sgRNA synthesis (Fig. 3, A and B). During the early phase of replication, in the absence of high levels of RNA-2, the replicase produces full-length complementary strand copies of RNA-1. Later, during production of progeny RNAs, RNA-2 becomes abundant. The trans-activator element on RNA-2 binds to the 8-nt element on RNA-1, possibly mediated by interactions with viral or host proteins (or both), to produce a structure that sterically prevents the replicase from completing synthesis of full-length complementary strands of RNA-1. Because the 5′-terminal sequences of the genomic RNA-1 and the sgRNA are highly conserved (Fig. 3A), this truncated complementary strand RNA may serve as a template for replication to produce the genomic-sense sgRNA, which is the translational template for CP. The presence of a double-stranded RNA species in RCNMV-infected tissue, corresponding in size to this replicative intermediate, supports this model (*5*, *20*). This mechanism for controlling the temporal expression of CP may have evolved to ensure that sufficient quantities of RNA-1 and RNA-2 are present before the commencement of virion assembly. This model for sgRNA synthesis differs from that proposed for most RNA plant viruses, as exemplified by brome mosaic virus (BMV). BMV sgRNA synthesis is initiated by binding of the viral polymerase complex to an internal sequence on the negative strand template followed by transcription of positive stranded mRNA for the CP (*23*). Our model is distinct because promoter specificity is not based solely on protein-RNA interactions but more on an 8-nt RNA-RNA interaction that occurs, probably with the aid of proteins, between two genomic-sense RNAs.

References and Notes

1. L. Jaeger, *Curr. Opin. Struct. Biol.* **7**, 324 (1997); B. Zhang and T. R. Cech, *Nature* **390**, 96 (1997).
2. S. A. Liebhaber, *Nucleic Acids Symp. Ser.* **36**, 29 (1997); D. R. Gallie, *Plant Mol. Biol.* **32**, 145 (1996).
3. M. D. Crespi *et al.*, *EMBO J.* **13**, 5099 (1994); C. J. Decker and R. Parker, *Curr. Opin. Cell Biol.* **7**, 386 (1995); E. R. Jupe *et al.*, *Cell Growth Differ.* **7**, 871 (1996).
4. R. C. Lee, R. L. Feinbaum, V. Ambros, *Cell* **75**, 843 (1993); B. Wightman, I. Ha, G. Ruvkin, *ibid.*, p. 855.
5. D. Giesman-Cookmeyer, K.-H. Kim, S. A. Lommel, in *Pathogenesis and Host-Parasite Specificity in Plant Diseases: Histopathological, Biochemical, Genetic and Molecular Basis*, U. S. Singh, R. P. Singh, K. Kohmoto, Eds. (Pergamon, Tarrytown, NY, 1995), vol. III, pp. 157–176.
6. M. Kozak, *J. Cell Biol.* **108**, 229 (1989).
7. A. A. Vaewhongs and S. A. Lommel, *Virology* **212**, 607 (1995).
8. Z. Xiong and S. A. Lommel, *ibid.* **182**, 388 (1991).
9. W. L. Chiu *et al.*, *Curr. Biol.* **6**, 325 (1996).
10. The sGFP sequence was amplified by polymerase chain reaction (PCR) from plasmid blue-sGFP-TYG-nos KS. The product was cleaved with Cla I–Mlu I and ligated into the infectious cDNA clone of RCNMV RNA-1 to produce clone R1sGFP. Infectious T7 RNA polymerase transcripts were synthesized in vitro and mechanically inoculated onto tobacco plants. Plants were inspected for sGFP expression 1 to 3 days after inoculation. All inoculations were repeated a minimum of three times on both *N. benthamiana* and *N. clevelandii* with at least two independent constructs. Experiments specific for MP^+ plants were repeated a minimum of three times with at least two independent constructs. All plants were maintained in a 20°C glasshouse with ambient lighting.
11. T. L. Kendall and S. A. Lommel, *J. Gen. Virol.* **73**, 2479 (1992).
12. T. L. Sit and S. A. Lommel, unpublished data.
13. Z. Ge, C. Hiruki, K. L. Roy, *J. Gen. Virol.* **73**, 2483 (1992); Z. Ge and C. Hiruki, *Proc. Jpn. Acad.* **69**, 113 (1993).
14. The wild-type GFP sequence in plasmid pGFP was cleaved with Age I, treated with Klenow fragment, and cleaved with Spe I. This GFP fragment was inserted into the infectious cDNA clone of RCNMV RNA-2 that had been cleaved with Nco I, treated with Klenow fragment, and cleaved with Xba I to produce clone R2GFP. Clone R2GΔH was produced by cleavage of R2GFP with Hpa I (within the GFP coding sequence) and Hind III (within the multiple cloning site of the plasmid) followed by insertion of the ApaL I–Hind III fragment from wild-type RCNMV RNA-2. Clone R2GΔHX was produced by cleavage of clone R2GΔH with Bgl II–Xba I followed by Klenow treatment and religation to yield a 120-nt deletion.
15. Plasmid pHST2. H. B. Scholthof, K.-B. G. Scholthof, A. O. Jackson, *Annu. Rev. Phytopathol.* **34**, 299 (1996).
16. The RNA-2 fragment was excised from the infectious cDNA clone with ApaL I–Xba I followed by Klenow treatment and ligation into the SnaB I site of pHST2 to yield clones pHST2-RC2.3 (+) and −RC2.4 (−). The 209-nt RNA-2 insert was produced by digestion of pHST2-RC2.3 with Bgl II–Xba I followed by Klenow treatment and religation to yield clone pHST2-ΔBX.
17. RNA secondary structure was predicted with M. Zuker's mfold server at http://www.ibc.wustl.edu/~zuker/rna/
18. The 5′-terminal 121-nt and 3′-terminal 91-nt regions of the 209-nt element were amplified by PCR. The products were cleaved with SnaB I–Xho I and ligated into similarly cleaved pHST2.
19. RCNMV RNA-2 inserts of 50 nt or less in pHST2 were produced by annealing of phosphorylated complementary oligonucleotides followed by ligation of the resulting double-stranded DNAs into pHST2 cleaved with SnaB I–Xho I.
20. S. K. Zavriev, C. M. Hickey, S. A. Lommel, *Virology* **216**, 407 (1996).
21. PCR-based mutagenesis of the RNA-1 sequence was performed as by S. Li and M. F. Wilkinson [*Biotechniques* **23**, 588 (1997)]. After amplification, the PCR product was gel-purified, cleaved with Xho I–Bgl II, and ligated into similarly cleaved R1sGFP to generate clone R1sGFP-M.
22. W. A. Miller, C. M. Brown, S. Wang, *Semin. Virol.* **8**, 3 (1997); W. A. Miller, personal communication.
23. W. A. Miller, T. W. Dreher, T. C. Hall, *Nature* **313**, 68 (1985); R. W. Siegel, S. Adkins, C. C. Kao, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11238 (1997).

