V-1, a Protein Expressed Transiently during Murine Cerebellar Development, Regulates Actin Polymerization via Interaction with Capping Protein

Masato Taoka†‡§, Tohru Ichimura†, Akiko Wakamiya-Tsuruta†, Yoshiaki Kubota‡, Takeshi Araki‡, Takashi Obinata‡, and Toshiaki Isobe†‡¶**

-from The Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University, Hachioji-shi, Tokyo 192-0397, Japan, Integrated Proteomics System Project, Pioneer Research on Genome the Frontier, Ministry of Education, Culture, Sports, Science and Technology, Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University, Hachioji-shi, Tokyo 192-0397, Japan, Department of Biology, Faculty of Science, Chiba University, Chiba 263-8522, Japan, and Division of Proteomics Research (ABJ-Millipore), The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 110-8639, Japan

V-1 is a 12-kDa protein consisting of three consecutive ANK repeats, which are believed to serve as the surface for protein-protein interactions. It is thought to have a role in neural development for its temporal profile of expression during murine cerebellar development, but its precise role remains unknown. Here we applied the proteomic approach to search for protein targets that interact with V-1. The V-1 cDNA attached with a tandem affinity purification tag was expressed in the cultured 293T cells, and the protein complex formed within the cells were captured and characterized by mass spectrometry. We detected two polypeptides specifically associated with V-1, which were identified as the α and β subunits of the capping protein (CP, alternatively called CapZ or β-actinin). CP regulates actin polymerization by capping the barbed end of the actin filament. The V-1-CP complex was detected not only in cultured cells transfected with the V-1 cDNA but also endogenously in cells as well as in murine cerebellar extracts. An analysis of the V-1-CP interaction by surface plasmon resonance spectroscopy showed that V-1 formed a stable complex with the CP heterodimer with a dissociation constant of $1.2 \times 10^{-7}$ M and a molecular stoichiometry of 1:1:1. In addition, V-1 inhibited the CP-regulated actin polymerization in vitro in a dose-dependent manner. Thus, our results suggest that V-1 is a novel component that regulates the dynamics of actin polymerization by interacting with CP and thereby participates in a variety of cellular processes such as actin-driven cell movements and motility during neuronal development.

The V-1 protein was originally identified in the murine cerebellum as one of the proteins expressed significantly at the initial stage of postnatal development (1), particularly in the regions where synaptic formation and neuronal migration occur actively during neurogenesis (2, 3). V-1 consists of 117 amino acids containing three contiguous repeats of the ANK motif, alternatively called the cdc10/SW16 motif (1, 2), which is crucial for a large number of protein-protein interactions (4). Previous studies suggested the potential role of V-1 in the signal transduction pathways leading to catecholamine synthesis or to cardiac hypertrophy. For example, Yamakuni et al. (5–7) demonstrated that the overexpression of V-1 caused a significant increase in the catecholamine level in PC12 cells, presumably through the transcriptional activation of the genes for catecholamine synthesis. In other reports (8–11), V-1 was designated as “myotrophin” and was shown to participate in the cell signaling pathways for the NFkB-mediated activation of protein synthesis in the myocytes. Thus, both of these studies (5, 9) suggested the roles of V-1 in the biological events taking place in the nucleus. However, no biological function has been attributed to V-1 in the cytoplasm in which this molecule predominantly resides within the cells and tissues (5, 9).

In this study, we screened for V-1-binding proteins by a novel proteomic approach that combined the tandem affinity purification (TAP) procedure (12) and mass spectrometry (MS). Following this strategy, we identified capping protein (CP) as a V-1-binding protein. We confirmed the existence of the V-1-CP complex not only in cultured cells transfected with the TAP-tagged V-1 but also endogenously in cells and rat cerebellar extracts. Furthermore, we found that V-1 inhibited the CP-regulated actin polymerization. On the basis of these results, the possible role of V-1 in neuronal development is discussed.

