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Unraveling ICAP-1 function : toward a new direction ?

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Abstract

Cell adhesion to either the extracellular matrix (ECM) or to neighbouring cells is of critical importance during both physiological and pathological situations. Integrins are a large family of cell adhesion receptors composed of two non-covalently linked α and β sub-units. They have a well identified dual function of mediating both firm adhesion and signalling. The short cytoplasmic domain of integrin can interact with cytoplasmic proteins that are either shared by several different integrins or specific for one type of integrin. ICAP-1 (Integrin Cytoplasmic domain Associated Protein-1) is a small cytoplasmic protein that specifically interacts with the β_1 integrin subunit. In this review we will discuss recent findings on ICAP-1, not only at the structural and functional level, but also its possible interconnection in other signalling pathways such as those that control cell proliferation.

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Introduction

Cell adhesion to the extracellular matrix (ECM) or to cellular counter-receptors is crucial for several cellular processes, such as migration, spreading, proliferation, apoptosis, differentiation and gene expression (Brakebusch et al., 2002; Hynes, 2002). These events contribute in turn to the maintenance of tissue integrity, embryogenesis, wound healing, while their deregulation leads to different types of pathogenesis (Bouvard et al., 2001; Brakebusch and Fassler, 2003). Among the numerous types of adhesion receptors, the integrin family plays an important role, not only by supporting cell adhesion but also by its capability to transmit and/or to modulate cellular signaling (outside-in signaling) by converting extracellular information (such as local concentration of growth factors or the nature of the ECM) into intracellular responses such as cell proliferation, differentiation and apoptosis (Giancotti and Ruoslahti, 1999; Keely et al., 1998). By providing mechanical anchorage, controlling actin dynamics and polarization within the cell, integrins are intimately involved in cell migration. These phenomena occur in tight connection with either diffusable factors (such as growth factors) or with insoluble proteins such as matricellular proteins or metalloproteases. On the other hand, integrins are submitted to a tight and dynamic control of their affinity state that will, in return, modulate their function (inside-out signaling). Integrin activation is achieved by rapid, reversible changes in the conformation of their extracellular domains through direct associations of the tails of the receptors with structural and/or signaling proteins which results in increased affinity for the ligand (Ginsberg et al., 2005). It has recently been shown that integrin activation can be dynamically regulated by the binding of talin to β integrin tails and that efficient binding of talin depends on both integrin occupancy by an extracellular ligand (Calderwood, 2004; Liddington and Ginsberg, 2002; Tadokoro et al., 2003) and PIP2/talin interaction (Martel et al., 2001). After attachment to

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extracellular matrix or after ligand binding, most integrins are clustered into sub-cellular structures and recruit not only structural proteins, but also a number of signaling proteins that are required for the activation of integrin-dependent cell signaling cascades. So far, 8 β subunits and 16 α subunits that can combine to form more than 24 different receptors have been described. Alternative splicing has also been reported for several integrin chains adding to the complexity of the family. Moreover, one type of integrin receptor can bind several different ligands, and the same ligand can be shared by several receptors (Plow et al., 2000). On most cells, more than one form of integrin heterodimers is expressed, implying that different integrins heterodimers can be redundant. However, a knockout approach in mouse has revealed that this redundancy is not observed *in vivo* nor, in most of the cases, *in vitro* as well (Bouvard et al., 2001). Therefore an important question still to be addressed is to understand how a cell is able to specifically regulate one type of integrin, and on the other side, how a specific integrin can trigger the activation of a specific signaling cascade. Some recent advances in this field have emerged with the identification of some putative cytosolic integrin regulators that bind a specific integrin tail and are therefore likely to control a certain integrin. For instance, TAP-20 interacts specifically with β_5 integrins, β_3-endonexin binds to β3 integrins, cytohesin-1 or -3 bind to β_2 integrins and ICAP-1 to β_1 integrins.

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Structure of ICAP-1

ICAP-1, integrin cytoplasmic domain associated protein-1, was first identified as a β_1 integrin binding partner in a two hybrid screen (Chang et al., 1997). It is a small cytoplasmic protein of 200 amino-acid residue length that can be divided into two separate domains. The N terminal moiety (1-60 aa) is rich in serine/threonine residues and contains several consensus sites for protein kinases such as PKC, PKA/PKG, PAKs and CaMKII (Ca2+/Calmodulin dependent protein kinase II). The C-terminal region (61-200 aa) has a phospho-tyrosine binding domain (PTB domain) that includes the binding site for the more distal NPxY motif of the β_1 integrin cytoplasmic tail (Chang et al., 2002). Using site directed mutagenesis and protein structure modeling, a number of key amino-acid residues on both β_1 integrin and ICAP-1 were proposed to be required for ICAP-1 integrin interaction. For instance, a valine in the -5 position relative to the NPxY motif of the integrin tail was shown to be crucial for ICAP-1 binding to β_1 and may restrict the specificity of ICAP-1 to the cytosolic tails of β_1.