24. We thank D. Cookmeyer, T. Petty, and M. Conkling for reviewing this manuscript; J. Sheen for the sGFP and a GFP plasmid; G. Payne for a GFP plasmid; T. Okuno for the SCNMV RNA-2 cDNA clone; and H. Scholthof for the TBSV pHST2 vector. Supported in part by NSF grant MCB-9419700 to S.A.L.

12 March 1998; accepted 25 June 1998

Role of IQGAP1, a Target of the Small GTPases Cdc42 and Rac1, in Regulation of E-Cadherin–Mediated Cell-Cell Adhesion

Shinya Kuroda, Masaki Fukata, Masato Nakagawa, Katsuhiko Fujii, Tomoko Nakamura, Tadashi Ookubo, Ichiro Izawa, Takahiro Nagase, Nobuo Nomura, Hideki Tani, Ikuo Shoji, Yoshiharu Matsuura, Shin Yonehara, Kozo Kaibuchi*

The small guanosine triphosphatases (GTPases) Cdc42 and Rac1 regulate E-cadherin–mediated cell-cell adhesion. IQGAP1, a target of Cdc42 and Rac1, was localized with E-cadherin and β-catenin at sites of cell-cell contact in mouse L fibroblasts expressing E-cadherin (EL cells), and interacted with E-cadherin and β-catenin both in vivo and in vitro. IQGAP1 induced the dissociation of α-catenin from a cadherin-catenin complex in vitro and in vivo. Overexpression of IQGAP1 in EL cells, but not in L cells expressing an E-cadherin–α-catenin chimeric protein, resulted in a decrease in E-cadherin–mediated cell-cell adhesive activity. Thus, IQGAP1, acting downstream of Cdc42 and Rac1, appears to regulate cell-cell adhesion through the cadherin-catenin pathway.

Dynamic rearrangement of cell-cell adhesion is a critical step for various cellular processes, including establishment of epithelial cell polarity and developmental patterning (*1*). Cell-cell adhesion mediated by a cadherin-catenin complex participates in the initial stages of association of polarized cells (*2*, *3*). Cadherins are Ca^{2+}-dependent adhesion molecules, and catenins such as α- and β-catenins are cadherin-associated cytoplasmic proteins that are required for cadherin-mediated cell-cell adhesion. The small GTPases Cdc42 and Rac1 regulate cellular properties such as cell shape, cell growth, and cell polarity (*4*). Rac1 (*5*) and Cdc42 (*6*), together with their exchange factor Tiam-1 (*7*), also regulate cadherin-mediated cell-cell adhesion. We now present evidence that IQGAP1 mediates these effects.

