Xenopus laevis* were loaded. The blots were hybridised with *Xcortactin* (upper panel) or  $\beta$ -actin (lower panel) cDNA probes. Positions of RNA marker are indicated on the left in kb.



**Fig. 3.** Two isoforms of the *Xcortactin* transcript. RT-PCR was performed to amplify the cortactin repeats coding region of the *Xcortactin* transcript. The amplified products from brain (lane 1), lung (lane 2), heart (lane 3), liver (lane 4), skeletal muscle (lane 5), and ovary (lane 6) total RNA were analyzed on 2.5% agarose. Larger band corresponds to the transcript for the isoform with 6.5 cortactin repeats. Smaller band corresponds to the isoform with 5.5 cortactin repeats. No product was amplified when reverse transcriptase was excluded from the reactions (not shown). Positions of size markers in bp are shown at the left.



## DISCUSSION

We isolated a homolog of the cortactin cDNA from *Xenopus laevis*. Xcortactin showed over 70% identity to human, mouse, and chicken cortactin, and was expressed in all the tissues examined; brain, lung, heart, liver, skeletal muscle, kidney, ovary, and testis. As previously reported for mouse and rat cortactin, Xcortactin has isoforms which differ in the number of cortactin repeats (Ohoka and Takai, 1998; Wu and Parsons, 1993). As the sixth cortactin repeat, which presents in the isoform with 6.5 cortactin repeats but missed in the isoform with 5.5 cortactin repeats, is encoded by a single exon of 111 bp flanked by 2.9- and 2.1-kb introns, these isoforms must be generated by alternative splicing.

Cortactin was originally designated as p80/85 because the human, mouse, and chicken cortactin proteins represent two bands with molecular masses of 80 and 85 kDa on SDS-PAGE, while their calculated molecular masses are 62.2, 61.0, and 63.3 kDa, respectively (Schuuring *et al.*, 1993; Wu *et al.*, 1991; Zhan *et al.*, 1993). Recently, it was shown that p80 is converted to p85 by a post-translational modification, most probably a protein phosphorylation (van Damme *et al.*, 1997). Western blot analysis using the anti-chicken cortactin antibody showed that Xcortactin has more varied forms than p80/85 (Fig. 5). Most of them showed much larger molecular masses on SDS-PAGE than the calculated molecular masses of the Xcortactin isoforms with 6.5 or 5.5 cortactin repeats (59.8 and 55.6 kDa, respectively). The profiles of the multiple forms of Xcortactin differ from tissue to tissue. Relative expression levels of the transcripts for two Xcortactin isoforms also varied among the tissues examined (Fig. 3). It is likely that the multiple forms of Xcortactin are due to the post-translational modification of the protein as well as to the alternative splicing of the transcript.

A recent study on rat cortactin isoforms revealed that the isoform with 6.5 cortactin repeats has F-actin cross-linking activity but the isoforms with 5.5 or 4.5 cortactin repeats do not (Ohoka and Takai, 1998). On the other hand, several lines of evidence have shown that the property of cortactin is regulated by phosphorylation. The conversion of p80 to p85, most probably by phosphorylation, is accompanied by a redistribution of cortactin from cytoplasm to cell-matrix contact sites (van Damme *et al.*, 1997). The F-actin cross-linking activity of cortactin is down-regulated by Src mediated tyrosine phosphorylation (Huang *et al.*, 1997). Substitutions of the tyrosine residues responsible for Src mediated phosphorylation impair cortactin to enhance cell migration, when it is ectopically expressed in an endothelial cell line (Huang *et al.*, 1998). We found that these tyrosine residues are conserved in Xcortactin (represented by asterisks in Fig. 1). Thus, it should be possible that each of the multiple Xcortactin forms has a different functional property in the tissue specific cytoskeletal regulation.

The dynamic reorganization of the cytoskeleton is essential for the active proliferation and migration of cells during ontogenesis. Especially in the cortical actin network, cortactin

should play an important role. The involvement of cortactin has been suggested in osteoclast and megakaryocyte differentiation (Hiura *et al.*, 1995; Zhan *et al.*, 1997). Investigation of Xcortactin in embryos utilizing microinjection of synthetic mRNA is currently under progress, and will provide further information on the role of cortactin in development.

## ACKNOWLEDGMENTS

We thank Dr. Makoto Asashima (University of Tokyo, Tokyo, Japan) for a kind gift of *Xenopus laevis*. We are also grateful to Dr. Michihiko Ito (Kitasato University, Sagami-hara, Japan) for samples of *Xenopus* mRNAs.