Functions of ICAP-1

It was postulated that ICAP-1 is a phosphoprotein that undergoes increase in phosphorylation upon cell adhesion on β_1 dependent ligands such as fibronectin (Zhang and Hemler, 1999). The phosphorylation sites, however, were not mapped in this study. ICAP-1 is a critical regulator of cell spreading on fibronectin (Bouvard and Block, 1998), and its over-expression increases cell migration in a modified Boyden chamber assay (Zhang and Hemler, 1999). By mutating the calcium and calmodulin dependent protein kinase II (CaMKII) consensus site located on the threonine 38 on ICAP-1 we were able to interfere with the capability of ICAP-1 to modulate cell spreading. Indeed, the expression of a phospho-mimetic mutant form of ICAP-1 (T38D) dramatically impairs cell spreading on fibronectin, while a non-

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phosphorylable form (T38A) displays an increased capability for spreading (Bouvard and Block, 1998). Thus, ICAP-1 function is modulated, at least in part by phosphorylation, and probably CaMKII, which has previously been shown to regulate integrin activation, is probably involved in this pathway (Bouvard et al., 1998).

ICAP-1 is never found in focal adhesions where integrins are clustered. This suggests that ICAP-1 acts differently compared to most of the other known integrin partners. Indeed over-expression of ICAP-1 in cells by transfection or micro-injection of the purified protein, leads to a disorganization or disruption of focal adhesion structures resulting often in the rounding up and detachment of the cells (Figure 2). Consistently, it was shown that ICAP-1 can inhibit talin binding to the β_1 cytoplasmic domain. Therefore one could speculate that ICAP-1 can disorganize focal adhesions and ultimately cell attachment by interfering with the binding of talin to the β_1 cytoplasmic domain (Bouvard et al., 2003).

ICAP-1 and regulation of integrin affinity

It was suggested that integrin dimer containing β_1 integrins are able to increase their affinity towards their extracellular ligands similarly to the platelet integrin $\alpha_{IIB}\beta_{3,}$ although direct demonstration of this has only recently been shown. Indeed, structural analysis of $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins either by crystallography or electronic microscopy analysis has visualized such a conformational change (Takagi et al., 2003; Vinogradova et al., 2002). According to theses analyses, integrin can shift from an inactive bent conformation to an active extended one. The molecular switch was suspected to be triggered from the cytosol due to an interaction of the cytosolic receptor tail with some effector. This inside-out signaling model was confirmed by Mark Ginsberg's laboratory after the finding that the head of talin acts as an activator of integrin function (Tadokoro et al., 2003). Talin is thus able to induce an extracellular conformational change of integrins that results in an increase in affinity. Once ICAP-1 is

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bound to the β_1 cytoplasmic domain, talin no longer binds. Therefore, by inhibiting the talin/integrin interaction, ICAP-1 may control or reverse the activated conformation of β_1 integrins and regulate integrin binding of talin. This property could explain why overexpression of ICAP-1 reduces cell spreading and disorganized focal adhesions (Bouvard et al., 2003). More recently we observed that, on ICAP-1 null cells, fibronectin receptors are in an active conformation, even when cells are in suspension, again emphasizing the role of ICAP-1 in the control of integrin activity. Lack of ICAP-1 expression results in an increase in β_1 dependent cell adhesion without any significant change in β_1 integrin surface expression (Bouvard et al., submitted). Analysis of ICAP-1 null cells also reveals an abnormal focal adhesion distribution pattern. However no direct evidence has yet been provided to determine whether this latter observation is a result of an increased β1 integrin activity (Bouvard et al., submitted).

ICAP-1 partners

Beside β_1 integrin, other partners of ICAP-1 have been identified, mainly by two hybrid screens. Using Krit-1 (K-rev interaction trapped 1) as a bait ICAP-1 has been identified by two independent laboratories (Zawistowski et al., 2002; Zhang et al., 2001). Krit-1 is a protein of 736 amino acid residues length originally described as a Rap1a binding protein. It contains several well-characterized structural motifs such as three ankyrin repeats, a 4.1, ezrin, radixin, moesin (FERM) domain, a NPxY motif involved in ICAP-1 binding and a putative Rap1A binding site. Although little is known regarding the function of Krit-1, mutation in the human *krit-1* gene is linked to a genetic disease that is characterized by cerebral cavernous malformations (CCM). Mice lacking Krit-1 die at mid-gestation from vascular defects, and mice heterozygous for *krit-1* in a *p53* null background develop a disease mimicking the human CCM pathology (Plummer et al., 2004; Whitehead et al., 2004). Immunofluorescence