IQGAP1, a target of Cdc42 and Rac1 (*8, 9*), is localized at sites of cell-cell contact in Madin-Darby canine kidney (MDCK) epithelial cells, where α-catenin is also localized (*9*). We therefore examined whether IQGAP1 contributes to cadherin-mediated cell-cell adhesion. IQGAP1 accumulated at sites of cell-cell contact in mouse L cells stably expressing E-cadherin (EL cells) (*3, 10*) (Fig. 1A), but it did not accumulate at such sites in L cells or in L cells stably expressing an E-cadherin mutant in which the cytoplasmic domain was deleted and replaced by the COOH-terminal domain of α-catenin (nEαCL cells). We confirmed that β-catenin did not accumulate at sites of cell-cell contact in nEαCL cells (*3*). These observations indicated that IQGAP1 accumulates at sites of cell-cell contact in a manner dependent on E-cadherin, β-catenin, or the NH_2-terminal domain of α-catenin.

Immunoprecipitation of IQGAP1 from EL cells resulted in the coprecipitation of β-catenin and E-cadherin (Fig. 1B) (*11, 12*). The stoichiometries of β-catenin and E-cadherin associated with immunoprecipitated IQGAP1 were ~0.2 and 0.03, respectively. Exposure of EL cells to a cross-linker, dithiobis(succinimidyl propionate) (DSP), before lysis resulted in an increase in the amounts of β-catenin and E-cadherin coprecipitated with IQGAP1, such that their stoichiometries relative to IQGAP1 became 1.0 and 0.4, respectively. These results suggested that IQGAP1 interacts simultaneously with β-catenin and E-cadherin. α-Catenin was not co-immunoprecipitated with IQGAP1 from EL cells that had been incubated in the absence or presence of DSP. Neither the E-cadherin mutant nor β-catenin was immunoprecipitated with IQGAP from nEαCL cells (*13*), possibly because of the small amounts of β-catenin present in these cells (*3*).

Maltose-binding protein (MBP)–E-cadherin (the cytoplasmic domain; amino acids 734 to 884), MBP–β-catenin, and MBP–α-catenin fusion proteins were individually incubated with beads coated with either glutathione S-transferase (GST) or a GST-IQGAP1 fusion protein (*14*). MBP–β-catenin and MBP–E-cadherin specifically interacted with GST-IQGAP1, whereas MBP–α-catenin did not (Fig. 2A). Binding of MBP–β-catenin and MBP–E-cadherin to GST-IQGAP1 was dose dependent and saturable; half-maximal binding was apparent at 20 and 400 nM, respectively. The stoichiometries of the β-catenin and E-cadherin bound to IQGAP1 were each ~1.0.

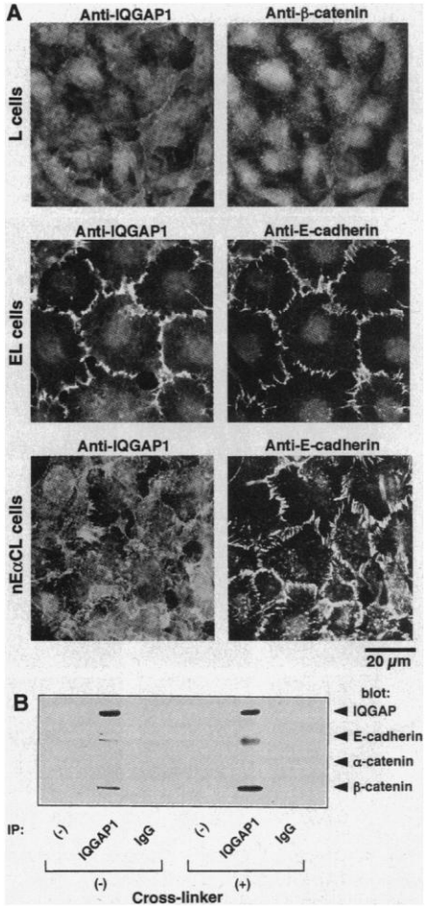

Fig. 1. Localization of IQGAP with E-cadherin at sites of cell-cell contact (A) and coimmunoprecipitation of β-catenin and E-cadherin with IQGAP1 (B). (**A**) Confluent L cells were fixed and doubly stained with antibodies to IQGAP1 (anti-IQGAP1) and anti–β-catenin, and confluent EL cells and nEαCL cells were fixed and doubly stained with anti-IQGAP1 and anti–E-cadherin (*10*). (**B**) Confluent EL cells were incubated in the absence or presence of DSP, and cell lysates were then subjected to immunoprecipitation (IP) with no antibody (−), with anti-IQGAP1, or with control immunoglobulin G (IgG) (*12*). The immunoprecipitates were separated by centrifugation and subjected to SDS-PAGE and immunoblot analysis with antibodies to the indicated proteins (*12*). Data are representative of three independent experiments.

