β-Catenin and plakoglobin are related proteins involved in the regulation of adherens junctions and desmosomes. Moreover, by binding to Tcf-4, they can act as transcriptional modulators of genes involved in embryonic development and tumorigenesis. However, they associate to distinct Tcf-4 subdomains causing opposing effects on Tcf-4 binding to DNA: whereas β-catenin does not affect this binding, plakoglobin prevents it. Both proteins are composed by two N- and C-tails and a central armadillo repeat domain. Interaction of Tcf-4, as well as other desmosomal or adherens junction components, with β-catenin or plakoglobin takes place through the central armadillo domain. Here we show that, as reported for β-catenin, plakoglobin terminal tails also interact with the central domain and regulate the ability of this region to bind to different cofactors. Moreover the specificity of the interaction of β-catenin and plakoglobin with different subdomains in Tcf-4 or with other junctional components resides within the terminal tails and not in the armadillo domain. For instance, a chimeric protein in which the central domain of β-catenin was replaced by that of plakoglobin presented the same specificity as wild-type β-catenin. Therefore, the terminal tails of these proteins are responsible for discerning among binding of factors to the armadillo domain. These results contribute to the understanding of the molecular basis of the interactions established by these key regulators of epithelial tumorigenesis.

β-Catenin is a multifunctional protein involved in a wide range of cell processes. Although these processes may appear to be unrelated, they form an interdependent network of events. β-Catenin has a structural role at cell-cell junctions and acts as a transcription factor in a signaling pathway. In adherens junctions, β-catenin links the cytosolic domain of the transmembrane protein E-cadherin to α-catenin, which in turn binds to the actin cytoskeleton (1–3). When released from E-cadherin and α-catenin, the cytosolic pool of β-catenin is tightly controlled by formation of a complex that includes the product of the tumor suppressor adenomatous polyposis coli (APC) gene, axin, and the Thr/Ser protein kinases CKIε and glycogen synthase kinase 3β (4, 5). In the absence of Wnt stimulation, this complex facilitates phosphorylation of the β-catenin N-terminal region. Initially β-catenin is first phosphorylated by CKIε on Ser-45 and then sequentially by glycogen synthase kinase 3β on Thr-41 and Ser-37, Ser-33, and Ser-29. Upon phosphorylation, β-catenin is polyubiquitinated and degraded by the proteasome system. The activity of the complex is controlled by a signaling pathway triggered by Wnt factors that inhibit the activity of glycogen synthase kinase 3β and stabilize cytosolic β-catenin (4).

β-Catenin can also translocate to the nucleus in a process that requires binding to pygopus and legless proteins (6, 7). Nuclear β-catenin binds to members of the Tcf family of transcription factors and promotes activation of a number of target genes involved in embryonic development and tumorigenesis (for reviews, see Refs. 4 and 8). In this complex Tcf binds to the DNA, and β-catenin provides the transactivation domain. In various types of tumors β-catenin degradation is frequently blocked as a consequence of inactivating mutations affecting proteins of the degradation complex. As a result, aberrant activation of β-catenin/Tcf downstream genes is enhanced (4, 8).

β-Catenin also interacts with factors of the basal transcription machinery, such as the TATA box-binding protein (TBP) (9) and p300/CBP (10, 11). p300/CBP may relax chromatin structure through its histone acetyltransferase activity, facilitating access of other factors to target gene promoters. However, β-catenin interaction with TBP and p300/CBP does not fully explain how β-catenin stimulates transcription of particular Tcf-regulated genes because some transactivating elements at the C terminus of β-catenin stimulate gene expression without binding to TBP or p300/CBP (9, 12). Therefore, to promote the activation β-catenin may interact with additional factors such as Brg-1 (13) or TIP49 (known as p53in52 or reptin52) (14, 15), an ATP-dependent DNA heli-

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§ The on-line version of this article (available at http://www.jbc.org) contains Supplemental Tables 1–3.

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†††† To whom correspondence maybe be addressed: Inst. Municipal d’Investigación Médica, c/ Dr. Aiguader 80, E-08003 Barcelona, Spain. Tel.: 34-93-221-1009; Fax: 34-93-221-3237; E-mail: agarcia@imim.es.

** To whom corresponding may be addressed: Unitat de Biofísica, Dept. Bioquímica i Biologia Molecular, Facultat de Medicina, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Spain. Tel.: 34-93-581-1870; Fax: 34-93-581-1907; E-mail: mireia.dunach@uab.es.
case found in complexes with chromatin-remodeling and histone-modifying proteins.