studies have localized Krit-1 at microtubule tips (Gunel et al., 2002). How Krit-1 is acting at the cellular level is still puzzling, but, since the interaction of ICAP-1 with either β_1 integrin or Krit-1 is mutually exclusive, Krit-1 might locally regulate ICAP-1 binding to β_1 integrin, or, alternatively Krit-1 might deliver ICAP-1 to the vicinity of β_1 integrin via microtubules in order to destabilize focal adhesions (Small and Kaverina, 2003). Recently, malcavernin (CCM-2) has been identified as a novel partner for Krit-1. Malcavernin has a PTB domain similarly to ICAP-1. Based on transfection experiments a model has been proposed in which ICAP-1 could redirect the localization of Krit-1 to the nucleus, whereas CCM-2 would favor its cytoplasmic localization (Zawistowski et al., 2005). The situation is still unresolved and likely more complex since ICAP-1 mice do not suffer of any obvious vascular phenotype such as it could be observed in *krit-1* deficient mice, suggesting that either ICAP-1 or Krit-1 has additional, yet unidentified, function. Alternatively, it remains to be determined whether or not some protein could be functionally redundant with ICAP-1 or Krit-1 that would explain these phenotypical differences.

ICAP-1 also interacts with the tumor suppressor Nm23-H2 (Fournier et al., 2002). Nm23 belongs to a protein family made up of 8 members in human (from H1 to H8) (Lacombe et al., 2000). The protein has a nucleoside diphosphate (NDP) kinase activity and might thereby provide NTP to NTP-dependent proteins such as dynamin or other small GTPases such as Rac1, Cdc42 and RhoA known to regulate cell migration and spreading (Raftopoulou and Hall, 2004; Ridley, 2001; Ridley et al., 2003). Interestingly, Nm23-H1 is able to bind Tiam1, a guanosine exchange factor (GEF) for Rac1, while Nm23-H2 interacts with Lcb which is a GEF for Rho. As for Tiam1 and Nm23-H1, interaction of Nm23-H2 with Lcb inhibits RhoA activation by an unknown mechanism (Iwashita et al., 2004; Otsuki et al., 2001). ICAP-1 and Nm23-H2 colocalize with β_1 integrin in cell ruffles in early stages of cells spreading. ICAP-1

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may therefore be required for localizing Nm23 within the cell or for targeting Nm23 to the cell edge (Fournier et al., 2002).

It has also been shown using pull down experiments that ICAP-1 interacts directly with the small GTPases Rac and Cdc42. From this work it was speculated that ICAP-1 could act as a RhoGDI (Rho GDP dissociating inhibitor) for Rac and Cdc42 but not for RhoA thereby sequestering the inactive form of those small GTPases (Degani et al., 2002). This inhibitory function could explain in part why ICAP-1 affects cell spreading when overexpressed. However, considering that Rho GDI proteins typically have an immunoglobin domain, this view is not favored by structural modeling, which strongly suggests that ICAP-1 folds into a PTB domain. Further work needs to be done to elucidate this apparent contradiction.

ICAP-1 and cell proliferation

Analysis of *Icap-1*$^{-/-}$ mice and cells has revealed that cell proliferation is reduced in the absence of ICAP-1 (Bouvard et al., submitted; Fournier et al., 2005). A functional nuclear localization sequence (NLS) has been reported on ICAP-1 and its deletion impairs nuclear localization of ICAP-1. Furthermore, cell proliferation is dependent on this NLS, since its deletion abolishes the capability of ICAP-1 to stimulate cell proliferation under low serum culture conditions. Translocation of ICAP-1 into the nucleus and the related cell proliferation clearly depend on engagement of β_1 integrin to the extracellular matrix, since ICAP-1 is redirected to the nucleus when the cell is spread and the overexpression of β_1 integrin inhibits nuclear localization of ICAP-1. How ICAP-1 stimulates proliferation needs further investigation, but Nm23-H2 may be involved since both it and ICAP-1 are able to stimulate the c-Myc promoter (Fournier et al., 2005).

Expression and gene structure of ICAP-1

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Northern blot analyses revealed that ICAP-1 is ubiquitously expressed in human. Using a mouse gene trapped line, Gruss's laboratory reported ICAP-1 expression during embryogenesis and in adult brain (Faisst and Gruss, 1998). Recently, we generated a mouse line where the *Icap-1* locus was targeted by homologous recombination (Bouvard et al., submitted). We inserted the *lacZ* gene to visualize the *in vivo* expression pattern during development and in adult mice. As it was previously reported, we also noticed a strong expression of ICAP-1 in adult brain, and more specifically in some areas such as the cerebellum, the smooth muscle cells of the intestine and in the testis. During embryogenesis ICAP-1 expression was first detected at embryonic day 8.5 (E8.5), where expression was restricted to the neural lips and the developing heart, while the caudal region of the embryo was negative for β-galactosidase staining. We observed increased staining as the embryo aged, and ICAP-1 appears to become ubiquitously expressed although the expression level varies depending on the cell type and the tissue.