EXPERIMENTAL PROCEDURES

Materials—Actin monomer was prepared from the acetone-dried powder of rabbit skeletal muscle according to the procedure of Spudich and Watt (13). Pyrene-labeled actin was purchased from Cytoskeleton Inc. (Denver, CO). The polyclonal antibody against the V-1 protein was raised in rabbits injected with the recombinant V-1 protein. The antibody was purified by ammonium sulfate fractionation (0–50% saturation) followed by affinity chromatography on Sepharose beads coupled with the recombinant V-1 protein. The anti-CP monoclonal antibody (14) was obtained from the Developmental Studies Hybridoma Bank, developed under the auspices of the National Institute of Child Health and Human Development, and maintained by the University of Iowa, Department of Biological Sciences. Oligonucleotides were purchased from Sigma.

Plasmid Constructions—To construct the cDNA for TAP-tagged V-1,
the TAP cDNA (pBS1479) (12) was first digested with BamHI and HindIII and was inserted into the BamHI and EcoRI sites of the mammalian expression vector pcDNA3 (Invitrogen) after blunting the HindIII and EcoRI sites (termed pcDNA3-TAP). The V-1 cDNA was then generated by PCR using the oligonucleotides 5′-GGGAAGCTTAGCCACTGTTGGAGAAGAGCGTTCCAGGAACTGGTTAGC-3′ and the rat V-1 cDNA. The PCR fragment was digested with BamHI and BamHI and was inserted into the cloning sites of the bacterial expression vector pGEX-3X (Amersham Biosciences). The V-1 cDNA was also amplified by PCR using the oligonucleotides 5′-TACCCTCACCAGGGATCTCTGCT-3′ and 5′-CTGGATTCGGAAGAGCCTGGATC-3′ and the rat V-1 cDNA. The PCR fragment was digested with NdeI and BamHI and was inserted into the cloning sites of the bacterial expression vector. The complex was subsequently incubated with 20 % formic acid and were concentrated by adsorption onto 0.1 mL of reversed-phase beads (POROS R2, Applied Biosystems, Foster, CA). The concentration of urea was then decreased to 150 mM Tris-HCl (pH 8.0) to release the protein complex into 150 mM NaCl, 10 mM NaF, and 5 mM Tris-HCl (pH 8.0), 10 mM EDTA, 2 μg/ml leupeptin, and 4 mM phenylmethylsulfonyl fluoride. The IgG beads (Amersham Biosciences) were washed twice with phosphate-buffered saline and were immediately scraped into 500 μl of lysis buffer containing 50 μM Tris-HCl (pH 8.0), 10% glycerol, 1% Triton X-100, 150 mM NaCl, 5 mM MnCl2, 1 mM Na3VO4, 10 mM EDTA, 2 μg/ml leupeptin, and 4 mM phenylmethylsulfonyl fluoride. The cell lysate was centrifuged at 100,000 × g for 20 min at 4 °C, and the supernatant (5–mg protein) was incubated with 20 μl of IgG-Sepharose beads (Amersham Biosciences) for 2 h at 4 °C. The IgG beads were washed five times with 500 μl of a buffer containing 20 μM Tris-HCl (pH 7.5), 5% glycerol, 0.1% Triton X-100, and 150 mM NaCl and 2 μl of 50 μg/ml tobacco etch virus protease (Invitrogen) at room temperature for 1 h in 50 mM Tris-HCl (pH 8.0) to release the protein complex into solution. The complex was subsequently incubated with 20 μl of calmodulin-agarose beads (Stratagene, La Jolla, CA) in a buffer containing 50 μM Tris-HCl (pH 8.0), 150 mM NaCl, and 1 mM CaCl2. After washing the beads twice with the same buffer, the bound proteins were eluted from the beads with this buffer containing 4 mM EDTA instead of 1 mM CaCl2.