IQGAP1 interacted with β-catenin, but not with α-catenin, under conditions in which E-cadherin interacted with both catenins (Fig. 2B) (*15*). IQGAP1 also inhibited the binding of α-catenin to immobilized β-catenin (*13*).

We next examined the interaction of fragments of IQGAP1 with β-catenin or E-cadherin (*16*). MBP–β-catenin interacted with GST-IQGAP1 (amino acids 1 to 1657) and to a lesser extent with GST–IQGAP1-N (amino acids 1 to 863) and GST–IQGAP1-C (amino acids 764 to 1657) (Fig. 3A). MBP–β-catenin did not interact with GST–IQGAP1-ΔC (amino acids 1 to 1504). MBP–E-cadherin also interacted with GST-IQGAP1 and to a lesser extent with GST–IQGAP1-N and GST–IQGAP1-C, but not with GST–IQGAP1-ΔC. The binding of IQGAP1-N or IQGAP1-C to β-catenin or E-cadherin was dose-dependent (*13*). These results suggest that the entire structure of IQGAP1 is important for high-affinity binding to β-catenin and E-cadherin. When expressed in amounts smaller

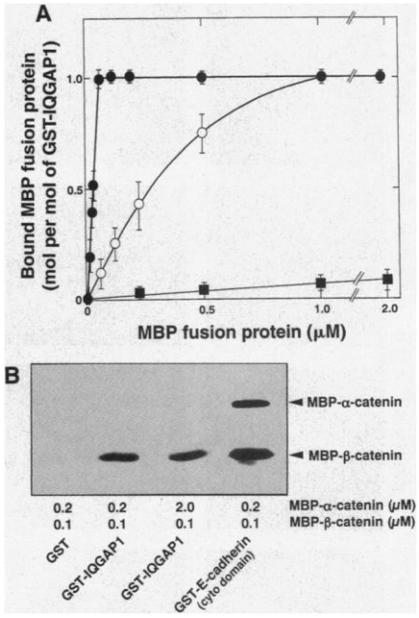

Fig. 2. Direct interaction of IQGAP1 with β-catenin and E-cadherin. (**A**) Saturable and stoichiometric interaction of MBP–β-catenin and MBP–E-cadherin with GST-IQGAP1. MBP–β-catenin (●), MBP–E-cadherin (○), or MBP–α-catenin (■) was incubated at the indicated concentrations with beads coated with GST-IQGAP1 (40 pmol), and their interactions with GST-IQGAP1 were examined (*14*). (**B**) Preferential binding of GST-IQGAP1 to MBP–β-catenin in the presence of MBP–α-catenin. MBP–β-catenin and MBP–α-catenin were mixed and incubated for 1 hour at 4°C. The mixture was then incubated with beads coated with GST, GST-IQGAP1, or GST–E-cadherin (cytoplasmic domain). Proteins eluted from the beads by glutathione were subjected to SDS-PAGE and immunoblot analysis with anti–β-catenin or anti–α-catenin (*15*). Data are representative of three independent experiments.

S. Kuroda, Division of Signal Transduction, Nara Institute of Science and Technology, Ikoma 630-0101, Japan, and Inheritance and Variation Group, PRESTO, Japan Science and Technology, Kyoto 619-0237, Japan. M. Fukata, Division of Signal Transduction, Nara Institute of Science and Technology, Ikoma 630-0101, Japan, and Department of Biochemistry, Hiroshima University School of Medicine, Hiroshima 734-8551, Japan. M. Nakagawa, K. Fujii, T. Nakamura, T. Ookubo, K. Kaibuchi, Division of Signal Transduction, Nara Institute of Science and Technology, Ikoma 630-0101, Japan. I. Izawa, Central Laboratories for Key Technology, Kirin Brewery, Yokohama 236-0004, Japan. T. Nagase and N. Nomura, Kazusa cDNA Research Group, Kazusa DNA Research Institute, Kisarazu 292-0812, Japan. H. Tani, I. Shoji, Y. Matsuura, Department of Virology II, National Institute of Infectious Diseases, Tokyo 162-0052, Japan. S. Yonehara, Institute for Virus Research, Kyoto University, Kyoto 606-8397, Japan.

*To whom correspondence should be addressed.

than that of the endogenous protein in EL cells, recombinant full-length IQGAP1, but none of the IQGAP1 mutants, accumulated at sites of cell-cell contact (Fig. 3B) (*17*), suggesting that the interaction of IQGAP1 with β-catenin or E-cadherin (or both) may be essential for its localization at these sites. IQGAP1-N was localized in the nucleus. Wild-type IQGAP1 and IQGAP1-C are able to interact with Cdc42 (*9, 13*).