In humans, it was reported that *Icap-1* is transcribed in two messages due to alternative splicing. By analysis of the genomic structure of *Icap-1* gene, we identified the exon 6 as the region that is spliced out and gives rise to ICAP-1β (Chang et al., 1997). ICAP-1β is a shorter form lacking an internal 50 amino acids (encompassing aa 127-177), which is unable to interact with the β_1 integrin (Figure 1). The function of this isoform is still unclear. In humans, the gene structure is slightly different when compared with the mouse counter-part in that we found an additional polyadenylation site in humans that may explain the presence of two bands in northernblot analysis whereas only a single band is seen in mouse tissue. However, in rodents, computer searches did not reveal any EST corresponding to this isoform which make it doubtful that the ICAP-1β protein is expressed in mice (Bouvard and Fassler, unpublished). *Icap-1* gene is located on chromosome 2 and chromosome 12 in human and mouse respectively.

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ICAP-1 deficient mice

Recently we generated a mouse line deficient in ICAP-1 expression by gene targeting. Surprisingly we did not observe any embryonic lethality in mutant animals. However, about 20 % of the mutant population was missing around birth. This partial perinatal lethality could be caused by an absence of feeding since we often observed mutant new born mice without milk within their stomach. Those pups will die rapidly within the first couple of days after birth and will be eaten by the mothers (Bouvard et al., submitted). Mutant mice display several phenotypes such as a moderate dwarfism and a severe craniofacial dysmorphism due to altered osteoblast function and severe neurological deficit with reduced overall activity and ataxia (Figure 3). While the mouse is aging, its appearance degenerates and finally the mouse is often moribund and dies prematurely (Bouvard, personal observations).

Interestingly most of the mice that have been targeted for β_1 integrin binding proteins exhibit a much more severe phenotype characterized by mostly peri-implantation lethality suggesting an important requirement of integrin at this developmental step. This difference suggests a particular and subtle role of ICAP-1 in β_1 integrin regulation, since no obvious homologous protein has been identified that could be redundant.

Conclusion

ICAP-1 is the first protein found to directly inhibit β_1 integrin function by interfering with the binding of other integrin partners. Furthermore, it could act as a messenger that relays information to the nucleus for controlling gene expression and cell proliferation in a β_1-integrin-dependent manner. Moreover, ICAP-1 deficient mice have a specific phenotype that largely differs from other related β_1 integrin binding proteins. Altogether, these results suggest that ICAP-1 constitutes a novel type of integrin binding protein, which could be

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involved in the fine tuning of integrin function and/or in novel aspects of integrin signaling pathways. One of them is being unraveled with the identification of the ICAP-1 partner Krit-1. A more detailed analysis of ICAP-1 deficient mice and cells will provide a significant input to understanding ICAP-1's roles during development and pathogenesis. Clearly, a lot of work is still to be done to elucidate this intriguing multifunctional protein, and one could speculate that many more surprises will come in the future. Indeed, while the direct binding of ICAP-1 onto the β_1 integrin cytoplasmic domain negatively regulates its adhesive function, ICAP-1 by binding to other proteins could also modulate additional signaling pathways (Figure 4).

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References:

Bouvard, D., Block, M. R. 1998. Calcium/calmodulin-dependent protein kinase II controls integrin alpha5beta1-mediated cell adhesion through the integrin cytoplasmic domain associated protein-1alpha. Biochem. Biophys. Res. Commun. 252, 46-50.
Bouvard, D., Brakebusch, C., Gustafsson, E., Aszodi, A., Bengtsson, T., Berna, A., Fassler, R. 2001. Functional consequences of integrin gene mutations in mice. Circ. Res. 89, 211-223.
Bouvard, D., Molla, A., Block, M. R. 1998. Calcium/calmodulin-dependent protein kinase II controls alpha5beta1 integrin-mediated inside-out signaling. J. Cell Sci. 111 (Pt 5), 657-665.
Bouvard, D., Vignoud, L., Dupe-Manet, S., Abed, N., Fournier, H. N., Vincent-Monegat, C., Retta, S. F., Fassler, R., Block, M. R. 2003. Disruption of focal adhesions by integrin cytoplasmic domain-associated protein-1 alpha. J. Biol. Chem. 278, 6567-6574.
Brakebusch, C., Bouvard, D., Stanchi, F., Sakai, T., Fassler, R. 2002. Integrins in invasive growth. J. Clin. Invest. 109, 999-1006.
Brakebusch, C., Fassler, R. 2003. The integrin-actin connection, an eternal love affair. Embo J. 22, 2324-2333.
Calderwood, D. A. 2004. Integrin activation. J. Cell. Sci. 117, 657-666.
Chang, D. D., Hoang, B. Q., Liu, J., Springer, T. A. 2002. Molecular basis for interaction between Icap1 alpha PTB domain and beta 1 integrin. J. Biol. Chem. 277, 8140-8145.
Chang, D. D., Wong, C., Smith, H., Liu, J. 1997. ICAP-1, a novel beta1 integrin cytoplasmic domain-associated protein, binds to a conserved and functionally important NPXY sequence motif of beta1 integrin. J. Cell Biol. 138, 1149-1157.
Degani, S., Balzac, F., Brancaccio, M., Guazzone, S., Retta, S. F., Silengo, L., Eva, A., Tarone, G. 2002. The integrin cytoplasmic domain-associated protein ICAP-1 binds and regulates Rho family GTPases during cell spreading. J. Cell Biol. 156, 377-387.
Faisst, A. M., Gruss, P. 1998. Bodenin: a novel murine gene expressed in restricted areas of the brain. Dev. Dyn. 212, 293-303.
Fournier, H. N., Dupe-Manet, S., Bouvard, D., Lacombe, M. L., Marie, C., Block, M. R., Albiges-Rizo, C. 2002. Integrin cytoplasmic domain-associated protein 1alpha (ICAP-1alpha) interacts directly with the metastasis suppressor nm23-H2, and both proteins are targeted to newly formed cell adhesion sites upon integrin engagement. J. Biol. Chem. 277, 20895-20902.
Fournier, H. N., Dupe-Manet, S., Bouvard, D., Luton, F., Degani, S., Block, M. R., Retta, S. F., Albiges-Rizo, C. 2005. Nuclear translocation of integrin cytoplasmic domain-associated protein 1 stimulates cellular proliferation. Mol. Biol. Cell 16, 1859-1871.
Giancotti, F. G., Ruoslahti, E. 1999. Integrin signaling. Science 285, 1028-1032.
Ginsberg, M. H., Partridge, A., Shattil, S. J. 2005. Integrin regulation. Curr. Opin. Cell Biol. 17, 509-516.
Gunel, M., Laurans, M. S., Shin, D., DiLuna, M. L., Voorhees, J., Choate, K., Nelson-Williams, C., Lifton, R. P. 2002. KRIT1, a gene mutated in cerebral cavernous malformation, encodes a microtubule-associated protein. Proc. Natl. Acad. Sci. U S A 99, 10677-10682.
Hynes, R. O. 2002. Integrins: bidirectional, allosteric signaling machines. Cell 110, 673-687.
Iwashita, S., Fujii, M., Mukai, H., Ono, Y., Miyamoto, M. 2004. Lbc proto-oncogene product binds to and could be negatively regulated by metastasis suppressor nm23-H2. Biochem. Biophys. Res. Commun. 320, 1063-1068.
Keely, P., Parise, L., Juliano, R. 1998. Integrins and GTPases in tumour cell growth, motility and invasion. Trends Cell Biol. 8, 101-106.

HAL author manuscript inserm-00166352, version 1

Lacombe, M. L., Milon, L., Munier, A., Mehus, J. G., Lambeth, D. O. 2000. The human Nm23/nucleoside diphosphate kinases. J. Bioenerg. Biomembr. 32, 247-258.
Liddington, R. C., Ginsberg, M. H. 2002. Integrin activation takes shape. J. Cell Biol. 158, 833-839.
Martel, V., Racaud-Sultan, C., Dupe, S., Marie, C., Paulhe, F., Galmiche, A., Block, M. R., Albiges-Rizo, C. 2001. Conformation, localization, and integrin binding of talin depend on its interaction with phosphoinositides. J. Biol. Chem. 276, 21217-21227.
Otsuki, Y., Tanaka, M., Yoshii, S., Kawazoe, N., Nakaya, K., Sugimura, H. 2001. Tumor metastasis suppressor nm23H1 regulates Rac1 GTPase by interaction with Tiam1. Proc. Natl. Acad. Sci. U S A 98, 4385-4390.
Plow, E. F., Haas, T. A., Zhang, L., Loftus, J., Smith, J. W. 2000. Ligand binding to integrins. J. Biol. Chem. 275, 21785-21788.
Plummer, N. W., Gallione, C. J., Srinivasan, S., Zawistowski, J. S., Louis, D. N., Marchuk, D. A. 2004. Loss of p53 sensitizes mice with a mutation in Ccm1 (KRIT1) to development of cerebral vascular malformations. Am. J. Pathol. 165, 1509-1518.
Raftopoulou, M., Hall, A. 2004. Cell migration: Rho GTPases lead the way. Dev. Biol. 265, 23-32.
Ridley, A. J. 2001. Rho family proteins: coordinating cell responses. Trends Cell Biol. 11, 471-477.
Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T., Horwitz, A. R. 2003. Cell migration: integrating signals from front to back. Science 302, 1704-1709.
Small, J. V., Kaverina, I. 2003. Microtubules meet substrate adhesions to arrange cell polarity. Curr. Opin. Cell Biol. 15, 40-47.
Tadokoro, S., Shattil, S. J., Eto, K., Tai, V., Liddington, R. C., de Pereda, J. M., Ginsberg, M. H., Calderwood, D. A. 2003. Talin binding to integrin beta tails: a final common step in integrin activation. Science 302, 103-106.
Takagi, J., Strokovich, K., Springer, T. A., Walz, T. 2003. Structure of integrin alpha5beta1 in complex with fibronectin. Embo J. 22, 4607-4615.
Vinogradova, O., Velyvis, A., Velyviene, A., Hu, B., Haas, T., Plow, E., Qin, J. 2002. A structural mechanism of integrin alpha(IIb)beta(3) "inside-out" activation as regulated by its cytoplasmic face. Cell 110, 587-597.
Whitehead, K. J., Plummer, N. W., Adams, J. A., Marchuk, D. A., Li, D. Y. 2004. Ccm1 is required for arterial morphogenesis: implications for the etiology of human cavernous malformations. Development 131, 1437-1448.
Zawistowski, J. S., Serebriiskii, I. G., Lee, M. F., Golemis, E. A., Marchuk, D. A. 2002. KRIT1 association with the integrin-binding protein ICAP-1: a new direction in the elucidation of cerebral cavernous malformations (CCM1) pathogenesis. Hum. Mol.Genet. 11, 389-396.
Zawistowski, J. S., Stalheim, L., Uhlik, M. T., Abell, A. N., Ancrile, B. B., Johnson, G. L., Marchuk, D. A. 2005. CCM1 and CCM2 protein interactions in cell signaling: implications for cerebral cavernous malformations pathogenesis. Hum. Mol. Genet. 14, 2521-2531.
Zhang, J., Clatterbuck, R. E., Rigamonti, D., Chang, D. D., Dietz, H. C. 2001. Interaction between krit1 and icap1alpha infers perturbation of integrin beta1-mediated angiogenesis in the pathogenesis of cerebral cavernous malformation. Hum. Mol. Genet. *10*, 2953-2960.
Zhang, X. A., Hemler, M. E. 1999. Interaction of the integrin beta1 cytoplasmic domain with ICAP-1 protein. J. Biol. Chem. *274*, 11-19.