**In-gel Digestion and Tandem Mass Spectrometry**—Protein bands (0.2 × 0.8 cm) were excised from the SDS gel, dehydrated with acetone, and reduced with 10 mM DTT. The gel pieces were dissolved in 1% SDS, 10% glycerol, 10 mM Tris-HCl (pH 8.8), and the proteins were in-gel digested overnight with 250 ng of trypsin (Promega, Madison, WI) at 37 °C. The peptide fragments were extracted from the gel with 100 μl of 50% acetonitrile in 5% formic acid and were concentrated by adsorption onto 0.1 μl of reversed-phase beads (POROS R2, Applied Biosystems, Foster, CA). After the beads were washed twice with 400 μl of 0.1% trifluoroacetic acid, the bound peptides were recovered with 2 μl of 50% methanol in 5% acetic acid. The peptide mixture was then analyzed by tandem mass spectrometry on a electrospray ionization quadrupole time-of-flight mass spectrometer (Q-TOF, Micromass, Manchester, United Kingdom) equipped with a nanospray tip. All of the MS/MS spectra were recorded with a nanospray tip. All of the MS/MS spectra were recorded. For the kinetic analysis, various concentrations of the CP heterodimer were loaded on the sensor surface to equilibrate the V-1/CP interaction, and the concentration of the complex ( Req ) was measured as the response units. The correlation among Req, the concentration of ligand (C), and the total binding capacity ( K d ) of the immobilized protein was Req = ( K d + C ) 1/2 (18). Thus, the association constant ( K d ) was determined from the plot of Req versus C. For the kinetic analysis, various concentrations of the CP heterodimer were loaded on the sensor surface to equilibrate the V-1/CP interaction, and the concentration of the complex ( Req ) was measured as the response units. The correlation among Req, the concentration of ligand (C), and the total binding capacity ( K d ) of the immobilized protein was Req = ( K d + C ) 1/2 (18). Thus, the association constant ( K d ) was determined from the plot of Req versus C.

**RESULTS**

**Identification of CP as a V-1-binding Protein**—To search for the V-1-binding protein, we carried out an affinity purification experiment using the TAP method (12). The TAP method utilizes two affinity modules (a calmodulin-binding peptide and a protein A epitope) separated by a cleavage site for the tobacco etch virus protease. Therefore, the purification produces much less background than the conventional single epitope tags such as Myc or hemagglutinin. To adapt this method for our purpose, we constructed a mammalian expression vector encoding V-1 with a carboxyl-terminal TAP tag, transiently transformed 293T cells with the vector, and purified the tagged V-1 with its binding proteins from the cell lysate by the TAP method. Fig. 1A, lane 2, shows the SDS-PAGE photographs of the purified protein complex. In addition to the tagged V-1 used as bait, the 36- and 33-kDa polypeptide bands were reproducibly detected in the cells transfected with a TAP-tagged V-1 but not in the control cells (Fig. 1A, lane 1), suggesting that these polypeptides were associated with the expressed V-1 protein. These bands (assigned as bands 1 and 2 in Fig. 1A) were excised from the gel and subjected to in-gel tryptic digestion, and the peptide...
fragments thus generated were analyzed by nanoelectrospray tandem mass spectrometry, respectively.

Two doubly charged peptide ions with m/z 599.40 and 786.00, were observed from band 1. The data base analysis of the MS/MS spectrum of the peptide ion with m/z 599.40 showed that it corresponded to the sequence LLLNNDNLLR of CP/H9251 (GenBank™ accession number P52907) at residues 38–47. The manual assignment of the fragment ions also yielded the same sequence (Fig. 1B). Likewise, the MS/MS spectrum of the other peptide ion with m/z 786.00 was assigned to the sequence FTITPPTAQVVGVLK of CP/H9251 at residues 179–193 (data not shown), confirming that band 1 contained CP/H9251. From band 2, two doubly charged peptide ions with m/z 586.20 and 677.30 were observed. The analysis of their collision-induced dissociation fragments indicated that one (m/z 586.20) corresponded to the sequence STLNEIYFGK of CP/H9252 (GenBank™ accession number P47756) at residues 226–235 (Fig. 1C) and the other (m/z 677.30) corresponded to the sequence SGSGTMNLGGSLTR of the same polypeptide at residues 182–195 (data not shown). The molecular weights of bands 1 and 2 estimated by SDS-PAGE (Fig. 1A) were also agreed with those of the respective CP subunits (32,902 and 31,331). Because CP is a stable heterodimer of α and β subunits (22, 23) and because these polypeptides appear in almost equimolar amounts in the captured V-1 complex (Fig. 1A), we anticipated that V-1 bound the CP heterodimer itself.

