Immunodominant T Cell Epitope from Signal Sequence

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R. A. Henderson *et al.* (*1*) suggest that signal peptides or fragments thereof may be presented by class I major histocompatability complex (MHC) antigens as T cell epitopes that are biologically relevant. This possibility is illustrated by the fact that a dominant target peptide presented by D^b and recognized by cytotoxic T cells specific for lymphocytic choriomeningitis virus (LCMV) in H-2^b mice is derived from the signal sequence of the viral glycoprotein precursor GP-C. The dominant immunogenic peptide is gp-a, which is contained within amino acids 32 through 42 (*2*). This peptide is part of the leader sequence (amino acids 1 to 58) of the GP-C protein, the signal peptide of which was deduced by the predictive algorithm of G. von Heijne (*3*) and confirmed directly by NH_2-terminal amino acid sequencing of the GP-1 glycoprotein (*4*). Moreover, investigation of the pathway of signal peptide epitope presentation may also yield information of general biological interest about the fate of cleaved signal peptides in eukaryotic cells.

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Plakoglobin and β-Catenin: Distinct But Closely Related

Catenins are cytoplasmic proteins that were originally identified in association with the cytoplasmic domain of the cell adhesion molecule uvomorulin (E-cadherin), but they also form complexes with other proteins of the cadherin family (*1, 2*). P. D. McCrea *et al.* (*3*) isolated cDNA for the *Xenopus* β-catenin and performed a sequence comparison that revealed it to be homologous to mammalian plakoglobin and to the product of the segment polarity gene *armadillo* of *Drosophila*. They also found that a monoclonal antibody to bovine plakoglobin recognized canine β-catenin in Madin-Darby canine kidney (MDCK) cells, which raises the possibility that β-catenin may be identical to plakoglobin. However, we had found previously that antibodies to plakoglobin did not recognize components of the isolated uvomorulin-catenin complex (*1*).

To resolve this apparent contradiction, we used a cDNA probe of human plakoglobin (*4*) to screen a mouse cDNA library prepared from embryonal carcinoma cells, PCC4. We used intermediate hybridization and washing conditions to detect different degrees of homology (*5*). Phage clones with strong and weaker signals were plaque-purified. Restriction sites in the largest insert found in each group, 3.4 and 2.8 kb, respectively, were mapped and the insert was sequenced (Fig. 1).

We found the primary structure encoded by the cDNA with strong hybridization signals to have a high homology (99% identity) to human plakoglobin. In contrast, the deduced amino acid sequence of the cDNA with the weaker hybridization signal showed a strong homology (97% identity) to *Xenopus* β-catenin and a weak homology (68%) to human plakoglobin. The cDNAs each detected a single copy gene in Southern blot (DNA) analysis of mouse genomic DNA (*6*).

These results indicate that mouse plakoglobin and β-catenin are distinct but related proteins. Out of 781 amino acids, β-catenin from mouse and from *Xenopus* differed only in 21 residues. Such evolutionary conservation indicates that β-catenin manifests basic cellular functions. To characterize β-catenin further and to demonstrate that it forms a complex with uvomorulin, we produced rabbit antibodies to a synthetic peptide specific for β-catenin (Fig. 1). These antibodies specifically recognized β-catenin from uvomorulin-catenin complexes collected by antibodies to uvomorulin (Fig. 2A, lane 3). The antibodies specific for β-catenin cross-reacted on immunoblots (*8*) with the homologous protein from human, bovine, mouse, chicken, and *Xenopus* cell lysates (Fig. 2C). When immunoblots of whole-cell lysates from different species were stained with antibodies to β-catenin and to plakoglobin, these proteins exhibited different electrophoretic mobilities (Fig. 2B, lanes 4 and 5); the