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Figures legends

Figure 1 :
Schematic structure of ICAP-1α and ICAP-1β protein. ICAP-1α is a protein of 200 amino acids and is characterized by the presence of a 60 amino acid rich in serine and threonine at the N terminal part of the protein. The remaining part (60 to 200) folds into a PTB domain that interacts with both the β_1 integrin cytoplasmic domain and Krit1. ICAP-1β is a smaller protein where an internal part (127 to 177) is spliced out. This region, called here the integrin binding domain, is important for interaction with β_1 integrin.

Figure 2 :
ICAP-1 dissociates focal adhesions. (A) Overexpression of an EGFP-ICAP fusion protein in fibroblast leads to a strong reduction in vinculin staining. Vinculin is stained in red using a monoclonal antibody and ICAP-1 appears in green by fluorescence of the EGFP. Arrows indicate a strong vinculin positive focal adhesion in non transfected cells, while arrow heads indicate very faint staining of vinculin in EGFP-ICAP-1 transfected cells. Bar is 10 µm. (B-C) Higher magnification view focusing on the focal adhesion structure. Note that in ICAP-1transfected cell (B) vinculin staining is less intense than in non transfected cells (B). Bar is 10 µm.

Figures 3 :
General aspect of a 30-day old wild-type and *Icap-1*$^{-/-}$ mouse. Both mice are male and come from the same litter. Note that the *Icap-1* deficient mouse is smaller and a cranio-facial abnormality is evident compared to the wild-type animal.

Figure 4 :
Schematic drawing of ICAP-1 functions. ICAP-1 is likely phosphorylated by protein kinases such as CaMKII. This phosphorylation would increase its capability to interact with the β_1 integrin cytoplasmic domain, thus preventing the binding of talin. This would lead to an overall reduction in the activation state of the integrin. ICAP-1 also binds to other proteins such as Krit1, Nm-23 or CDC42, those interactions would modulate specific signaling pathway, such as the recruitment of Krit-1 into the nucleus, modulation of the activity of small GTPases like Cdc42, or localization of Nm23-H2.

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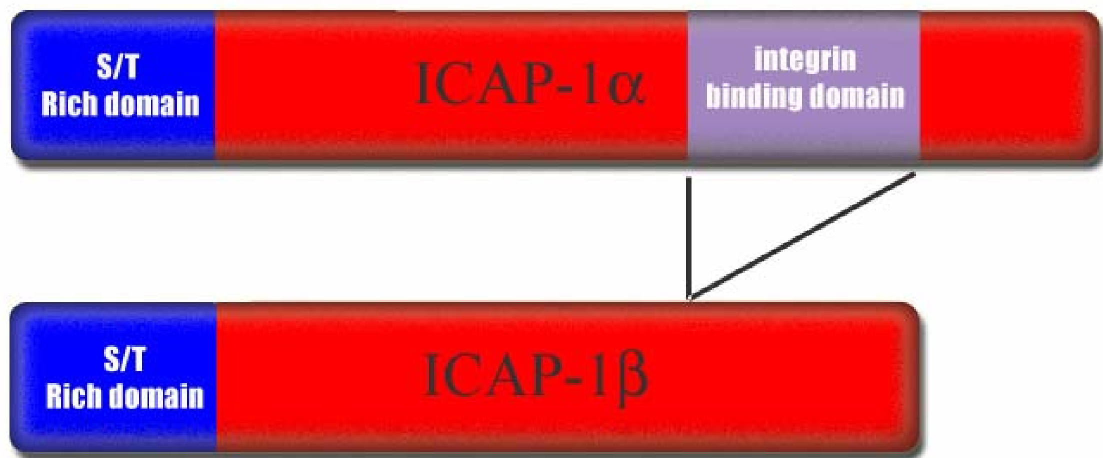

