sophilids, 8° to 15°C; lizards, 10°C) (*8, 14, 22*), so that, under these conditions, the dominant entraining cue could be the temperature cycle. Data presented here provide insight on how physiologically and ecologically relevant temperature steps and pulses act to reset a day-phase circadian oscillator. More generally, the data provide another example in which highly conserved and plainly adaptive behaviors of a circadian system can be understood in terms of the straightforward responses of clock components to factors in the environment of the organism.

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27. The further delay of phases with decreasing temperature is not due to a long time being needed for warming of the culture; warming of the race tubes from 4° to 30°C occurs in less than 20 min. To confirm this, in another set of experiments, we first transferred the cultures to 30°C LL for 20 min before the light was turned off, and the same amount of phase delay was observed (*19*).
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30. Cultures of *bdA* were inoculated into 14 sets of six race tubes each prepared as previously described (*18, 24*). The cultures were grown in constant light for ~24 hours and then were transferred into constant darkness either at 21°C (seven sets) or 28°C (seven sets). After 48 hours and at five 4-hour intervals thereafter, groups of race tubes were reciprocally shifted from 21° to 28°C or vice versa; for two control sets, there was no temperature step given after they were transferred into darkness. As a result of the difference in temperature, there were slight differences in the period length between the two sets: the average period length was 22.3 hours at 21°C and 20.5 hours at 28°C.
31. The *bdA* (wild-type clock) strain was used in all the experiments described in this study. Conditions used for liquid culture experiments were as described (*3, 24*). For the experiment shown in Fig. 1A, after the cultures were grown in LL at 25°C for a few hours, they were transferred from L to D and from 25°C to either 21° or 28°C at hour 0. Thirteen hours later and, subsequently, at 5- to 6-hour intervals, samples were collected and used as a source for RNA and protein (*3, 17, 23, 24*). Equal amounts of total RNA (40 μg) or protein (100 μg) were loaded onto agarose or acrylamide gels for electrophoresis as previously described (*3, 18, 23*), and the gels were blotted and probed as appropriate either with a *frq* RNA-specific probe (*3*) or with an antibody to FRQ (*17*). Equal loading among lanes was confirmed by probing the RNA blot with a ribosomal DNA probe (*3*) and by staining the protein blot with amido black (*18*). After developing the blots, densitometry was performed (*17, 18*).

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RNA-Mediated Trans-Activation of Transcription from a Viral RNA

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The red clover necrotic mosaic virus genome is composed of two single-stranded RNA components, RNA-1 and RNA-2. The viral capsid protein is translated from a subgenomic RNA (sgRNA) that is transcribed from genomic RNA-1. Here, a 34-nucleotide sequence in RNA-2 is shown to be required for transcription of sgRNA. Mutations that prevent base-pairing between the RNA-1 subgenomic promoter and the 34-nucleotide trans-activator prevent expression of a reporter gene. A model is proposed in which direct binding of RNA-2 to RNA-1 trans-activates sgRNA synthesis. This RNA-mediated regulation of transcription is unusual among RNA viruses, which typically rely on protein regulators.

RNA performs many of the functions that were once thought to be restricted to proteins. RNA molecules perform various enzymatic reactions in addition to catalyzing peptide bond formation (*1*). Given this diversity of functions, it is not surprising that gene expression can be regulated posttranscriptionally by the structure or stability of an mRNA (*2*). Noncoding RNAs as well as the 3′ untranslated regions (3′ UTRs) of cellular mRNAs function as trans-acting regulators of cell division and differentiation (*3*). In the nematode *Caenorhabditis elegans*, the small noncoding lin-4 RNAs alter the stability or translatability (or both) of lin-14 mRNAs by interacting with their 3′ UTRs (*4*). However, RNA-mediated regulation of transcription from an RNA molecule has not been observed.