Detection of the Endogenous V-1-CP Complex—The overexpression of a particular protein sometimes induces artificial interactions among proteins, which do not occur under physiological conditions. To exclude the possibility that the observed interaction between V-1 and CP might be artificial, we sought to detect the endogenous V-1-CP complex in cultured 293T cells as well as in rat cerebella. Soluble extracts were prepared from 293T cells or from rat cerebella at a postnatal day 12, and the V-1 protein was immunoprecipitated from the extracts with the anti-V-1 IgG immobilized on Sepharose beads. The precipitate was then analyzed by SDS-PAGE followed by immunoblotting with the antibody against the CPβ subunit. As shown in Fig. 2, the CPβ was clearly detected in the precipitates derived both
from the 293T cells and the rat cerebella, suggesting that the V-1/CP complex was present under physiological conditions in vivo.

**Biochemical Properties of the Interaction between V-1 and CP**—To study the biochemical characteristics of the V-1/CP complex in further detail, we prepared a number of materials including V-1, GST-tagged V-1, and the CP/H9251 and H9252 subunits. These proteins were expressed in the E. coli cells and were purified to near homogeneity as observed by SDS-PAGE (Fig. 3A). First, we studied the complex formation of V-1 and CP by SPR. For the direct binding assay on the SPR biosensor, the GST-V-1 protein was attached to the sensor surface via the GST antibody, and the recombinant CP was passed over the sensor chip. As shown in Fig. 3B, positive binding signals were detected when the CP heterodimer, reconstituted from the recombinant CPα and β subunits, was introduced to the sensor chip. Interestingly, however, the purified CPα or CPβ alone gave no SPR signals (Fig. 3B) even with repeated binding experiments using different subunit preparations, suggesting that V-1 bound specifically to the heterodimeric CP molecule.

To obtain kinetic data of the complex formation, the steady-state resonance was measured using various concentrations of CP (Fig. 3C). The results showed that the immobilized V-1 was saturable with respect to the CP binding and the association constant calculated from the slope of the straight line of the corresponding Scatchard plot (inset) was $8.4 \times 10^{-6}$ M. The $K_a$ value was not influenced by the amount of immobilized V-1 protein (data not shown). The equilibrium dissociation constant ($K_d$) calculated from $1/K_a$ was $1.2 \times 10^{-7}$ M, indicating that the V-1-CP complex is relatively stable.

To clarify the formation of the V-1-CP complex and to determine the molecular stoichiometry, the "native-PAGE assay"
Interaction of V-1 and Capping Protein

**Fig. 4. Inhibition of CP-mediated actin nucleation and capping by the V-1 protein.** A, effect of V-1 on the CP-mediated actin nucleation. Actin and the indicated proteins were incubated for 10 min at room temperature, and the actin polymerization was initiated by adding the salt solution (50 mM KCl and 1 mM MgCl₂) into the mixture. The actin nucleation was monitored by UV absorption at 237 nm. The time-course representation (a) and the statistical evaluation (b) of the V-1 activity on CP-nucleated actin polymerization are shown. The rate of actin nucleation in <b><i>n</i></b> was measured as the rate of increase in absorbance for the first 100 s. Each value represents the ratio to the control experiment (b, No addition) and the mean ± S.E. of three independent measurements. Asterisks indicate significant difference by Student’s <i>t</i> test (<i>p</i> < 0.005) from CP (†) and CP+V-1 (‡). B, effect of V-1 on the capping activity of CP in the F-actin depolymerization. The F-actin was prepared as described under “Experimental Procedures,” and the depolymerization was initiated by dilution with the buffer containing CP and V-1 at the molar ratios indicated in the figure. The fluorescence changes in the pyrene-labeled actin versus time after dilution are shown.

**Fig. 5. V-1 induced inhibition of the CP/F-actin interaction.** The actin monomer was polymerized in the presence of CP with [lane 1] or without the V-1 protein [lane 2]. After centrifugation, the precipitate (2.5-μg protein) was analyzed by SDS-PAGE to detect the CP that co-sedimented with the actin filament (CBB staining).