Figure 1

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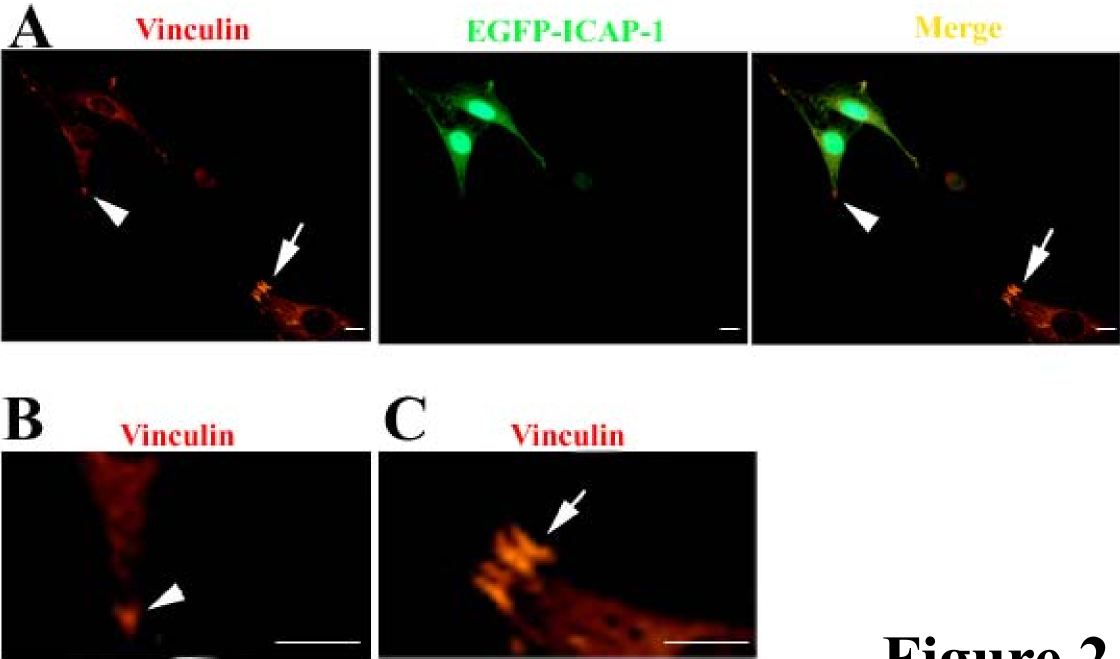

Figure 2

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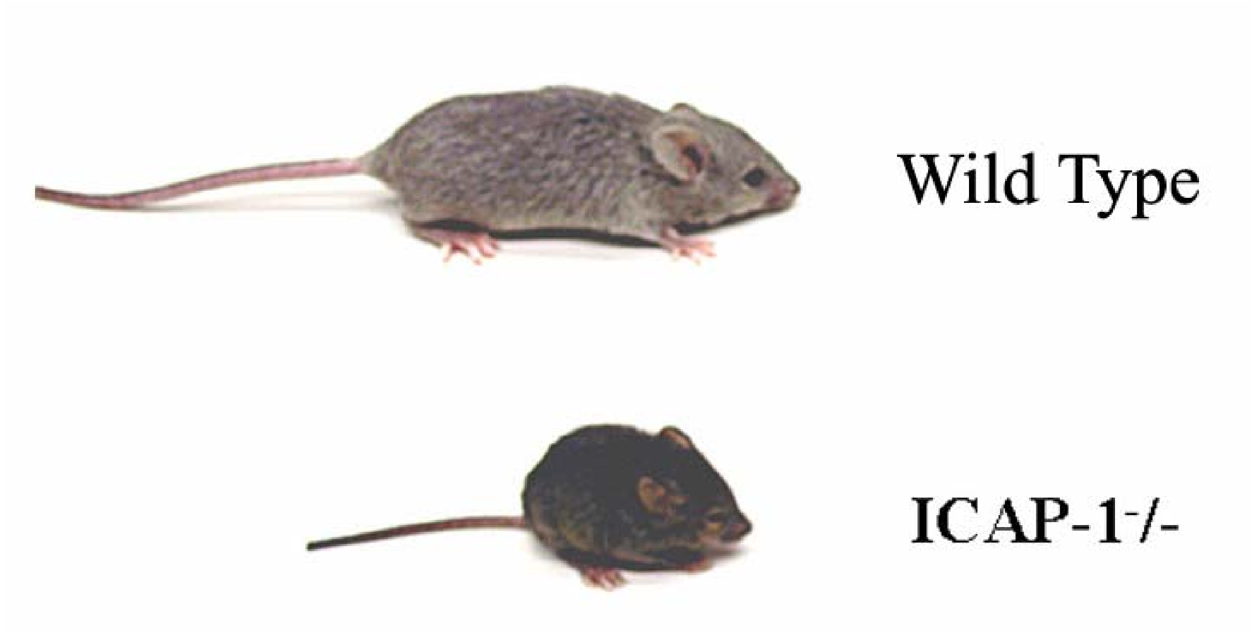