Fig. 1 (right). Alignment of the mouse β-catenin (Mouse-Beta) and mouse plakoglobin (Mouse-Plako) protein sequences with the corresponding sequences of *Xenopus* β-catenin (Xenopus-Beta) and human plakoglobin (Human-Plako). The amino terminus of mouse plakoglobin (around 124 residues) is missing, so the residue number is provisional. Amino acids identical in three or four protein sequences are boxed; dots represent gaps. A synthetic peptide of the amino acid sequence, denoted by an overline, was used for antibody production. Abbreviations for the amino acid residues are as follows. A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

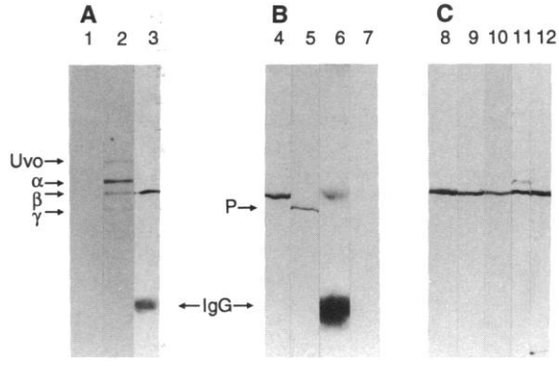
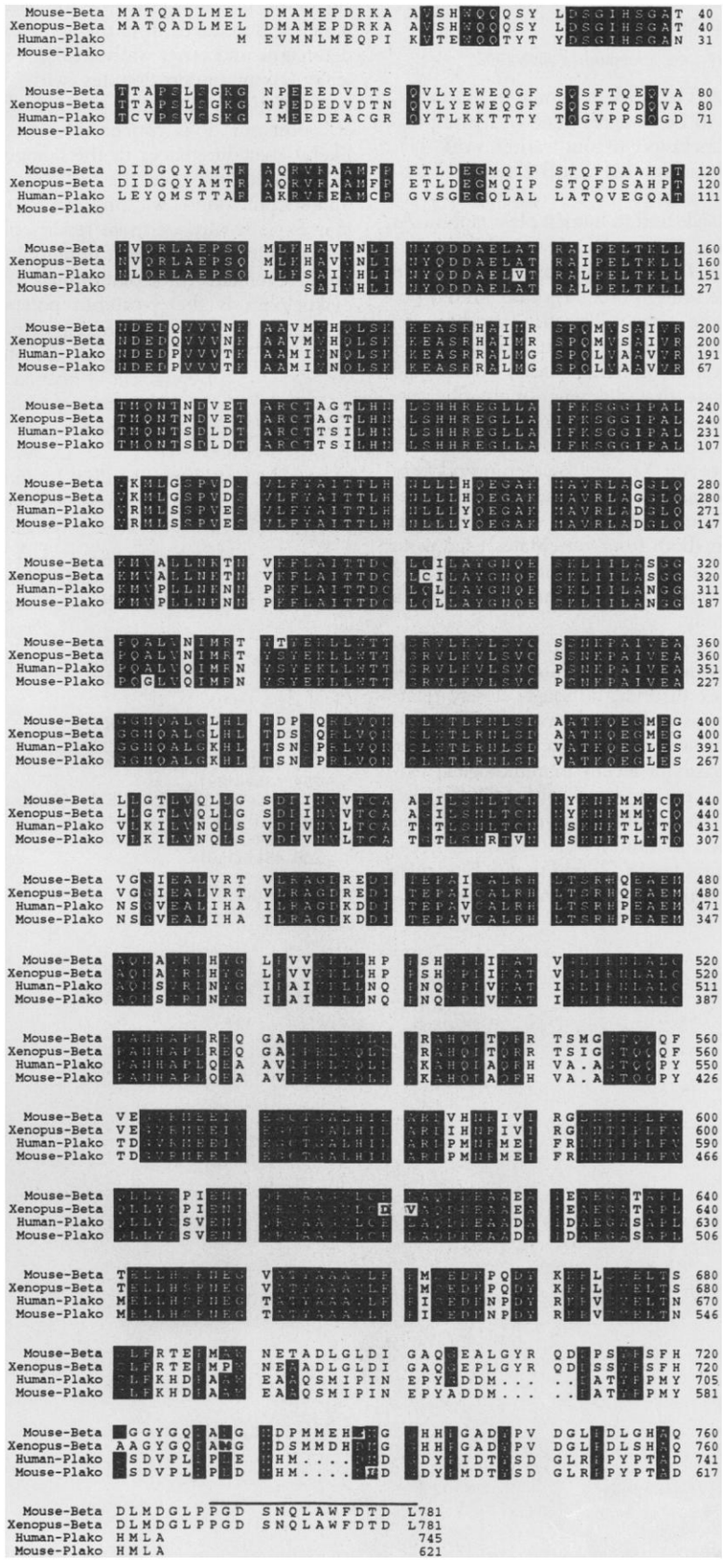