Plakoglobin (also known as γ-catenin) is closely related to β-catenin and can replace β-catenin in adherens junctions. It is also a component of the desmosomes where it mediates the link of desmosomal cadherins desmocollin and desmoglein to desmoplakin and the intermediate filament cytokeratin (16, 17). This role of plakoglobin is specific and cannot be exerted by β-catenin even though both proteins are structurally similar. Moreover plakoglobin has also been implicated in the regulation of the Wnt pathway. Kolligs et al. (18) observed that plakoglobin can induce transformation of RK3E cells with a higher efficiency than β-catenin. However, data from other laboratories, including ours, indicated that binding of plakoglobin decreases the affinity of Tcf-4 for DNA (19, 20), suggesting that the positive effect of plakoglobin on Tcf-mediated transcription can be explained by increased transport of β-catenin to the nucleus (21). It has been observed that β-catenin and plakoglobin bind to different sites on Tcf-4 (20). Moreover other data (for a review, see Ref. 21) indicate that plakoglobin overexpression is normally associated with tumor suppression contrary to what happens with β-catenin.

As mentioned above, the physiological roles of β-catenin and plakoglobin involve interactions with many factors. In some cases, these interactions are modulated by post-translational modifications. For instance, we have described that phosphorylation of β-catenin residues Tyr-654 and Tyr-142 specifically decreases its interaction with E-cadherin and α-catenin, respectively (22, 23). Structural data support these results because these two Tyr residues are involved in E-cadherin binding (Tyr-654 by establishing an ionic pair with E-cadherin Asp-667) (24) and in the stabilization of the β-catenin structure implicated in α-catenin binding (Tyr-142) (25). The Tyr kinases that catalyze these modifications have been identified with Fer and Fyn being responsible for β-catenin Tyr-142 phosphorylation (23), whereas Tyr-654 is modified by epidermal growth factor receptor or related proteins. A similar modulation has been proposed for plakoglobin action on desmosomes and adherens junctions. Tyrosine phosphorylation of plakoglobin after epidermal growth factor receptor activation has been related with loss of association with desmoplakin but not with desmoglein (26). Addition of the inhibitor of Tyr(P) phosphatases peroxovanadate inhibits the interaction of plakoglobin with α-catenin and E-cadherin, an effect that can be reverted by Tyr(P) phosphatase (27). Data from our group indicate that, although β-catenin and plakoglobin are closely related, the same kinases phosphorylate different residues in each. Moreover phosphorylation of equivalent residues has different effects on the interaction of plakoglobin and β-catenin with their cellular partners (28).

Both plakoglobin and β-catenin can be structurally divided into three subdomains. The central domain is composed of 12 repetitions of 42 amino acids each, named the armadillo repeat domain after the β-catenin ortholog in Drosophila, armadillo. This region, very similar in both proteins (with 83% identity) (29), has a basic pI, and its structure has been determined; it folds into a super helix composed of 36 small α-helices (three per armadillo repeat) (30). Tyr-654 lies on the last repeat of this domain, and Tyr-142 lies at the limit of this domain and the N-terminal tail. The armadillo repeat domain is flanked by more divergent N- and C-terminal regions, mainly acidic. The similarity between β-catenin and plakoglobin tails is low, especially for the C-tail (57 and <15% respectively, for the N- and C-tails) (29). The structure of the entire protein with the two β-catenin terminal domains is still unknown. In previous reports we have described that the β-catenin N- and C-tails interact with the armadillo domain and limit its ability to bind E-cadherin and other cofactors (31). Moreover binding of the two β-catenin tails is mutually dependent because the C-tail is necessary for the association of the N-tail to the armadillo domain. As a consequence of the interconnection of the tails, different cofactors interact coordinately with distant binding sites on the β-catenin molecule (32).

Here we report that the terminal tails of plakoglobin also interacted with its armadillo domain and restricted binding of interacting factors to this domain. The general organization of β-catenin and plakoglobin seems to be similar, although the plakoglobin C-tail bound to the armadillo domain more tightly than the β-catenin C-tail did. Moreover we demonstrated that the tails determine the differential specificity and affinity of each protein for different cofactors. Therefore, specificity was unaffected when the armadillo domain of β-catenin was replaced by that of plakoglobin, whereas it was totally altered when the tails were swapped.