Red clover necrotic mosaic *Dianthovirus* (RCNMV) contains two RNA components, a polycistronic RNA-1, which encodes the viral polymerase and capsid protein (CP), and RNA-2, which encodes the viral movement protein

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(MP) required for the cell-to-cell spread of infection (*5*) (Fig. 1, A and B). CP is translated from a subgenomic RNA (sgRNA) transcribed from RNA-1 because the CP open reading frame (ORF) on RNA-1 is inaccessible to the eukaryotic translational machinery (*6*). RNA-1 alone is infectious in plant protoplasts (*5*) and can produce a local infection on the inoculated leaves of transgenic *Nicotiana benthamiana* plants expressing the RCNMV MP (MP^+ plants) (*7*). However, CP synthesis and subsequent systemic infection are observed only when RNA-1 is co-inoculated with wild-type RNA-2 or mutant RNA-2 transcripts that are incapable of MP synthesis (*7*).

To investigate the requirement for RNA-2 in sgRNA synthesis, we engineered an RCNMV RNA-1 cDNA clone, from which infectious transcripts can be synthesized (*8*), to express a synthetic mutant form of the *Aequorea victoria* green fluorescent protein (sGFP) (*9*) in place of the CP (clone R1sGFP) (*10*) (Fig. 1A). Inoculation of R1sGFP transcripts onto MP^+ plants resulted in the accumulation of sGFP only when wild-type or mutant RNA-2 transcripts were also present in the inoculum. RNA-2 transcripts from the related *Dianthoviruses* carnation ringspot virus (CRSV) (*11*, *12*) and sweet clover necrotic mosaic virus (SCNMV) (*13*) also induced sGFP expression. These findings suggest that a conserved region of RNA-2 is required to elicit efficient transcription of sgRNA from RNA-1.

A chimeric RNA-2 clone with the wild-type GFP ORF in place of the MP ORF (clone R2GFP) (*14*) (Fig. 1B) was unable to replicate on MP^+ plants when co-inoculated with wild-type RNA-1 transcripts. Replacement of the 3′-terminal 300 nucleotides (nt) from the GFP insert with the 3′-terminal 324 nt from the MP ORF (clone R2GΔH) (*14*) (Fig. 1B) restored RNA-2 replication. This RNA-2 construct was also able to elicit sGFP expression when co-inoculated with R1sGFP transcripts, suggesting that the 324-nt region contained cis-acting elements required for RNA-2 replication along with trans-acting sequences required for eliciting RNA-1 sgRNA synthesis. Deletion of the 3′-terminal 120 nt of the MP ORF from clone R2GΔH (clone R2GΔHX) (*14*) abolished replication of this RNA-2 construct, so we turned to another strategy for delimiting the trans-acting sequences.

To uncouple the trans-acting sequences required for RCNMV sgRNA expression from the cis-acting sequences required for RNA-2 replication, we used a heterologous RNA viral vector. An infectious cDNA clone of the monopartite tomato bushy stunt virus (TBSV) was previously engineered to allow transcription of foreign sequences from the CP subgenomic promoter (*15*) (Fig. 1C). The 324-nt fragment from RCNMV RNA-2, having the trans-activator activity, was cloned into the TBSV vector in both the genomic- and complementary-sense orientation (*16*). Transcripts of these constructs were co-inoculated with R1sGFP transcripts onto *N. benthamiana* and *Nicotiana clevelandii* plants. sGFP expression was induced only when the RNA-2 insert was present and in the RCNMV genomic-sense orientation (Table 1).

A smaller 209-nt RCNMV RNA-2 insert (representing the sequence present in clone R2GΔHX) (*16*) in the TBSV vector also induced sGFP expression when co-inoculated with R1sGFP transcripts. CP was expressed when this TBSV construct was co-inoculated with wild-type RCNMV RNA-1 transcripts (Fig. 2A). This expression suggests that the trans-activation effect is not gene-specific and that sGFP is a valid reporter to identify and delimit the cis- and trans-acting signals for CP sgRNA synthesis.