Figure 3

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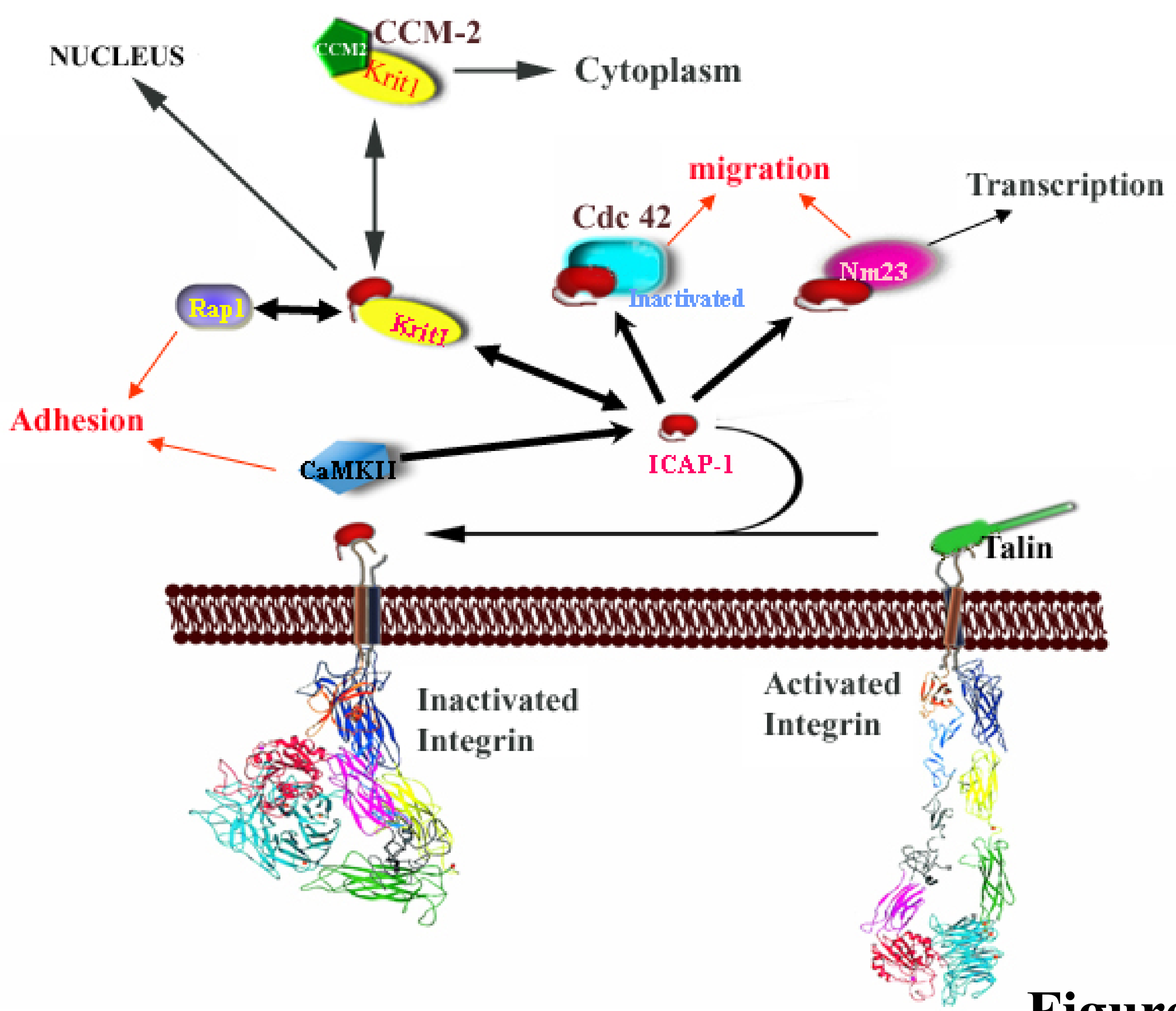

Figure 4