To obtain an enriched population of EL cells expressing recombinant IQGAP1, we transfected EL cells with plasmids encoding wild-type or mutant IQGAP1 together with a plasmid encoding tAic2A, an interleukin-3β1 receptor lacking its cytoplasmic domain. The IQGAP1-expressing cells were collected immunologically with an antibody to Aic2 (*18, 19*). The amount of recombinant IQGAP1 expressed in these cells was two to three times that of endogenous IQGAP1 (*13*). In cell dissociation assays (*3, 19*), the IQGAP1-expressing cells were largely dissociated, with many single cells apparent, whereas control cells or IQGAP1-ΔC–expressing cells remained as aggregates (Fig. 4A). Thus, the adhesive activity of IQGAP1-expressing cells appeared reduced relative to that of control cells or of cells expressing IQGAP1-ΔC. Overexpression of neither IQGAP1-N nor IQGAP1-C altered the adhesive properties of EL cells (*13*). When these experiments were repeated with nEαCL cells, the IQGAP1-expressing cells showed an adhesive activity similar to that of control cells. Therefore, the effect of IQGAP1 on cell adhesion appeared to be mediated by β-catenin or E-cadherin.

When the dissociation assay was performed with EL cells expressing both IQGAP1 and $Cdc42^{Val12}$, a Cdc42 mutant that is defective in GTPase activity and is thought to exist constitutively in the GTP-bound form in cells, the cells did not dissociate, remaining as aggregates (Fig. 4B). Expression of IQGAPI with either $Cdc42^{Asn17}$, a mutant that preferentially binds GDP rather than GTP and is thought to exist constitutively in the GDP-bound form in cells, or $RhoA^{Val14}$ did not inhibit cell dissociation (*13*). Thus, Cdc42 inhibits IQGAP1

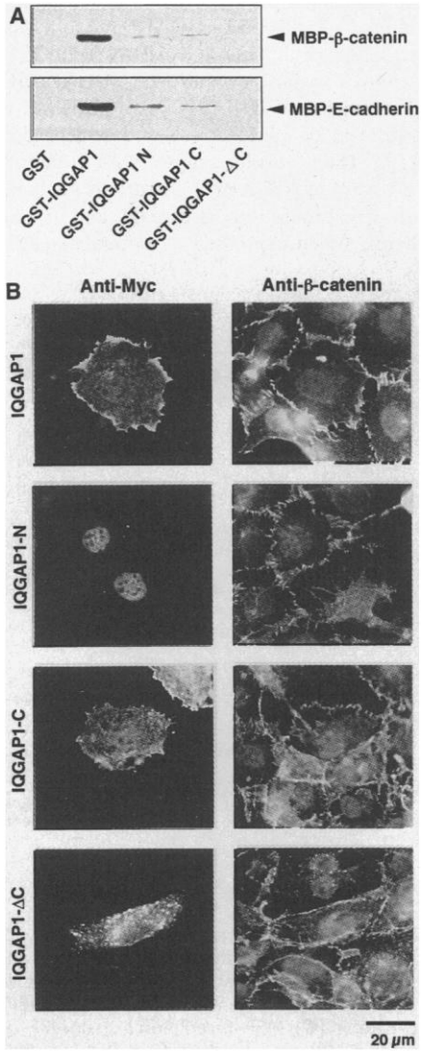

Fig. 3. Interaction of IQGAP1 mutants with β-catenin and E-cadherin (A) and their localization in EL cells (B). (**A**) MBP–β-catenin (50 nM) or MBP–E-cadherin (250 nM) was mixed with beads coated with the indicated GST fusion proteins. Proteins eluted from the beads by glutathione were subjected to SDS-PAGE and immunoblot analysis with anti–β-catenin or anti–E-cadherin, as indicated (*16*). (**B**) The indicated plasmids were microinjected into the nuclei of EL cells. After 5 hours, the cells were fixed and doubly stained with the indicated antibodies (*17*). Data are representative of three independent experiments.