Interaction between the V-1/CP Complex and F-actin—We studied whether the V-1/CP complex bound F-actin by using a co-sedimentation assay. The actin monomer was polymerized and co-sedimented with CP in the presence or absence of the V-1 protein, and aliquots of the precipitates were analyzed by SDS gel electrophoresis. As shown in Fig. 5, CP co-sedimented with F-actin only in the absence of the V-1 protein. Thus, V-1 appeared to form a stable complex with CP and to prevent CP binding to F-actin.

**DISCUSSION**

CP is one of the F-actin-binding proteins that caps the barbed ends of actin filaments (14, 23, 25, 26). To examine the effects of V-1 protein on the activities of CP, we assayed the initial rates of polymerization with the CP-nucleated actin and depolymerization of the CP-capped F-actin in the presence and absence of the V-1 protein. For the CP-nucleated actin polymerization, we monitored the changes in optical absorption to measure the initial rate of actin polymerization. As shown in Fig. 4Ba, CP markedly increased the initial rate of actin polymerization and the V-1 protein canceled this CP function. The difference in the initial rate of polymerization between CP-nucleated actin in the presence and absence of the V-1 protein was statistically significant (Fig. 4Ab). The V-1 protein reduced the nucleation activity of CP in a dose-dependent manner, whereas V-1 alone had no effect on the actin polymerization (data not shown). Besides this activity, we also measured the effects of V-1 on the dilution-induced depolymerization of the CP-capped actin filaments by monitoring the changes in fluorescence (Fig. 4B). After the preincubation with V-1, CP reduced its capping activity to depolymerize the actin filaments. Thus, the capping activity of CP was dependent on the concentration of V-1, whereas V-1 alone had no effect on the actin depolymerization. These results demonstrate that the V-1 protein suppressed the CP activities of both actin nucleation and F-actin capping.

Suppression of CP Activity by V-1—CP nucleates actin polymerization and caps the barbed ends of actin filaments (14, 23, 25, 26). To examine the effects of V-1 protein on the activities of CP, we assayed the initial rates of polymerization with the CP-nucleated actin and depolymerization of the CP-capped F-actin in the presence and absence of the V-1 protein. For the CP-nucleated actin polymerization, we monitored the changes in optical absorption to measure the initial rate of actin polymerization. As shown in Fig. 4Ba, CP markedly increased the initial rate of actin polymerization and the V-1 protein canceled this CP function. The difference in the initial rate of polymerization between CP-nucleated actin in the presence and absence of the V-1 protein was statistically significant (Fig. 4Ab). The V-1 protein reduced the nucleation activity of CP in a dose-dependent manner, whereas V-1 alone had no effect on the actin polymerization (data not shown). Besides this activity, we also measured the effects of V-1 on the dilution-induced depolymerization of the CP-capped actin filaments by monitoring the changes in fluorescence (Fig. 4B). After the preincubation with V-1, CP reduced its capping activity to depolymerize the actin filaments. Thus, the capping activity of CP was dependent on the concentration of V-1, whereas V-1 alone had no effect on the actin depolymerization. These results demonstrate that the V-1 protein suppressed the CP activities of both actin nucleation and F-actin capping.

**DISCUSSION**

CP is one of the F-actin-binding proteins that caps the barbed end of actin filaments and nucleates the actin polymerization in a Ca²⁺-independent (22, 23, 27, 28) and a phosphatidylinositol 4,5-bisphosphate-dependent manner (29–32).

---

² M. Taoka, unpublished result.
This activity is thought to be functionally significant, because the actin-based movement of Dictostelium is proportional to the expression level of CP (33) and because CP is essential for the in vitro reconstitution of the cell movement (34, 35). In this study, we have shown that the V-1 protein forms a stable stoichiometric complex with CP in vitro as well as in vivo (Figs. 2 and 3) and inhibits the CP-mediated nucleation of actin polymerization. Therefore, we assume that V-1 participates in the regulation of actin dynamics in the cells via the interaction with CP.

Our strategy to identify the V-1-interacting molecules was based on the tandem affinity purification of the V-1 complex with a TAP tag followed by protein identification by mass spectrometry. The TAP method was originally developed to analyze interactions among yeast proteins (12). We constructed a mammalian expression vector for the TAP method and applied it to the mammalian 293T cell line. Even though the V-1 protein is a rather minor cellular component and a transient expression system was used for the assay, the method enabled us to isolate a sufficient amount of the V-1-CP complex for characterization by nanospray tandem mass spectrometry (Fig. 1). This affinity-tag technique coupled with mass spectrometry is useful to detect novel protein interactions not only in yeast but also in mammalian cells.

