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This article has been withdrawn by the authors. Errors were identified in several figures. Evaluation by the Journal with image analysis software determined that images were reused to represent different experimental conditions in the GST immunoblots in Fig. 2, A and C, and the N-tail immunoblots in Fig. 3B. The raw data are no longer available to validate the information. The authors have expressed the opinion that none of these errors affect the final conclusions of this article that, according to them, have been validated.

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**β-Catenin N- and C-terminal Tails Modulate the Coordinated Binding of Adherens Junction Proteins to β-Catenin**

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**β-Catenin plays a central role in the establishment and regulation of adherens junctions because it interacts with E-cadherin and, through α-catenin, with the actin cytoskeleton. β-Catenin is composed of three domains: a central armadillo repeat domain and two N- and C-terminal tails. The C-tail interacts with the armadillo domain and limits its ability to bind E-cadherin and other cofactors. The two β-catenin tails are regulated because the binding of the N-tail to the armadillo domain restricts the interaction of the C-tail with E-cadherin. Depletion of either of the two tails has consequences for the binding of factors at the other end: deletion of the N-tail limits the ability of E-cadherin and other armadillo repeats. As for the tails, the association of α-catenin with the N-tail, through consensus motif for the C-tail, facilitates the interaction of α-catenin with E-cadherin. These results indicate that different cofactors coordinate their binding to the terminal ends of β-catenin.**

β-Catenin is a multifunctional protein that exerts two essential functions in epithelial cells. It is necessary for the maintenance of adherens junctions because it binds E-cadherin and connects it, through α-catenin, to the actin cytoskeleton (1, 2). On the other hand, when not bound to E-cadherin, β-catenin can move to the nucleus and act as a transcriptional co-activator, through the interaction with members of the T cell transcription factor family (3). In addition to T cell transcription factor proteins, several other transcriptional factors have been reported to interact with β-catenin, presumably modulating its positive activity on transcription in the presence of several target genes (4). On the other hand, a complex of proteins containing the product of the adenomatous polyposis coli, axin, glycogen synthase kinase 3β, Eβ-transfase, and the N-terminal tail binds to β-catenin and presumably modulates its activity (4). The costs of publication of this article were defrayed in part by the agency of the publisher in the public interest to encourage publication of such articles.

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The abbreviations used are: TBP, TATA-binding protein; arm, β-catenin armadillo domain; GST, glutathione S-transferase; cytoE-cadherin, cytosolic domain of E-cadherin; mAb, monoclonal antibody; Tyr(P), phosphotyrosine; N-tail, N-terminal tail; C-tail, C-terminal tail.
the N terminus also interacts with the armadillo domain. This interaction is potentiated by the C-tail. The functional relevance of this interconnection between the terminal tails is evidenced by the fact that α-catenin, which binds to the N-tail of β-catenin, also affects the association of E-cadherin to the armadillo repeats.

**EXPERIMENTAL PROCEDURES**

**Reagents**—The following β-catenin monoclonal antibodies were used in this study: β-catenin C terminus (Transduction Laboratories, Lexington, KY); the epitope recognized by this antibody has been mapped to residues 750–775 using different deletion mutants of this protein (not shown); β-catenin C-end (Calbiochem), epitope recognized, 769–781 (according the manufacturer); β-catenin armadillo core, epitope recognized, 422–685 (according the manufacturer) and β-catenin N terminus, raised against the first 100 amino acids (both from Alexis Biochemicals, San Diego, CA).