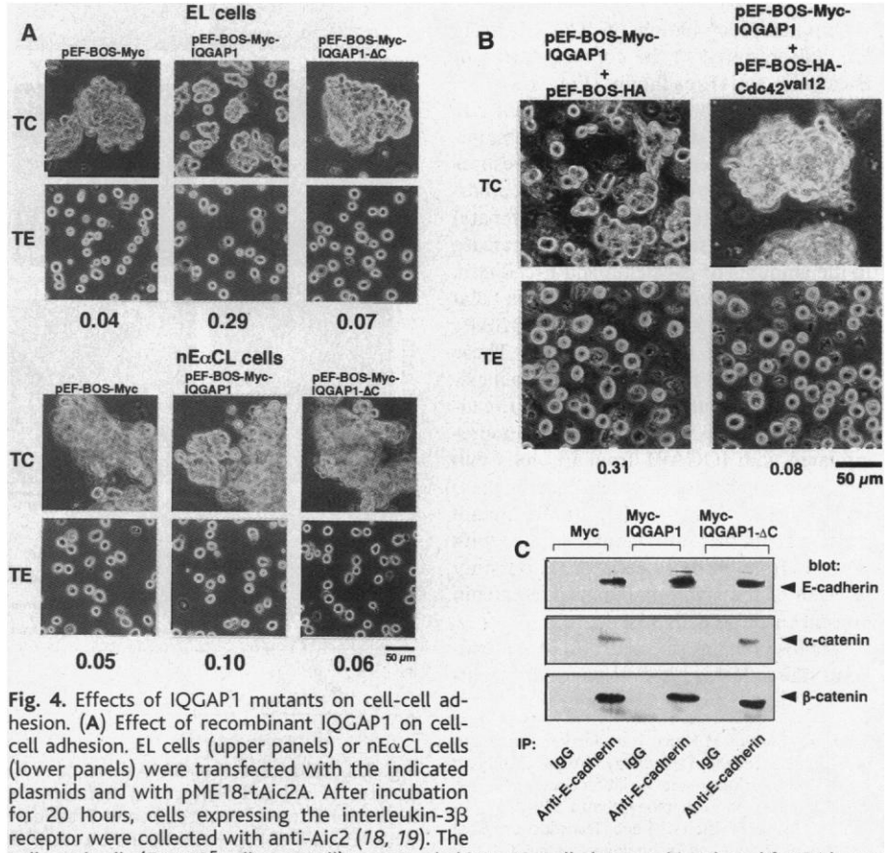

Fig. 4. Effects of IQGAP1 mutants on cell-cell adhesion. (**A**) Effect of recombinant IQGAP1 on cell-cell adhesion. EL cells (upper panels) or nEαCL cells (lower panels) were transfected with the indicated plasmids and with pME18-tAic2A. After incubation for 20 hours, cells expressing the interleukin-3β receptor were collected with anti-Aic2 (*18, 19*). The collected cells (5×10^5 cells per well) were seeded into 48-well plates and incubated for 24 hours. A dissociation assay was then performed after treatment of the cells with trypsin in the presence of either Ca^{2+} (TC treatment) or EGTA (TE treatment) as described (*3, 19*). The total particle numbers after TC or TE treatment were designated N_{TC} and N_{TE}, respectively. The cell dissociation index (N_{TC}/N_{TE}) was scored and is shown below the panels. (**B**) Effect of Cdc42 on cell-cell adhesion. EL cells were transfected with pEF-BOS-Myc-IQGAP1 and pME18-tAic2A plasmids together with either pEF-BOS-HA or pEF-BOS-HA-$Cdc42^{Val12}$. The cells were collected and subjected to the dissociation assay (*3, 19*). (**C**) Dissociation of α-catenin from a cadherin-catenin complex by IQGAP1 in vivo. EL cells were transfected with pEM18-tAic2A and either pEF-BOS-Myc, pEF-BOS-Myc-IQGAP1, or pEF-BOS-Myc–IQGAP1-ΔC (*19, 20*). The cells were isolated and transferred to new plates. After incubation for 24 hours, the cells were treated with DSP, lysed, and subjected to centrifugation (*12, 20*). The resulting supernatant was subjected to immunoprecipitation (IP) with either anti–E-cadherin or control IgG, and the immune complexes were isolated by centrifugation and subjected to SDS-PAGE and immunoblot analysis with antibodies to the indicated proteins. Data are representative of three independent experiments.

function, possibly through direct interaction with IQGAP1.

The amount of α-catenin, but not that of β-catenin, associated with E-cadherin was reduced when IQGAP1 (but not IQGAP1-ΔC) was overexpressed in EL cells (Fig. 4C) (*20*). Overexpression of IQGAP1 or IQGAP1-ΔC did not affect the amounts of E-cadherin, α-catenin, or β-catenin expressed in these cells, and the amounts of recombinant IQGAP1 and IQGAP1-ΔC were similar (*13*). Thus, overexpression of IQGAP1 appeared to induce dissociation of α-catenin from the cadherin-catenin complex. The dissociation of α-catenin from β-catenin may be responsible for the in vivo action of IQGAP1.

Treatment of cells with pervanadate results in the dissociation of α-catenin from the cadherin-catenin complex, and the dissociation of α-catenin from the complex correlates with the decrease in cadherin activity (*21*). Dissociation of α-catenin from the cadherin-catenin complex also occurs during the passage of human breast epithelial cells (*22*). Our data suggest that IQGAP1 might participate in these processes.