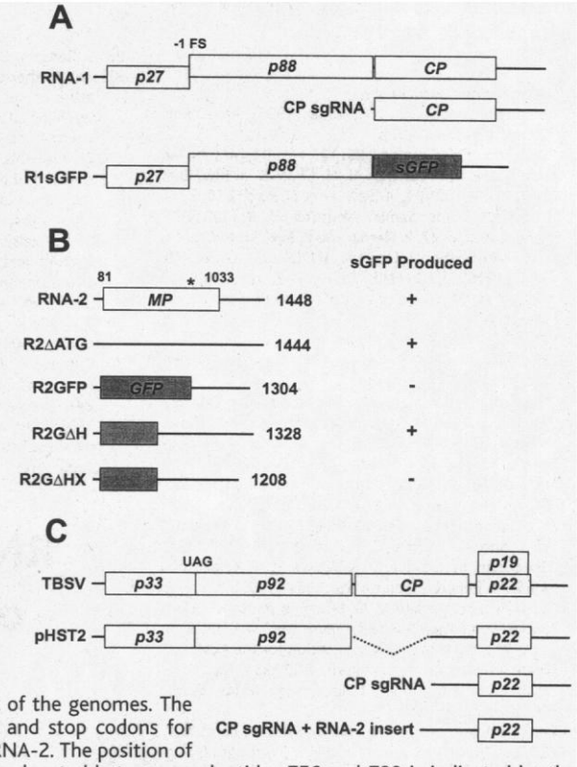

Fig. 1. Genome organization of RCNMV and TBSV. (**A**) Schematic diagram of RCNMV RNA-1, CP sgRNA, and R1sGFP. ORFs for p27 (polymerase component), the −1 ribosomal frameshift (FS) product p88, and the CP are labeled. R1sGFP is a construct in which the CP is replaced with the sGFP ORF (shaded box). sGFP is expressed from this modified genomic RNA-1 only when a subgenomic RNA is generated. (**B**) RCNMV RNA-2 and RNA-2 derivatives. R2ΔATG contains a deletion of the MP start codon and does not produce functional MP. R2GFP is a construct in which the MP is replaced with the wild-type GFP ORF (shaded box). R2GΔH is a construct derived from R2GFP in which 300 3′-terminal nucleotides of the GFP sequence are replaced by 324 3′-terminal nucleotides from the MP ORF. R2GΔHX is a construct derived from R2GΔH with a 120-nt deletion of the 3′-terminal MP ORF sequence. The length of transcripts in nucleotides is indicated to the right of the genomes. The nucleotide positions of the start and stop codons for the MP ORF are indicated above RNA-2. The position of the 34-nt trans-activator sequence located between nucleotides 756 and 789 is indicated by the asterisk. (**C**) Map of the wild-type TBSV genome, TBSV expression vector pHST2, and the CP sgRNA it produces, which was engineered to accept and express foreign genes. The deleted region of TBSV CP in pHST2 is indicated by the dotted line. RCNMV RNA-2 sequences are inserted 55 nt from the 5′-terminus of the sgRNA.

Table 1. Expression of sGFP from RCNMV R1sGFP depends on RNA-2 sequences. R1sGFP was co-inoculated with transcripts from the TBSV replicon containing segments from RCNMV RNA-2 in order to delimit the minimal trans-activator element. See Fig. 1B for nucleotide positions on RNA-2. Complementary sequence is indicated by (−). Primer sequences used for PCR and color images of GFP expression can be viewed at www.sciencemag.org/feature/data/981282.shl

Construct	Position of RNA-2 sequence	Length of RNA-2 inserts (nucleotides)	SGFP produced*
RCNMV RNA-2	1–1448	1448	+++
pHST2	–	–	–
pHST2-RC2.3	708–1031	324	+
pHST2-RC2.4	708–1031(–)	324	–
pHST2-ΔBX	708–916	209	++
pHST2-707	707–837	121	+
pHST2-828	828–918	91	–
pHST2-SL2	756–789	34	+++
pHST2-TA38	755–792(–)	38	–
pHST2-20	762–782	21	+
pHST2-L12	767–775	9	–

*sGFP production relative to induction by RNA-2: +++, 100%; ++, 50%; +, 25%; –, not detected.