Expression of Recombinant Proteins—The preparation of all the plasmids coding for the different β-catenin forms (deletion and point mutants) as glutathione S-transferase (GST) fusion proteins has been described previously (7, 11) except for the following cases: GST-arm-(120–683) and GST-β-catenin (120–750); DNA fragments corresponding to amino acids 120–683 or 120–750 of murine β-catenin were amplified by PCR using oligonucleotides corresponding to the nucleotide sequences 358–372 and 2035–2047 or 358–372 and 2260–2242 and containing BamHI and XhoI sites at their ends. The 1.7- or 1.9-kbp amplification fragments were digested with BamHI and XhoI and cloned in the same sites of pGEX-6P3 plasmid, and expressed in E. coli. These relevant E-cadherin amino acids are conserved in Escherichia coli; GST-ΔNβ-catenin was prepared cutting pGEX-β-cat with SacI/NcoI, purifying the 0.66-kbp fragment and inserting it in pGEX-arm-(120–683) cut with the same enzymes; GST-ΔCβ-catenin was prepared cutting pGEX-β-cat with BamHI/SacI, purifying the 1.75-kbp fragment, inserting it in pGEX-arm-(120–683) cut with the same enzymes.

ΔCβ-catenin (Y86E) was prepared identically to GST-ΔCβ-catenin using the BamHI/SacI fragment obtained from pGEX-arm-(120–683) cut with the same enzymes; GST-ΔCβ-catenin was prepared cutting pGEX-β-cat with BamHI/SacI, purifying the 1.75-kbp fragment, inserting it in pGEX-arm-(120–683) cut with the same enzymes.

When indicated, GST was harvested from the media (Amersham Biosciences). The relevant E-cadherin amino acids are conserved in E. coli. GST fusion proteins were separated by SDS-PAGE and electroblotted onto nitrocellulose membranes. Free membrane-binding sites were blocked in TTBS buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Triton X-100) plus 0.1% bovine serum albumin. Membranes were incubated with 5–10 μg/ml of recombinant protein in TTBS with 1% bovine serum albumin for 90 min at room temperature. After extensive washing with TTBS, the nitrocellulose membranes were incubated with 1% bovine serum albumin with the corresponding specific antibody, followed by a peroxidase-conjugated secondary antibody. Immunoblots were developed with peroxidase-conjugated secondary antibody followed by enhanced chemiluminescence detection system (ECL, Pierce). The autoradiograms were scanned and the values obtained were either compared with known amounts of recombinant proteins included as reference in the same blot (β-catenin binding assays) or with the value obtained for wild-type full-length β-catenin (pull-down assays). Duplicate samples were always introduced. The amount of each experiment was performed at least three times. Values obtained from the scanning of the gels were compared with a control and the average percentage of binding calculated ± S.D.

Protein Sensitivity of β-Catenin—5.5 pmol of the different forms of β-catenin were incubated in the presence of trypsin (30 ng, when analyzing the C terminus; 0.1 ng for the N terminus analysis) at 24 °C in a final volume of 100 μl, in a buffer containing 90 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Triton X-100, 1.4 mM CaCl₂, 0.1% dithiothreitol. Alternatively, 17 pmol of cytoE-cad, α-catenin, or GST was incubated in a final volume of 50 μl in 50 mM Tris-HCl, pH 7, 150 mM NaCl, 3 mM MgCl₂, 0.1% Triton X-100, 1 mM dithiothreitol. Complexes were digested with carboxypeptidase Y (Roche Molecular Biochemicals) (17.5 ng) at 25 °C. Reactions were stopped at the indicated digestion times from 1 to 90 min with electrophoresis loading buffer and boiled for 4 min. Extent of the digestion was determined analyzing the samples by SDS-PAGE and Western blot with mAbs against β-catenin C terminus, N terminus, or C-end. A quantification of the reaction was performed scanning the autoradiograms and representing the amount of full-length β-catenin remaining at the different times of incubation relative to the initial time. The experiments, performed in duplicate, were repeated twice, and the average β-catenin (± range) remaining at different times was represented. In all the cases, the results were reproduced when a higher concentration of trypsin was used, although all the forms presented an accelerated rate of degradation.