The V-1 protein consists of three consecutive ANK repeats with an additional short stretch of sequence (1, 2, 37). The ANK repeat is a structural motif found in many proteins (36) and mediates specific interactions with a diverse array of protein targets (4). In the tertiary structure of V-1 determined by NMR spectroscopy (37), the ANK repeats comprise the hairpin-helix-loop-helix modules where the α helices lie along one side providing a structural framework and the hairpins protrude on the other side of the molecule. The hairpins and the surface of the α helices form a groove-like structure, which is believed to be responsible for the contact with the target molecule. This study identified CP as a potential target of V-1. Interestingly, V-1 bound to the functional CP heterodimer consisting of the α and β subunits but did not bind each of the two subunits. This finding is comparable with previous observations that each of the CP subunits was unstable and did not bind actin in vitro and in vivo (26, 38, 39). Thus, it seems likely that V-1 recognizes the structural interface of the CP heterodimer by its groove-like ANK repeats and covers the F-actin binding surface located in the carboxyl-terminal region of the Cβ subunit (26). However, whether this is the molecular mechanism by which V-1 interacts the interaction of CP and F-actin awaits further structural investigation.

Two distinct proteins, carmil (40) and twinfilin (24), are known to bind CP. Carmil is a scaffold protein containing the CP-binding site, the myosin I-binding site, the short sequence commonly found in several actin monomer-binding proteins, and the acidic stretch that can activate Arp2/3-dependent actin nucleation (40). The NH2-terminal region of carmil binds CP, but its activity with CP is unknown. Twinfilin is a ubiquitously actin monomer-binding protein composed of two ADF/cofilin-like domains connected by a short linker region and has a role in the regulation of actin turnover (41). Twinfilin also forms a stable complex with CP but does not affect its activity. V-1 is neither a scaffold protein nor an actin-binding protein, and it lacks structural homology to either carmil or twinfilin. Thus, V-1 belongs to a novel CP-binding protein category. Recently, Ena/VASP was reported as an anti-capping molecule, which promotes actin filament elongation by associating with the barbed ends of actin and shielding them from CP (42). The actin cytoskeleton in the Ena/VASP-deficient cell contained shorter, more highly branched filaments than those in the control cells. V-1 resembles Ena/VASP in the activity of CP-regulated actin polymerization, but whether a similar phenotype can be attributed to the V-1-deficient cell is currently unknown.

Previous studies suggested the potential roles of V-1 in nuclear events such as the transcriptional activation of a set of enzymes involved in catecholamine synthesis (5–7) or the regulation of drosophila protein synthesis (8–10, 43). This study suggests for the first time that V-1 functions in the molecular events taking place in the cytoplasm in which the major portion of V-1 resides within the cells as revealed by previous immunohistochemical studies (5, 9). V-1 also appeared to be a typical cytoplasmic protein in terms of amino acid sequence with no apparent nuclear localization signal. Our previous studies revealed the characteristic temporal profile of V-1 expression in the developing mouse cerebellum at postnatal days 7–12 (2). Namely, V-1 expression is particularly significant during the migration of progenitor granule cells from the external to internal granular layer to make synaptic contacts with the target Purkinje cells. Likewise, the dynamics of actin polymerization play a pivotal role in the maturation of granule cells, because the modulation of the barbed ends of actin filaments with cytoscalasins changed the behavior of the growth cone (44) and the migration of granule cells (45). These observations coupled with the results reported here suggest that V-1 may have a role in the CP-mediated actin-driven cell movements and motility such as granular cell migration and synapse formation.