**EXPERIMENTAL PROCEDURES**

**Reagents**—The following monoclonal antibodies were used: β-catenin C terminus (the epitope recognized by this antibody has been mapped to residues 696–750), plakoglobin C terminus (epitope recognized, residues 553–745), E-cadherin, α-catenin, and TATA box-binding protein (all from BD Transduction Laboratories, Lexington, KY); β-catenin armadillo core (epitope recognized, residues 422-6685) (from Alexis Biochemicals, San Diego, CA); plakoglobin C-end (epitope recognized, residues 698–745) (from Santa Cruz Biotechnology, Santa Cruz, CA); and Tcf-4 (clone 6H5-3, from Upstate Biotechnology, Lake Placid, NY). Antibody against glutathione S-transferase (GST) was from Amersham Biosciences.

**Generation of β-Catenin-Plakoglobin Chimeras**—The β-catenin and plakoglobin chimeras used in this study are depicted; the diagram indicates which parts of the molecules they comprise.
Chimera were produced, an additional AflIII site was generated at the beginning of the armadillo domain in plakoglobin (amino acid 140). β-Catenin has a restriction site for this enzyme in the nucleotide sequence corresponding to amino acid 150. The same procedure as that described above was followed using oligonucleotides 5'-ATGCAATGGTACCTACCATGTCAT-3' (sense) and 5'-TGGGGCGGTAGGTACCATCCATGTCAT-3' (antisense) for β-catenin and oligonucleotides 5'-ATGCCGAGCTGGCCACACGTGCC-CTGC-3' (sense) and 5'-TGCCGAGCAGCTGGCCACACGTGCC-CTGC-3' (antisense) for plakoglobin. After verifying that the KpnI site had been modified by cloning the two tails obtained by digesting the inverse catenin with KpnI and AflIII, purifying the fragments corresponding to the nucleotide sequences 331–348 and 2235–2217 and containing BamHI and EcoRV sites at their ends. The 2.1-kbp amplification fragment was digested with BamHI and cloned in the same line that contains pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 1.9-kbp amplification fragment corresponding to amino acids 1–18 and 2016–1999 and containing BamHI and XhoI sites at their ends. The 1.9-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends. The 2.1-kbp amplification fragment was digested with these enzymes and cloned in pGEX-6P3 cut with BamHI and XhoI sites at their ends.
performed. Thus, in this case, after quantitation the representative of those obtained in the several experiments the rest of the results presented below, were reproducible and the cytosolic domain of E-cadherin. These results, as well as the ability of the armadillo domain of plakoglobin to bind to the protein lacking only the N-tail (lane 5) presented a very slightly decreased association to the cytosolic domain of E-cadherin. Therefore, plakoglobin-C-tail restricts the interaction of E-cadherin to the armadillo repeats similarly to what we reported for β-catenin (although to a lower extent) (32), and the N-tail does not appear to exert a major effect on this binding.

The tails play a role also in the interaction of plakoglobin with α-catenin, another component of the adherens junctions. The region of plakoglobin involved in α-catenin binding has been located at the end of the N-tail and the first armadillo repeat between amino acids 109 and 137 (33). Since the integrity of this sequence was not preserved in the armadillo domain or in the ΔN-tail mutant, these constructions did not show binding to α-catenin (not shown). Deletion of the distant C-tail (lane 4) presented a very slightly decreased association to the cytosolic domain of E-cadherin. Therefore, plakoglobin-C-tail restricts the interaction of E-cadherin to the armadillo repeats similarly to what we reported for β-catenin (although to a lower extent) (32), and the N-tail does not appear to exert a major effect on this binding.

Binding of plakoglobin to its nuclear partner TBP was also analyzed (Fig. 2, C and D). Full-length plakoglobin interacted with TBP much more strongly than with β-catenin (Fig. 2C). Whereas in β-catenin the tails appeared to hinder the interaction, the plakoglobin armadillo domain bound TBP to a lower extent than the full-length protein (Fig. 2C). However, with respect to the bare armadillo domain, a plakoglobin mutant that also contained the C-tail (ΔN-tail mutant) presented a lower binding to TBP (Fig. 2D).

More striking results were obtained when the interaction to Tcf-4 was analyzed. The armadillo repeats of β-catenin and plakoglobin contain the binding domains for Tcf-4 (19, 34). As reported previously (20), only β-catenin, but not plakoglobin, interacts with the most amino-terminal sequence of Tcf-4 (amino acids 1–51); whereas plakoglobin binds to the Tcf-4 sequence comprised of amino acids 51–90. However, removal of the N- and C-terminal tails of both proteins completely abolished this specificity; the armadillo domains of plakoglobin and β-catenin bound indistinctly to both Tcf-4 sequences (Fig. 3).