Two conserved stem-loop structures were predicted on the basis of alignments with the CRSV and SCNMV RNA-2 sequences, in conjunction with a computer-predicted secondary structure model of the 209-nt RCNMV RNA-2 sequence (*12*, *17*) (Fig. 2B). Inserts from the regions containing each predicted stem loop were tested separately for their ability to trans-activate sGFP expression. Only the TBSV replicon containing the 5′-terminal 121 nt from this region of RNA-2 (*18*) induced sGFP expression from co-inoculated R1sGFP transcripts (Table 1). We next focused on the highly conserved 34-nt stem-loop sequence within this 121-nt region (Fig. 2B). The 34-nt RNA-2 sequence expressed from the TBSV replicon (*19*) in the genomic- but not the complementary-sense orientation was sufficient for sGFP expression at levels near those obtained by wild-type RNA-2 co-inoculation (Table 1). Northern blot hybridization analysis showed that the sgRNA containing the sGFP sequence is produced only when the 34-nt RNA-2 sequence is present (Fig. 2C). The terminal 21 nt of the stem-loop structure conserved among the *Dianthoviruses* (Fig. 2B) also elicited sGFP expression but at a much lower level. However, a 9-nt insert containing the 8 nt in the loop of the 34-nt element was not sufficient to induce sGFP expression (Table 1).

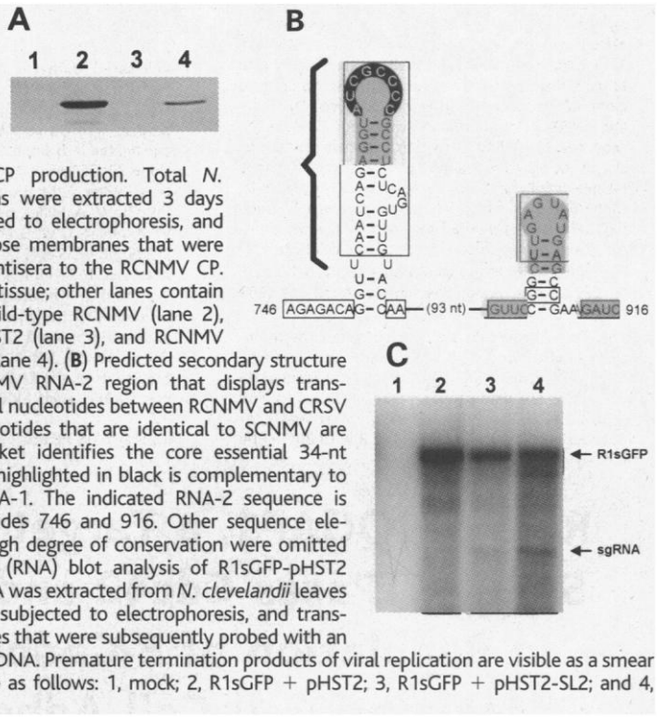

Fig. 2. Analysis of RCNMV RNA-1 sgRNA synthesis and CP gene expression with RCNMV RNA-2 sequences expressed from the TBSV replicon. (**A**) Immunoblot analysis of CP production. Total *N. benthamiana* leaf proteins were extracted 3 days after inoculation, subjected to electrophoresis, and transferred to nitrocellulose membranes that were probed with polyclonal antisera to the RCNMV CP. Lane 1, mock-inoculated tissue; other lanes contain tissue inoculated with wild-type RCNMV (lane 2), RCNMV RNA-1 plus pHST2 (lane 3), and RCNMV RNA-1 plus pHST2-ΔBX (lane 4). (**B**) Predicted secondary structure within the 209-nt RCNMV RNA-2 region that displays trans-activator activity. Identical nucleotides between RCNMV and CRSV are shaded in gray; nucleotides that are identical to SCNMV are boxed. The vertical bracket identifies the core essential 34-nt trans-activator. The loop highlighted in black is complementary to an 8-nt element on RNA-1. The indicated RNA-2 sequence is located between nucleotides 746 and 916. Other sequence elements not displaying a high degree of conservation were omitted for clarity. (**C**) Northern (RNA) blot analysis of R1sGFP-pHST2 co-inoculations. Total RNA was extracted from *N. clevelandii* leaves 3 days after inoculation, subjected to electrophoresis, and transferred to nylon membranes that were subsequently probed with an sGFP-specific ^{32}P-labeled DNA. Premature termination products of viral replication are visible as a smear in lanes 2 to 4. Lanes are as follows: 1, mock; 2, R1sGFP + pHST2; 3, R1sGFP + pHST2-SL2; and 4, R1sGFP + RNA-2.