## REFERENCES

- Bhattacharya S, Fu C, Bhattacharya J, Greenberg S (1995) Soluble ligands of the alpha v beta 3 integrin mediate enhanced tyrosine phosphorylation of multiple proteins in adherent bovine pulmonary artery endothelial cells. *J Biol Chem* 270: 16781–16787
- Dehio C, Prevost MC, Sansonetti PJ (1995) Invasion of epithelial cells by *Shigella flexneri* induces tyrosine phosphorylation of cortactin by a pp60<sup>c-src</sup>-mediated signalling pathway. *EMBO J* 14: 2471–2482
- Hiura K, Lim SS, Little SP, Lin S, Sato M (1995) Differentiation dependent expression of tensin and cortactin in chicken osteoclasts. *Cell Motil Cytoskeleton* 30: 272–284
- Huang C, Liu J, Haudenschild CC, Zhan X (1998) The role of tyrosine phosphorylation of cortactin in the locomotion of endothelial cells. *J Biol Chem* 273: 25770–25776
- Huang C, Ni Y, Wang T, Gao Y, Haudenschild CC, Zhan X (1997) Down-regulation of the filamentous actin cross-linking activity of cortactin by Src-mediated tyrosine phosphorylation. *J Biol Chem* 272: 13911–13915
- Huang C, Tandon N, Greco N, Ni Y, Wang T, Zhan X (1997) Proteolysis of platelet cortactin by calpain. *J Biol Chem* 272: 19248–19252
- Katsube T, Takahisa M, Ueda R, Hashimoto N, Kobayashi M, Togashi S (1998) Cortactin associates with the cell-cell junction protein ZO-1 in both *Drosophila* and mouse. *J Biol Chem* 273: 29672–29677
- Liu M, Qin Y, Liu J, Tanswel, AK, Post M (1996) Mechanical strain induces pp60<sup>src</sup> activation and translocation to cytoskeleton in fetal rat lung cells. *J Biol Chem* 271: 7066–7071
- Maa MC, Wilson LK, Moyers JS, Vines RR, Parsons JT, and Parsons SJ (1992) Identification and characterization of a cytoskeleton-associated, epidermal growth factor sensitive pp60<sup>c-src</sup> substrate. *Oncogene* 7: 2429–2438
- Migliarese MR, Mannion-Henderson J, Wu H, Parsons JT, Bender TP (1994) The protein tyrosine kinase substrate cortactin is differentially expressed in murine B lymphoid tumors. *Oncogene* 9: 1989–1997
- Ohoka Y, Takai Y (1998) Isolation and characterization of cortactin isoforms and a novel cortactin-binding protein, CBP90. *Genes Cells* 3: 603–612
- Schmidt A, Hall MN (1998) Signaling to the actin cytoskeleton. *Annu Rev Cell Dev Biol* 14: 305–338
- Schuuring E, Verhoeven E, Litvinov S, Michalides RJ (1993) The product of the *EMS1* gene, amplified and overexpressed in human carcinomas, is homologous to a v-src substrate and is located in cell-substratum contact sites. *Mol Cell Biol* 13: 2891–2898
- Thomas SM, Brugge JS (1997) Cellular functions regulated by Src family kinases. *Annu Rev Cell Dev Biol* 13: 513–609
- Thorn JM, Armstrong NA, Cantrell LA, Kay BK (1999) Identification and characterisation of *Xenopus* moesin, a Src substrate in *Xenopus laevis* oocytes. *Zygote* 7: 113–122

- Unger TF, Steele RE (1992) Biochemical and cytological changes associated with expression of deregulated pp60<sup>src</sup> in *Xenopus* oocytes. *Mol Cell Biol* 12: 5485–5498
- van Damme H, Brok H, Schuurin-Scholtes E, Schuurin E (1997) The redistribution of cortactin into cell-matrix contact sites in human carcinoma cells with 11q13 amplification is associated with both overexpression and post-translational modification. *J Biol Chem* 272: 7374–7380
- Vize PD, Melton DA, Hemmati-Brivanlou A, Harland RM (1991) Assays for gene function in developing *Xenopus* embryos. *Methods Cell Biol* 36: 367–387
- Wu H, Parsons JT (1993) Cortactin, an 80/85-kilodalton pp60<sup>src</sup> substrate, is a filamentous actin-binding protein enriched in the cell cortex. *J Cell Biol* 120: 1417–1426
- Wu H, Reynolds AB, Kanner SB, Vines RR, Parsons JT (1991) Identification and characterization of a novel cytoskeleton-associated pp60<sup>src</sup> substrate. *Mol Cell Biol* 11: 5113–5124
- Zhan X, Haudenschild CC, Ni Y, Smith E, Huang C (1997) Upregulation of cortactin expression during the maturation of megakaryocytes. *Blood* 89: 457–464
- Zhan X, Hu X, Hampton B, Burgess WH, Friesel R, Maciag T (1993) Murine cortactin is phosphorylated in response to fibroblast growth factor-1 on tyrosine residues late in the G1 phase of the BALB/c 3T3 cell cycle. *J Biol Chem* 268: 24427–24431

(Received October 4, 2000 / Accepted December 18, 2000)