**RESULTS**

β-Catenin protein is composed of three well differentiated domains: a central core, denominated armadillo repeat domain, and two N- and C-tails (see Introduction and Fig. 1). We have reported recently that the C-tail can interact with the armadillo domain, and this interaction restricts the ability of E-cadherin to associate with the armadillo repeats (11). Because the crystal structure of the armadillo region of β-catenin complexed with cytosolic domain of E-cadherin has recently been elucidated (9) and the amino acids involved in this interaction identified, we looked for sequence analogies between this E-cadherin arm re-
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### Table I

**Analogy between the armadillo-binding sequences of E-cadherin and the β-catenin C-tail**

The sequence of region II of the cytosolic domain of E-cadherin is shown. The amino acids of this sequence that interact with relevant amino acids of the armadillo domain of β-catenin (indicated below) are indicated in **bold**, according to Huber and Weis (9). Equivalent amino acids in the C-tail of β-catenin to those present in E-cadherin are shown in **boldface** type in the upper row. Other amino acids pairs showing similar properties in these two regions are underlined.

| β-cat (760-end) | ----- QDLMDGLPPGDSNQLAW-FDSDL | E-cadh (655-677) | ----- NFIDELNKAAADSDPTAAPPYDSSL----- |
|-----------------|--------------------------------|-----------------|-------------------------------------|
| Interaction with |                                | Interaction with |                                |
| the arm domain  |                                | the arm domain  |                                |
| A₆₆₆            |                                | A₆₆₆            |                                |
| A₆₆₆            |                                | Y₆₅₄            |                                |
| T₅₅₆            |                                | N₅₄₂            |                                |
| R₄₇₄            |                                | K₄₃₅            |                                |
| H₄₇₀ R₄₆₉       |                                | H₄₇₀ R₄₆₉       |                                |

The last part of β-catenin C-tail (amino acids 760-end) (Table I). Therefore, it is possible that the interaction of the C-tail with the armadillo domain is mainly mediated through the last amino acids of the molecule, 760–781. To check this possibility, binding of different C-tail fragments to the GST-arm was checked. As shown in Fig. 2A, **upper panel**, deletion of the last 31 amino acids of the C-tail greatly reduced its ability to bind this fusion protein. Further supporting the existence of this interaction, a peptide corresponding to the last 22 amino acids of β-catenin could interact *in vitro* with the armadillo repeats (Fig. 2B). Moreover, addition of an excess of this peptide (amino acids 760–781) inhibited the association of the complete C-tail (696–781) to the arm domain (Fig. 2C).

Analysis of this association using overlay assays gave the same results. Moreover, as evidenced in Fig. 2A, **bottom panel**, the C-tail to the armadillo domain was produced by the presence of the N-tail (N-tail-C-tail), suggesting that interaction of the N terminus to the complete C-tail is dependent on the presence of the C-tail. This result suggested that the conformation of the C-tail is important for the interaction of the N terminus to the armadillo domain. The last 31 amino acids of β-catenin do not seem to be involved in the interaction with the N-tail because this tail interacts similarly with ΔN-tail-β-catenin proteins containing the entire C-tail and with those missing the last 31 amino acids (ΔN-tail(120–750)) (Fig. 3A).

We have reported previously (7) that tyrosines 86, located in the N-tail, and 654, in the last armadillo repeat, can be phosphorylated *in vitro* by pp60*src* tyrosine kinase. In order to individually analyze the relevance of these phosphorylation in the N-tail-armadillo domain interaction, we have generated different fusion proteins with Tyr-86 and Tyr-654 replaced by Glu (Y₆₆₆ and Y₆₅₄, respectively). The mutations are considered to mimic the effect of the phosphorylation. As shown in Fig. 3A, Y₆₅₄E mutation in ΔN-tail-β-catenin did not modify N-tail binding. This mutation, located in the last armadillo repeat, has been shown to alter the association of last amino acids of the C-tail (760–781) with the armadillo domain (see Ref. 9 and data not shown). Therefore, this result reinforces our conclusion that the end of the C-tail does not play an important role in the potentiation of N-tail binding to the central core. On the other hand, N-tail (Y₆₆₆) interacted better than the wild-type N-tail, both with the armadillo domain and with ΔN-tail β-catenin (Fig. 3B). This result indicates that an increase in the negative charge of the N-tail potentiates the interaction to the arm domain and suggests that establishment of ionic pairs are probably relevant in the interaction between this two domains.