All these results indicate that, as previously reported for β-catenin, plakoglobin tails also restrict the ability of the protein to bind other cofactors and determine its specificity. We next asked whether, as in the case of β-catenin, plakoglobin tails interact with the central armadillo domain. Overlay binding assays between the plakoglobin N- or the C-tails and the armadillo domain showed that both plakoglobin tails interacted with the armadillo domain of plakoglobin (Fig. 4, A and B). Furthermore they also interacted with a similar affinity with the armadillo domain of β-catenin. Reciprocally β-catenin...
C-tail bound to plakoglobin armadillo domain with a similar affinity as that to β-catenin armadillo domain (Fig. 4C). However, the mutual dependence on binding of the two tails to the armadillo domain was not the same in β-catenin and plakoglobin. As we have reported previously (32), the presence of the N-tail decreased the affinity of β-catenin C-tail for its armadillo domain (Fig. 4D). This effect was not observed for the binding of the plakoglobin C-tail: it interacted equally with the armadillo domain and with a form also comprising the N-terminal domain of plakoglobin (ΔC-plakoglobin) (Fig. 4E). The same results were obtained when binding of plakoglobin C-tail was analyzed on the heterologous proteins β-catenin armadillo domain and ΔC-β-catenin. Both proteins interacted similarly with the C-tail (data not shown). These results suggest that although plakoglobin terminal tails also interact with the armadillo domain, the interconnections established between these tails are not the same as those occurring in β-catenin, and this difference is caused by the different properties of both C-tails.

To further identify the role of terminal tails, chimeric pro-

**FIG. 4. Interaction of the N- and C-terminal tails with the plakoglobin and β-catenin armadillo domains.** 8 pmol of the armadillo domains of β-catenin or plakoglobin was separated by SDS-PAGE and transferred to nitrocellulose membranes. A, binding of recombinant proteins was determined by overlay assays as described under “Experimental Procedures” in which blots were incubated with recombinant GST-plakoglobin-N-tail (amino acids 1–113) (0.25 nmol/ml). Binding was analyzed by incubating with a mAb against GST that tagged the overlaid N-tail domain. B and C, the same procedure was repeated, incubating the membrane with a 0.25 nmol/ml concentration of either GST-plakoglobin-C-tail (667–end) and blotted with a mAb against this plakoglobin C-tail (B) or GST-β-catenin-C-tail (696–end) and blotted with a mAb against this β-catenin C-tail (C). D and E, 8 pmol of the armadillo domain of β-catenin and β-catenin-ΔC-tail (amino acids 1–683) (D) or of plakoglobin and plakoglobin-ΔC-tail (amino acids 1–672) (E) was separated by SDS-PAGE and transferred to nitrocellulose membranes. The same overlay assay as described above was performed, incubating the blot with recombinant GST-β-catenin-C-tail (D) or GST-plakoglobin-C-tail (E) (0.25 nmol/ml). Binding was analyzed with a mAb against the corresponding C-tail of each catenin. 25 ng of the corresponding terminal tails that were included as internal reference in the blots (St). To verify that equal amounts of proteins were loaded in the gel, membranes were stained with phenol red to detect the proteins used as baits (not shown). The figure shows a representative experiment of three performed in each case. BSA, bovine serum albumin; WB, Western blot.
Determinants of β-Catenin and Plakoglobin Specificity

Fig. 5. Binding of chimeras to E-cadherin, α-catenin, and TBP. 7 pmol of recombinant plakoglobin, β-catenin, or the indicated chimera fused to GST was incubated with 200 µg of BW11 total cell extracts. The complexes were purified with glutathione-Sepharose and analyzed by Western blot with a mAb against TBP, α-catenin, or E-cadherin. The numbers below the lanes correspond to the amount of bound protein relative to the value obtained for wild-type β-catenin. The membranes were rebotted using an antibody against GST, and similar quantities of each protein were found (lower panel). The figure shows a representative experiment of three performed in duplicate. The average ± S.D. of these data is presented in Supplemental Table 2. WB, Western blot.