References and Notes

1. M. Takeichi, *Curr. Opin. Cell Biol.* **7**, 619 (1995); D. G. Drubin and W. J. Nelson, *Cell* **84**, 335 (1996); B. M. Gumbiner, *ibid.*, p. 345.
2. M. Ozawa, H. Baribault, R. Kemler, *EMBO J.* **8**, 1711 (1989); S. Hirano, N. Kimoto, Y. Shimoyama, S. Hirohashi, M. Takeichi, *Cell* **70**, 293 (1992); K. A. Knudsen and M. J. Wheelock, *J. Cell Biol.* **118**, 671 (1992); M. Peifer, P. D. McCrea, K. J. Green, E. Wieschaus, B. M. Gumbiner, *ibid.*, p. 681.
3. A. Nagafuchi, S. Ishihara, S. Tsukita, *J. Cell Biol.* **127**, 235 (1994).
4. A. J. Ridley, H. F. Paterson, C. L. Johnston, D. Diekmann, A. Hall, *Cell* **70**, 401 (1992); R. Kozma, S. Ahmed, A. Best, L. Lim, *Mol. Cell. Biol.* **15**, 1942 (1995); O. A. Coso *et al.*, *Cell* **81**, 1137 (1995); A. Minden, A. Lin, F.-X. Claret, A. Abo, M. Karin, *ibid.*, p. 1147; C. S. Hill, J. Wynne, R. Treisman, *ibid.*, p. 1159; M. F. Olson, A. Ashworth, A. Hall, *Science* **269**, 1270 (1995); C. D. Nobes and A. Hall, *Cell* **81**, 53 (1995); L. Stowers, D. Yelon, L. J. Berg, J. Chant, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5027 (1995).
5. V. M. M. Braga, L. M. Machesky, A. Hall, N. A. Hotchin, *J. Cell Biol.* **137**, 1421 (1997); K. Takaishi, T. Sasaki, H. Kotani, H. Nishioka, Y. Takai, *ibid.* **139**, 1047 (1997).
6. S. Kuroda *et al.*, *Biochem. Biophys. Res. Commun.* **240**, 430 (1997).
7. P. L. Hordijk *et al.*, *Science* **278**, 1464 (1997).
8. M. J. Hart, M. G. Callow, B. Souza, P. Polakis, *EMBO J.* **15**, 2997 (1996); S. Brill *et al.*, *Mol. Cell. Biol.* **16**, 4869 (1996); S. J. McCallum, W. J. Wu, R. A. Cerione, *J. Biol. Chem.* **271**, 21732 (1996).
9. S. Kuroda *et al.*, *J. Biol. Chem.* **271**, 23363 (1996).
10. L cells, EL cells, and nEαCL cells were cultured as described (*3*). Confluent cells were fixed and stained with polyclonal antibodies to IQGAP1 and with a monoclonal antibody to E-cadherin (ECCD-2) or a monoclonal antibody to β-catenin (Transduction Laboratories, Lexington, KY). Antibodies to IQGAP1 were generated against the NH_2-terminal domain of human IQGAP1 (amino acids 1 to 216).
11. L. Hinck, I. S. Nathke, J. Papkoff, J. W. Nelson, *J. Cell Biol.* **125**, 1327 (1994).
12. Cross-linking experiments were performed essentially as described (*11*). Cells were suspended in phosphate-buffered saline in the absence or presence of 0.75 mM DSP, and incubated for 20 min at room temperature. DSP activity was quenched by addition of 50 mM glycine in phosphate-buffered saline. The cells were then lysed in a solution containing 50 mM tris-HCl (pH 7.5), 1 mM EDTA, 50 mM NaCl, 10 μM (*p*-amidinophenyl)methanesulfonyl fluoride, leupeptin (10 μg/ml), 0.25% (w/v) Triton X-100, and 1 mM $CaCl_2$, and the lysates were incubated for 2 hours at 4°C in the absence or presence of the indicated antibodies. The resulting immunoprecipitates were subjected to SDS–polyacrylamide gel electrophoresis (PAGE) and immunoblot analysis with the indicated antibodies. About 50% of IQGAP1 was immunoprecipitated from EL cells incubated in the absence or presence of DSP (*13*).
13. S. Kuroda *et al.*, unpublished data.
14. Complementary DNAs encoding mouse α-catenin and mouse β-catenin, as well as a cDNA fragment encoding the cytoplasmic domain of mouse E-cadherin, were inserted individually into the Bam HI site of pMAL C-2. The encoded MBP fusion proteins were expressed in and purified from *Escherichia coli*. GST-IQGAP1 was purified from overexpressing insect cells as described (*23*). Various concentrations of MBP fusion proteins were mixed with affinity beads coated with GST or GST-IQGAP1. The beads were washed, and bound MBP fusion proteins were coeluted with GST or GST-IQGAP1 by the addition of reduced glutathione. The eluates were subjected to SDS-PAGE and immunoblot analysis with antibodies to MBP (New England Biolabs, Beverly, MA).
15. Various concentrations of MBP–α-catenin and MBP–β-catenin were mixed and incubated for 1 hour at 4°C. The mixture was then incubated with beads coated with GST, GST-IQGAP1, or GST–E-cadherin (the cytoplasmic domain). The interaction of MBP fusion proteins with GST fusion proteins was examined (*14*).
16. GST–IQGAP1-N and GST–IQGAP1-C were produced and purified as described (*23*). GST–IQGAP1-ΔC was purified from overexpressing insect cells. MBP–β-catenin (50 nM) or MBP–E-cadherin (250 nM) was mixed with affinity beads coated with the indicated GST fusion proteins (40 pmol each). The beads were washed, and the interaction of MBP fusion proteins with GST fusion proteins was examined (*14*).
17. The plasmids pEF-BOS-Myc-IQGAP1, pEF-BOS-Myc–IQGAP1-N, pEF-BOS-Myc–IQGAP1-C, and pEF-BOS-Myc–IQGAP1-ΔC were constructed (*9*, *13*) and microinjected (0.01 mg/ml) into nuclei of subconfluent EL cells. After 5 hours, the cells were fixed and doubly stained with antibodies to Myc and to β-catenin. Similar results were obtained by transfection (*13*).
18. S. Yonehara *et al.*, *Int. Immunol.* **2**, 143 (1990); N. Itoh *et al.*, *Science* **247**, 324 (1990).
19. EL cells or nEαCL cells were seeded into 10-cm dishes and transiently transfected with the indicated plasmids with the use of Lipofectamine (Gibco-BRL, Rockville, MD). After incubation for 20 hours, the cells were scraped from 5 to 10 dishes and transferred to a plastic dish coated with antibodies to Aic2 (HC) (*18*). The adherent cells (5×10^5) were then transferred to 48-well culture dishes and incubated for 24 hours. These various immunoisolated cells showed the same confluency. Recombinant IQGAP1 and Cdc42 were expressed in ~80% of the collected cells. The dissociation assay was performed in the presence of either Ca^{2+} or EGTA as described (*3*).
20. Immunoisolated cells (5×10^6) were transferred to six-well culture dishes (*19*), and, after incubation for 24 hours, they were treated with DSP (*12*) and lysed. The lysates were centrifuged at 100,000*g* for 30 min, and the resulting supernatant was mixed with antibodies to E-cadherin (Transduction Laboratories). After incubation at 4°C for 1 hour, the immunoprecipitate was separated by centrifugation and subjected to SDS-PAGE and immunoblot analysis with the indicated antibodies.
21. M. Ozawa and R. Kemler, *J. Biol. Chem.* **273**, 6166 (1998).
22. Y. Tsukatani, K. Suzuki, K. Takahashi, *J. Cell. Physiol.* **173**, 54 (1997).
23. M. Fukata *et al.*, *J. Biol. Chem.* **272**, 29579 (1997).