Our binding assays showed that the association of the N-tail to the armadillo repeats was reduced by the C-tail and suggest that the contribution of both tails in the native protein is the sequential addition of ionic pairs. This hypothesis, experiments of this nature confirmed, and the disappearance or addition of the C-tail at Tyr-654 Glu mutation accelerates degradation of the C-tail, because it hinders interaction of the C-tail with the armadillo domain. On the other hand, β-catenin Tyr-86 Glu mutant was clearly more sensitive to trypsin than the wild-type β-catenin, because it hinders interaction of the C-tail with the armadillo domain. On the other hand, β-catenin Tyr-86 Glu mutation is more sensitive to trypsin than the wild-type β-catenin, because it hinders interaction of the C-tail with the armadillo domain. On the other hand, β-catenin Tyr-86 Glu mutation is more sensitive to trypsin than the wild-type β-catenin, because it hinders interaction of the C-tail with the armadillo domain. On the other hand, β-catenin Tyr-86 Glu mutation is more sensitive to trypsin than the wild-type β-catenin, because it hinders interaction of the C-tail with the armadillo domain. On the other hand, β-catenin Tyr-86 Glu mutation is more sensitive to trypsin than the wild-type β-catenin, because it hinders interaction of the C-tail with the armadillo domain. On the other hand, β-catenin Tyr-86 Glu mutation is more sensitive to trypsin than the wild-type β-catenin, because it hinders interaction of the C-tail with the armadillo domain. On the other hand, β-catenin Tyr-86 Glu mutation is more sensitive to trypsin than the wild-type β-catenin, because it hinders interaction of the C-tail with the armadillo domain. On the other hand, β-catenin Tyr-86 Glu mutation is more sensitive to trypsin than the wild-type β-catenin, because it hinders interaction of the C-tail with the armadillo domain. On the other hand, β-catenin Tyr-86 Glu mutation is more sensitive to trypsin than the wild-type β-catenin, because it hinders interaction of the C-tail with the armadillo domain.
to the armadillo repeats, N-tail hinders C-tail-armadillo interaction. In this regard it is particularly illustrative of the fact that a mutation that increases binding of the N-tail to the armadillo and, therefore, retards the degradation of the N terminus accelerates the proteolysis of the C-end. This inter-dependent binding of both tails suggested to us that one of them could modulate the ability of β-catenin to attach other cofactors, even though they do not interact through this domain.