Fig. 6. Binding of chimeras to different N-terminal domains of Tcf-4. 1 pmol of GST, GST-Tcf-4-(1–51) (upper panels), or GST-Tcf-4-(51–110) (lower panels) was incubated with 1 pmol of β-catenin, plakoglobin, or the indicated chimeras. The amount of bound β-catenin, plakoglobin, or chimeras Nβ-armpl-Cβ, Nβ-armβ-Cpl and Nβ-armβ-Cpl was determined using mAbs specific for β-catenin N-tail (left panels) or plakoglobin C-tail (right panels). In all panels, the autoradiograms were scanned, and the results were referred to known amounts of wild-type β-catenin (10 ng) or plakoglobin (5 ng) included in the gels, respectively, for the left or right panels. The numbers below the lanes indicate the amount of bound protein (ng) estimated by this method. The figure shows a representative experiment of three performed in each case in duplicate. The average ± S.D. of these data is presented in Supplemental Table 3. WB, Western blot.

Determinants of β-Catenin and Plakoglobin Specificity

Teins were generated in which the armadillo domain of one protein was fused to the terminal tails of the other catenin (Fig. 1). Analysis of the binding ability of the corresponding fusion proteins rendered results totally compatible with the above mentioned assumption. As shown in Fig. 5, substitution of the armadillo domain of plakoglobin with the equivalent region of β-catenin decreased only slightly the affinity for TBP (compare binding of TBP to plakoglobin and to the chimera Npl-armβ-Cpl, upper panel, lanes 4 and 5). However, when the two tails of plakoglobin were replaced by those of β-catenin (in the chimera Nβ-armpl-Cβ), binding of TBP was barely detectable (lane 3). More precisely, the N-terminal tail seems to be the key determinant of the TBP binding specificity since the swapping of the N-tail between the chimeras Npl-armβ-Cpl and Nβ-armβ-Cpl resulted in a dramatic inhibition of the association in the latter (upper panel, lanes 4 and 2).

We next examined the interaction with α-catenin. In this case, β-catenin presented a higher affinity than plakoglobin (Fig. 5, second upper panel, lanes 1 and 5), and again the specificity was conferred by the protein tails. Thus, the Nβ-armpl-Cβ and full-length β-catenin presented a similarly high affinity for α-catenin, whereas α-catenin bound to Npl-armβ-Cpl as weakly as full-length plakoglobin. Also in this case, the N-tail was the major contributor to this specificity: substitution of the N-tail between Npl-armβ-Cpl and Nβ-armβ-Cpl resulted in a more than 5-fold increase in the affinity for α-catenin in the latter (second upper panel, compare lanes 4 and 2).

Similar specificity results were obtained for E-cadherin, although the difference in the affinity for this protein between plakoglobin and β-catenin was smaller (Fig. 5, second bottom panel). However, in this case the C-tail played a more relevant role since replacement of the plakoglobin C-tail for that of β-catenin originated a chimera that presented an affinity for E-cadherin close to that of plakoglobin and lower than that of β-catenin (compare lanes 1, 2, and 5).

To further explore the role of the C-tails, we next studied the affinity of the chimeras for the two different binding domains present in Tcf-4. We took advantage of the high specificity that β-catenin and plakoglobin show for these sites with the former binding only to the Tcf-4-(1–51) amino acids and the latter binding only to the Tcf-4-(51–110) (see Fig. 3). While replacement of the armadillo domain of β-catenin with that of plakoglobin did not modify association to the Tcf-4-(1–51) sequence, substitution of the C-tail abolished this interaction almost completely (Fig. 6, left upper panel). On the other hand, the Tcf-4-(51–110) sequence, which contains the plakoglobin binding site, interacted similarly with full-length plakoglobin, with the Nβ-armβ-Cpl chimera, and even with Nβ-armβ-Cpl (right bottom panel), further supporting that the C-tail is responsible for conferring the binding specificity. Accordingly substitution of the β-catenin armadillo domain by that of plakoglobin did not increase the affinity of the protein for Tcf-4-(51–110) (left bottom panel).

To study in more detail the molecular basis of the different C-tail specificity between β-catenin and plakoglobin, we next carried out experiments of limited trypsinolysis. Fig. 7A shows that both β-catenin and plakoglobin C-tails were digested at almost identical rates when they were expressed as a fusion to
GST. On the other hand, degradation of the C-terminal epitope was faster in β-catenin than in plakoglobin, suggesting that in the entire protein plakoglobin C-tail was less accessible to the protease (Fig. 7, B and C). Remarkably the protein chimeras containing the heterologous armadillo domains, Nβ-arm pl-Cβ and Npl-arm β-Cpl, presented identical sensitivity to the wild-type proteins β-catenin and plakoglobin, respectively (Fig. 7B).