Sequence alignments revealed that the 8-nt loop from RNA-2 is complementary to an 8-nt sequence element on RNA-1 located 2 nt upstream from the start site of sgRNA synthesis and within a region broadly mapped to be the CP subgenomic promoter (*20*). To determine if these sequences formed base pairs, we mutated the 8-nt element on RNA-1 at three positions that would not alter the amino acid coding sequence of the p88 polymerase (*21*). Co-inoculation of transcripts from this mutant clone (R1sGFP-M) with TBSV transcripts containing the 34-nt RNA-2 trans-activator element did not elicit sGFP synthesis (Table 2). When compensatory mutations were incorporated into the loop sequence of the 34-nt RNA-2 element (pHST2-SL2-M) (*19*) to restore complementarity with the mutated RNA-1 element, sGFP expression was restored (Table 2).

These results establish that a 34-nt sequence, predicted to form a simple stem loop, trans-activates sgRNA synthesis on another RNA. Furthermore, mutation data support the conclusion that trans-activation is accomplished by base-pairing between the 8-nt loop region of the RNA-2 trans-activator and an 8-nt element within the CP subgenomic promoter on RNA-1. It is unclear whether the interaction of RNA-1 and RNA-2 requires proteins. Sequence elements nearly identical to the RCNMV RNA-1 8-nt element are also present at the same location in CRSV and SCNMV RNA-1, suggesting that this mechanism for sgRNA synthesis is conserved among the *Dianthoviruses*.

We propose the following model (*22*) for

Table 2. Expression of sGFP from compensatory mutations between the predicted loop sequence of the RCNMV RNA-2 trans-activator and the complementary RCNMV RNA-1 8-nt element in the CP subgenomic promoter. Mutated nucleotides are in bold typeface and underlined.

RCNMV RNA-1/TBSV inoculum	RNA-1 8-nt element	RNA-2 TA loop sequence	sGFP produced*
R1sGFP/pHST2-SL2	5′-GGGGCGAT-3′	5′-ATCGCCCC-3′	+++
R1sGFP-M/pHST2-SL2	**C**GG**C**CG**T**T	ATCGCCCC	–
R1sGFP/pHST2-SL2-M	GGGGCGAT	A**A**CG**G**CC**G**	–
R1sGFP-M/pHST2-SL2-M	**C**GG**C**CG**T**T	A**A**CG**G**CC**G**	+++

*sGFP production relative to induction by RNA-2: +++, 100%; –, not detected.

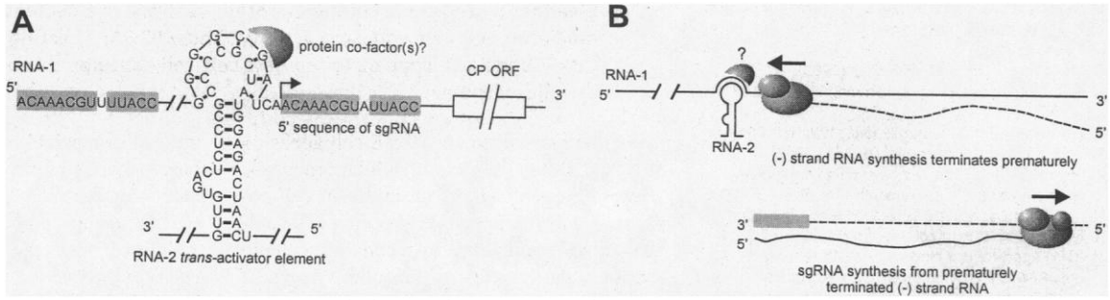

Fig. 3. Proposed components and model of the RCNMV trans-activation mechanism. (**A**) Sequences involved in trans-activation. Gray shaded nucleotides represent conserved sequences between genomic RNA-1 and the sgRNA that are likely to be + strand promoters. The loop region of the 34-nt trans-activator is shown base-pairing with the complementary 8-nt element, 2 nt upstream from the sgRNA start site (right angle arrow) on RNA-1. (**B**) Model for the generation of sgRNA. Complementary strands are depicted as dashed lines.

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.

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

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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.