We first analyzed the relevance of this domain in E-cadherin

Fig. 2. Amino acids 760–781 are involved in the interaction of the C-tail to the β-catenin armadillo domain. A, C-tail fragments (83 pmol) were incubated with 3.5 pmol of GST-arm or GST as a control as indicated under “Experimental Procedures.” Protein complexes were affinity-purified with glutathione-Sepharose and analyzed by Western blot (WB) with a mAb against β-catenin C-tail. The numbers below the lanes indicate the amount of bound protein (in fmol); these values were calculated comparing the results of the scanning of the corresponding lanes with known amounts of β-catenin proteins that were included as internal references (St) in the same blots. B, 8.3 pmol of the arm domain were incubated with 1 μg of C-tail (760-end) bound to glutathione-Sepharose or with the same amount of glutathione-Sepharose alone. The amount of associated β-catenin armadillo domain was determined using a mAb specific for this domain. C, binding assay was performed in the conditions described in A with the presence of a 100-fold molecular excess of C-tail-(760-end) when indicated. Addition of a similar excess of an irrelevant peptide did not modify binding of the C-tail-(696-end) to GST-arm (not shown). D, recombinant proteins (8.3 pmol) containing the indicated domains of β-catenin were separated by SDS-PAGE and transferred to nitrocellulose membranes. Binding of recombinant proteins was determined by overlay assays as described under “Experimental Procedures” incubating blots with β-catenin C-tail-(696-end) (0.52 nmol/ml). Binding was analyzed incubating with a mAb against the C-tail that recognized this overlaid β-catenin domain. A, C, and D, in order to verify that equal amounts of proteins were loaded in the gel, blots were stripped and rebotted with mAbs detecting the proteins used as bait (lower panels). The figure shows a representative experiment of three performed in each case, and the graphics at the left of A and D show the average (± S.D.) percentage of binding to GST-arm of C-tail-(696-750) relative to C-tail-(696-end) (A), or to the C-tail of ΔC-tail β-catenin relative to arm domain (D). These values were obtained after the densitometry of the three experiments performed.
binding. As mentioned previously, E-cadherin binds exclusively to the armadillo domain (see Ref. 9, and data not shown). As observed in Fig. 5A, deletion of the C-tail greatly increased the amount of the cytosolic domain of E-cadherin (cytoE-cadh) capable of attaching to β-catenin (compare results for full-length and ΔN-tail (B)). Binding was analyzed incubating with a mAb that recognized the overlaid β-catenin domain (upper analysis in every panel). The numbers below the lanes indicate the amount of bound protein (in fmol); these values were calculated comparing the results of the scanning of the corresponding lanes with known amounts of β-catenin proteins that were included as internal references (St) in the same blot. In the graphics, the relative binding to the N-tail of the different fragments analyzed is represented with respect to the control (binding of the arm domain). The figure shows the results of one representative experiment of three performed in both cases, and the graphics illustrate the average ± S.D. of the relative binding obtained in the three experiments.

FIG. 3. C-tail potentiates N-tail binding to β-catenin armadillo domain. Recombinant proteins (17 pmol) containing the indicated domains of β-catenin were separated by SDS-PAGE and transferred to nitrocellulose membranes. Binding of recombinant proteins was determined by overlay assays incubating blots with 1.1 nmol/ml of β-catenin N-tail-(1–106) (A) or 0.3 nmol/ml of either arm domain (amino acids 120–683) or ΔN-tail (B). Binding was analyzed incubating with a mAb that recognized the overlaid β-catenin domain (upper analysis in every panel). The numbers below the lanes indicate the amount of bound protein (in fmol); these values were calculated comparing the results of the scanning of the corresponding lanes with known amounts of β-catenin proteins that were included as internal references (St) in the same blot. In the graphics, the relative binding to the N-tail of the different fragments analyzed is represented with respect to the control (binding of the arm domain). The figure shows the results of one representative experiment of three performed in both cases, and the graphics illustrate the average ± S.D. of the relative binding obtained in the three experiments.

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Binding of α-catenin was also studied. The region of β-catenin involved in the interaction with α-catenin has been identified between amino acids 118 and 146 (13), residues located at the end of the N-tail and the first armadillo repeat. The integrity of this sequence seems to be required because neither the arm domain (amino acids 120–683) nor the ΔN-tail mutant significantly bound α-catenin (Fig. 5B). On the other hand, the ΔC-tail mutant associated with α-catenin slightly better than the wild-type form (lanes 2 and 3). This result also demonstrated an interconnection between the two tails and suggests that loosening of the N-tail interaction with the arm repeats favors α-catenin binding. No significant differences were found between binding of α-catenin to wild-type and Y86E forms of β-catenin (data not shown).
Similar results were obtained when the binding of $\beta$-catenin to a third partner, TBP, was analyzed (Fig. 5C). This protein binds to $\beta$-catenin through different sequences, although the main interaction was localized at the last repeats of the armadillo domain and the C-tail (see Ref. 14 and data not shown). Our results clearly indicate that deletion of N-tail facilitates binding of the protein, suggesting that this tail modulates the association of TBP to the C-terminal part of the molecule (Fig. 5C, compare lanes 1 and 4). Accordingly, further deletion of the C-tail markedly decreases binding of TBP (compare lanes 2 and 4).