REFERENCES

1. Tsak, M., Yamakuni, T., Song, S. Y., Yamakawa, Y., Seto, K., Okuyama, T., and Isobe, T. (1992) Eur. J. Biochem. 207, 615–620.
2. Tsak, M., Isobe, T., Okuyama, T., Watanabe, M., Hondo, H., Yamakawa, Y., Ozawa, F., Hishinuma, F., Kobota, M., Minegishi, A., Song, S. Y., and Yamakuni, T. (1994) J. Biol. Chem. 269, 9646–9651.
3. Fujigasagi, H., Song, S. Y., Kobayashi, T., and Yamakuni, T. (1996) Mol. Brain Res. 40, 203–213.
4. Sedgwick, S. G., and Smerdon, S. J. (1999) Trends Biochem. Sci. 24, 311–316.
5. Yamakuni, T., Yamamoto, T., Hoshino, S., Song, S. Y., Yamamoto, H., Kunikata-Sumimoto, M., Minegishi, A., Kobota, M., Ito, M., and Konishi, S. (1998) J. Biol. Chem. 273, 27551–27554.
6. Suzuki, T., Inagaki, H., Yamakuni, T., Naganatsu, T., and Ichihone, H. (2002) Biochem. Biophys. Res. Commun. 293, 962–968.
7. Suzuki, T., Yamakuni, T., Hagawara, M., and Ichihone, H. (2002) J. Biol. Chem. 277, 40768–40774.
8. Gupta, S., and Sen, S. (2002) Biochim. Biophys. Acta 1589, 247–260.
9. Knuefermann, P., Chen, P., Misra, A., Shi, S. P., Abdellatif, M., and Sivasubramanian, N. (2000) J. Biol. Chem. 275, 23888–23897.
10. Sivasubramanian, N., Adhikary, G., Silt, P. C., and Sen, S. (1996) J. Biol. Chem. 271, 2812–2816.
11. Silt, P. C., Kanda, M., and Sen, S. (1998) Circ. Res. 82, 1173–1188.
12. Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M., and Seraphin, B. (1999) Nat. Biotechnol. 17, 1030–1032.
13. Spudich, J. A., and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
14. Staber, D. A., Jennings, P. B., and Cooper, J. A. (1996) J. Cell Biol. 135, 169–179.
15. Fujiyama, S., Hayano, T., Miura, Y., Isobe, T., and Takahashi, N. (2002) J. Biol. Chem. 277, 23773–23780.
16. Ishimura, T., Uchiyama, Y., Kunihiro, O., Ito, M., Horigome, T., Omata, S., Shinkai, K., Kaji, H., and Isobe, T. (1995) J. Biol. Chem. 270, 28515–28518.
17. Sorensen, Y., Abe, H., Kimura, S., Maruyama, K., and Ohnata, T. (1998) J. Muscle Res. Cell Motil. 19, 639–646.
18. Banik, U., Wang, G. A., Wagner, P. D., and Kaufman, S. (1997) J. Biol. Chem. 272, 26219–26225.
19. Nishiguchi, S., and Oosawa, F. (1965) J. Mol. Biol. 12, 843–865.
20. Itagaki, C., Isobe, T., Tsak, M., Natsuume, T., Nomura, N., Horigome, T., Omata, S., Ichinose, H., Nagatou, T., Greene, L. A., and Ichimura, T. (1999) Biochemistry 38, 15673–15680.
21. Tsak, M., Makawakia, A., Nakayama, H., and Isobe, T. (2000) Electrophoresis 21, 1872–1879.
22. Maruyama, K., Kima, S., Ishii, T., Kuroda, M., Ohashi, K., and Muramatsu, S. (1977) J. Biochem. 81, 215–223.
23. Caldwell, J. E., Heiss, S. G., Merrall, V., and Cooper, J. A. (1989) Biochemistry 28, 8506–8514.
24. Vanabergen, S. J., and Pollard, T. D. (1985) Biochemistry 24, 793–799.
25. Hug, C., Miller, T. M., Torres, M. A., Casella, J. F., and Cooper, J. A. (1992) J. Cell Biol. 116, 923–931.
26. Maruyama, K., Kurokawa, H., Oosawa, M., Shimakawa, S., Yamamoto, H., and Ito, M. (1990) J. Biol. Chem. 265, 8712–8715.
27. Casella, J. F., Maack, D. J., and Lin, S. (1986) J. Biol. Chem. 261, 10915–10921.
28. Heis, S. G., and Cooper, J. A. (1991) Biochemistry 30, 8755–8758.
Interaction of V-1 and Capping Protein