**DISCUSSION**

As indicated in the introduction, β-catenin and plakoglobin are two closely related proteins required for the formation of adherens junctions and desmosomes. Moreover, through its binding to Tcf-4, β-catenin can act as a transcriptional activator. The activity of the β-catenin/Tcf-4 complex is required for the initial steps of epithelial tumor development (8). Probably for this reason, β-catenin is normally found to be overexpressed in epithelial tumors (8). The role of plakoglobin as a transcriptional regulator is still a matter of discussion, but experiments using transgenic animals suggest that plakoglobin acts as an inhibitor of tumorigenesis (21). A plausible explanation for this contrary effect of plakoglobin and β-catenin comes from its different binding to Tcf-4; plakoglobin interacts with a different sequence of Tcf-4 than β-catenin and precludes binding of the complex to DNA (20). Surprisingly, as we will discuss below, the amino acids of β-catenin involved in the interaction with Tcf-4 (present in the armadillo domain) (34) are almost totally conserved in plakoglobin. The results presented in this article may explain why both proteins interact with different regions of Tcf-4: although both sites are present in both proteins, they are hidden by the N- and C-tails. The different folding of these tails in the conformation of the native proteins is the reason that only one of the sites is accessible in each protein.

In addition to Tcf-4, many other proteins that interact with β-catenin and plakoglobin do so through the central armadillo repeat domain (19). We now show here, as we previously reported for β-catenin, that the protein tails also determine the specificity of the interactions of plakoglobin with different cofactors. In all the cases studied, deletion of one of the tails in plakoglobin resulted in alterations in the binding of factors directly associated to the central armadillo domain. These results are in accordance with our previous observations, indicating that the two tails interact with the central armadillo domain in a way that deletion of one of the tails affects the association of the other (32). Therefore, our model is not limited to β-catenin and can be extended to at least another armadillo repeat domain, p120-catenin and APC (35), may also be regulated in a fashion similar to that which occurs in β-catenin and plakoglobin.

Moreover, our results suggest that, at least in the case of β-catenin and plakoglobin, the armadillo domains are totally exchangeable without modifying their specificity of interaction.
for the different Tcf-4 sequences or their distinct affinity for TBP, α-catenin, or E-cadherin. Thus, the binding site for the Tcf-4-(51–110) sequence, i.e. the plakoglobin binding site, is also present in β-catenin but held cryptic in the full-length protein. However, the role of the N- and C-terminal tails in determining the specificity of interaction is different for the distinct cofactors. For instance, for the binding of TBP and α-catenin, the N terminus seems to be the key determinant of the difference of affinity displayed by plakoglobin and β-catenin. In this sense, substitution of the N-tail between Nβ-armβ-Cpl and Npl-armβ-Cpl dramatically affected the affinity for these cofactors, whereas the equivalent exchange of C-tails did not. On the other hand, in the case of binding to E-cadherin or to the different sequences in Tcf-4, the C-tail plays the more relevant role. Replacement of the C-tail in β-catenin by that of plakoglobin in the Nβ-armβ-Cpl chimera conferred the ability to interact with the plakoglobin binding site of Tcf-4-(51–110) and practically abolished the interaction with the β-catenin binding site (Tcf-4-(1–51)).

In all cases, our results showed that the armadillo domain was not responsible for the specificity of binding of plakoglobin and β-catenin, although many factors physically interact through this domain. Moreover differences between the interaction of the tails and the armadillo domains of both proteins were due to the different properties of the tails and not of the armadillo domains. To this respect, the study of the interaction of β-catenin and plakoglobin with Tcf-4 is particularly illustrative. Both proteins bound Tcf-4, but β-catenin bound to Tcf-4 amino acids 1–51, and plakoglobin bound to amino acids 51–110. The complex formed by the armadillo repeat domain of β-catenin and the Tcf-4 region that binds to it (amino acids 1–49) has been crystalized (34). It is remarkable that practically all the β-catenin amino acids involved in this interaction are conserved in plakoglobin, and the only variations are conservative changes: substitutions of Pro-606 and Ile-607 by Ser and Ala, respectively. This model validates the prediction that conservative changes: substitutions of Pro-606 and Ile-607 by Ser and Ala, respectively. This model validates the prediction that

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