These results confirm our hypothesis that both tails coordinately modulate the interaction of $\beta$-catenin partners either to the central armadillo domain or to the other tail of $\beta$-catenin. Effects of the tails were detected on the interaction with E-
cadherin and α-catenin, two proteins that can simultaneously associate with β-catenin in the adherens junctions. We examined the possibility that the binding of one of these β-catenin cofactors was modifying the binding of the other and thus affecting the interaction of the tails with the armadillo repeat domain. Pre-association of β-catenin to E-cadherin did not increase the amount of α-catenin that can be bound to the complex (Fig. 6B). However, binding of β-catenin to α-catenin raised the affinity of the armadillo repeats to E-cadherin (Fig. 5). This effect required the presence of the C-tail, because it was not detected when the β-catenin ΔC-tail mutant was used (Fig. 6A). Similar stimulation by α-catenin in E-cadherin binding was observed when a Y86E β-catenin mutant was analyzed (Fig. 6A).

Conformational changes of the β-catenin C-tail induced by E-cadherin binding were also studied by controlled proteolysis assays. β-Catenin was incubated with cytoE-cadherin, α-catenin, or equivalent amounts of irrelevant proteins, and resistance to proteolysis of an epitope situated in the C-end (amino acids 769–781) was monitored. As shown in Figs. 5B and C, there was a significant increase in the amount of the remaining epitope when α-catenin was present compared with when it was absent (Fig. 5C). In contrast, when the Y86E β-catenin mutant was used, no such increase was observed (Fig. 5C).
E-cadherin.

Regarding E-cadherin, the H9251 interaction with the complete protein is weaker (11, 23) than the complete C-tail (amino acids 696–781), which involves the armadillo domain. The N-tail affects C-tail binding but in a different fashion. In the absence of N-tail, the C-tail is more closely packed with the arm domain as indicated by data showing that the sequence of the N-tail interacts with each other negatively; thus, a better interaction of C-tail responsible for this effect could be allocated to amino acids 683–750, because a β-catenin protein consisting of the armadillo domain (amino acids 120–750) binds the N-tail similarly to a protein covering the complete C-tail (amino acids 120–end). Therefore, in the C-tail two different sub-domains can be distinguished: segment 683–750, which enhances N-tail binding to the armadillo domain, and the last residues of the protein (comprising at least residues 760–781) involved in the direct binding to the arm repeats.

The N-tail also affects C-tail binding but in a distinct fashion. In the absence of N-tail, the C-tail is more closely packed against the arm domain as indicated by data showing that the C-tail from ΔN-β-catenin is less sensitive to trypsin treatment than the same domain from wild-type β-catenin. Therefore, it can also be concluded that the two sub-domains of the C-tail interact with each other negatively; thus, a better interaction of 683–750 with the N-tail would involve a lower association of 750–781 with the armadillo domain.

Different consequences can be inferred from this mutually dependent association of the tails to the central arm domain. First, binding of β-catenin to factors that associate with primary sequences located far from one end might be affected by this tail. We have proved this hypothesis by analyzing the binding to β-catenin deletion mutants of three proteins: E-cadherin, α-catenin, and TBP. These three proteins attach to different amino acid sequences within β-catenin, but in all the cases deletion of the tails more distant from the place of interaction affects the binding.

Another consequence is that the association of two different proteins to separate binding sites on the β-catenin molecule might be interdependent. Thus, binding of the first partner

DISCUSSION

β-Catenin has been shown to interact with many different proteins. In most cases, binding takes place totally or partially through the central part of the protein, the armadillo repeat domain (3, 13–23). When analyzed in vitro, this central domain has a high affinity for various molecular partners, but the interaction with the complete protein is weaker (11, 23–24). Regarding E-cadherin, the β-catenin C-tail is responsible for the restriction of its association to the full-length protein. The C-tail end-fragment (amino acids 762–781) is very similar to the E-cadherin sequence involved in the interaction with the armadillo domain (amino acids 657–677) (9) (see Table I). As shown above, a peptide corresponding to the last 22 amino acids of β-catenin can bind the armadillo domain. Further support for the relevance of amino acids 750–781 in this interaction is indicated by experiments showing that a C-tail fragment corresponding to amino acids 696–750 binds less efficiently to armadillo than the complete C-tail (696–781), which covers up to the end of the protein.