30. Haus, U., Hartmann, H., Trommler, P., Noegel, A. A., and Schleicher, M. (1991) Biochem. Biophys. Res. Commun. 181, 833–839
31. Barkalow, K., Witke, W., Kwiatkowski, D. J., and Hartwig, J. H. (1996) J. Cell Biol. 134, 395–399
32. Dinhahle, M. J., and Huang, S. (1997) Biochim. Biophys. Acta 1358, 261–278
33. Hug, C., Jay, P. Y., Reddy, I., McNally, J. G., Bridgman, P. C., Elson, E. L., and Cooper, J. A. (1996) Cell 84, 591–600
34. Leislin T., P., Boujemaa, R., Pantaloni, D., and Carlier, M. F. (1999) Nature 401, 613–616
35. Pantaloni, D., Boujemaa, R., Didry, D., Gounon, P., and Carlier, M. F. (2000) Nat. Cell Biol. 2, 385–391
36. Rubin, G. M., Yandell, M. D., Wortman, J. R., Gabor Miklos, G. L., Nelson, C. R., Harharian, I. K., Fortini, M. E., Li, P. W., Apweiler, R., Fleischmann, W., Cherry, J. M., Henikoff, S., Skupski, M. F., Mirza, S., Ashburner, M., Birney, E., Boguski, M. S., Brody, T., Brokstein, P., Celukin, S. E., Cher- vitz, S. A., Coates, D., Cravchik, A., Gabrielsen, A., Galle, R. F., Gelbart, W. M., George, R. A., Goldstein, L. S., Gong, F., Guan, P., Harris, N. L., Hay, B. A., Hoskins, R. A., Li, J., Li, Z., Hynes, R. O., Jones, S. J., Rues, F. M., Lemaitre, B., Littleton, J. T., Morrison, D. K., Mungall, C., O’Farrell, P. H., Pickeral, O. K., Shae, C., Vosshall, L. B., Zhang, J., Zhao, Q., Zheng, X. H., and Lewis, S. (2000) Science 287, 2204–2215
37. Yang, Y., Nanduri, S., Sen, S., and Qin, J. (1998) Structure 6, 619–626
38. Amatruda, J. F., Guttermeier, D. J., Karpova, T. S., and Cooper, J. A. (1992) J. Cell Biol. 119, 1151–1162
39. Hart, M. C., and Cooper, J. A. (1999) J. Cell Biol. 147, 1287–1298
40. Jung, G., Hemmert, K., Wu, X., Vokesy, J. M., and Hammer, J. A., III (2001) J. Cell Biol. 153, 1479–1497
41. Palmgren, S., Vartiainen, M., and Lappalainen, P. (2002) J. Cell Sci. 115, 881–886
42. Bear, J. E., Svistina, T. M., Krause, M., Schaefer, D. A., Loureiro, J. J., Strasser, G. A., Maly, I. V., Chaga, O. Y., Cooper, J. A., Borisy, G. G., and Gertler, P. B. (2002) Cell 109, 509–521
43. Schroder, H. C., Kraiko, A., Batel, R., Skorokhod, A., Pahler, S., Kruse, M., Muller, I. M., and Muller, W. E. (2000) FASEB J. 14, 2022–2031
44. Zmuda, J. F., and Rivas, R. J. (2000) J. Cell Sci. 113, 2797–2809
45. Rivas, R. J., and Hatten, M. E. (1995) J. Neurosci. 15, 981–989

Downloaded from http://www.jbc.org/ by guest on July 25, 2018
V-1, a Protein Expressed Transiently during Murine Cerebellar Development, Regulates Actin Polymerization via Interaction with Capping Protein
Masato Taoka, Tohru Ichimura, Akiko Wakamiya-Tsuruta, Yoshiaki Kubota, Takeshi Araki, Takashi Obinata and Toshiaki Isobe

J. Biol. Chem. 2003, 278:5864-5870.
doi: 10.1074/jbc.M211509200 originally published online December 16, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M211509200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 23 of which can be accessed free at http://www.jbc.org/content/278/8/5864.full.html#ref-list-1