24. We thank A. Nagafuchi and S. Tsukita for providing L, EL, and nEαCL cells; cDNAs encoding E-cadherin, α-catenin, or β-catenin; and antibodies to E-cadherin (ECCD-2) or to α-catenin. We also thank M. Takeichi for providing ECCD-2 and Kazusa DNA Research Institute for support of a cDNA Research Program. Supported by grants-in-aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (1997), by the Japan Society for the Promotion of Science Research for the Future, by the Human Frontier Science Program, and by grants from Kirin Brewery Co. Ltd.

21 April 1998; accepted 24 June 1998

Positive Selection Through a Motif in the αβ T Cell Receptor

B. Thomas Bäckström,* Urs Müller, Barbara Hausmann, Ed Palmer†

The two lineages of T cells, αβ and γδ, differ in their developmental requirements: only αβ T cells require major histocompatibility complex recognition, a process known as positive selection. The αβ T cell receptor (TCR), but not its γδ counterpart, contains a motif within the α-chain connecting peptide domain (α-CPM) that has been conserved over the last 500 million years. In transgenic mice expressing an αβ TCR lacking the α-CPM, thymocytes were blocked in positive selection but could undergo negative selection. Thus, the α-CPM seems to participate in the generation of signals required for positive selection.

Positive selection of αβ thymocytes generates a T cell repertoire that is self–major histocompatibility complex (MHC) restricted (*1–4*), whereas negative selection ensures that the immune system is self-tolerant (*5–7*). Although the αβ TCR mediates both forms of thymic selection, the distinction between positive and negative selection signals has been difficult to define. The TCR α-chain has an amino acid motif in its connecting peptide domain (α-CPM) that is conserved in all α-chains from bony fish to humans (*8*). Although the α-CPM has been conserved over the last 500 million years (*9*), it is not found within the γδ TCR. αβ TCRs containing a defective α-CPM are unresponsive to antigens and are aberrantly associated to the CD3 complex (*8*). To test whether the α-CPM