Our results also clearly indicate that the two tails are closely located in the conformation adopted by the entire β-catenin molecule. For instance, the C-tail is necessary for an efficient binding of the N-tail to the armadillo domain. In the absence of C-tail, binding of the N-tail to the central domain is low. However, no direct binding of the two tails was detected in our

Fig. 6. Modulation of β-catenin-E-cadherin association by α-catenin. A, 0.8 pmol of GST-α-catenin were incubated with 1.4 pmol of wild-type or ΔC-tail (1–683) β-catenin. B, 0.5 pmol of GST-α-catenin were incubated with 1.4 pmol of wild-type β-catenin in the presence of 3.5 pmol of N-tail or 20 pmol of cytoE-cadh. In all cases the amount of associated numbers below armadillo core. The numbers below the lanes indicate the amount of bound GST-α-catenin (in fmol) as internal standards (St). In the graphics, the relative horizontal position of the indicated additions is represented with respect to the control (binding to full-length β-catenin). The relative binding obtained in the four experiments.

Our results also clearly indicate that the two tails are closely interdependent. Thus, binding of the first partner...
The influence of α-catenin on E-cadherin binding to β-catenin was also examined. As repeatedly observed, previous association to α-catenin increases the affinity of β-catenin for E-cadherin. This effect was only detected when the β-catenin C-tail was present. This is consistent with results showing that α-catenin binding accelerates carboxypeptidase proteolysis of the C-tail. According to our model, α-catenin association would modulate the C-tail conformation, inducing a tighter attachment of the 683–750 sub-domain to the N-tail and a looser binding of the C-end to the arm repeats. Therefore, regarding this system, changes in the N-tail elicited by binding of cofactors do transduce efficiently into alterations in the C-tail structure. The influence of the changes in the N-tail on the C-tail conformation versus changes in the C-tail on the N-tail is also probably related to the much more evident effect of β-catenin deletions in the binding of E-cadherin versus α-catenin. For instance, deletion of the C-tail only increases α-catenin binding 2.5-fold, whereas deletion of the N-tail practically blocks E-cadherin association (Fig. 5A, lane 5). Thus, E-cadherin binding is much more responsive to changes in the C-tail than α-catenin to those in the N-tail. We cannot rule out the possibility that other factors binding through the N-tail are much more sensitive to conformational changes in this domain than α-catenin, the binding being more pronouncedly affected by change in the C-tail.

The influence of α-catenin on E-cadherin binding to β-catenin might modulate the association of the second, through conformational changes induced in the tails. We have examined this possibility analyzing the binding of E-cadherin and α-catenin to β-catenin. These two proteins are present in the same complex with β-catenin. As mentioned above, binding of E-cadherin competes with the association of the C-tail to the armadillo domain. However, disruption of the binding of the last amino acids of the C-tail to the armadillo domain does not promote a better association of α-catenin to the other end of β-catenin (to amino acids 118–146). We think that the interaction of the C-tail sub-domain 683–750 to the N-tail and to the arm domain is not modified after association with E-cadherin. One possible explanation would be that the E-cadherin sequence 655–677 replaces almost perfectly the C-tail amino acids 760–781 in the structure of the complex; therefore, binding of E-cadherin would only unfold the last amino acids of β-catenin without altering amino acids 683–750. Thus, substitution of one peptide for the other does not bring about a conformational change in the N-tail. On the other hand, total deletion of the C-tail (depletion of 683–750) causes the unfolding of the N-tail and the acquisition of a new conformation of this domain that binds α-catenin slightly better.

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