Fig. 2. Detection of β-catenin in uvomorulin-catenin complexes by antibodies to β-catenin and recognition of the homologous proteins in human, bovine, canine, chicken, and *Xenopus* cell lysates by SDS-PAGE analysis. (**A**) Uvomorulin-catenin complexes immunoprecipitated by antibodies to uvomorulin (lane 2) from cell lysates of mouse epithelial cells labeled with ^{35}S-methionine, CMT. Lane 1 is a control with an unrelated antibody. Lane 3 is an immunoblot of lane 2 stained with antibodies to β-catenin. (**B**) Whole-cell lysates (lanes 4 and 5) from MDCK cells, or immunoprecipitates collected with antibody to uvomorulin (lanes 6 and 7), were probed in immunoblots with antibodies to β-catenin (lanes 4 and 6) or to plakoglobin (lanes 5 and 7). (**C**) Whole-cell lysates from mouse embryonal carcinoma cells, F9 (lane 8), bovine MDBK cells (lane 9), human JAR cells (lane 10), chicken fibroblast cells (lane 11), and *Xenopus* epithelial cells, A6 (lane 12) were probed with antibodies to β-catenin.

antibodies reacted only with the respective proteins, which allowed us to investigate the reactivity of both antibodies to components of the uvomorulin-catenin complex.



We investigated the possible association of plakoglobin with the uvomorulin-catenin complex in bovine and canine cells because the monoclonal antibody to bovine plakoglobin is of mouse origin. Immunocomplexes collected from MDCK and Madin-Darby bovine kidney (MDBK) cell lysates with rabbit antibodies to uvomorulin were blotted and stained with antibodies to either β-catenin or to plakoglobin. Whereas antibodies to plakoglobin were negative, β-catenin was easily detectable in the complex (Fig. 2B, lanes 6 and 7). Therefore, in spite of the high degree of homology, plakoglobin and β-catenin exhibited distinct functional properties. These results are in agreement with pulse-chase experiments in which β-catenin was found to associate with the uvomorulin precursor polypeptide (*9*), which suggests that only β-catenin can interact directly with uvomorulin. Nevertheless, a possible association of plakoglobin with the uvomorulin-catenin complex cannot be ruled out. Different solubilization procedures, with other detergents or ionic strengths, could result in soluble protein complexes of various sizes. It seems possible that under certain solubilization conditions plakoglobin could interact with the uvomorulin-catenin complex. In immunofluorescence tests on permeabilized canine MDCK and bovine MDBK cells, both proteins localized at the plasma membrane. Moreover, in epithelial cells, plakoglobin is a component of the desmosomal dense plaque, but it is also localized in the adherens-type junctions, where uvomorulin and catenins are found (*10*). These findings suggest a close colocalization and, together with the structural homology, indicate that both proteins may be involved in similar cellular processes.

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5. To obtain cDNA clones coding for mouse plakoglobin or mouse β-catenin, we screened 5 × 10^5 clones of a mouse λ gt11 cDNA library prepared from embryonal carcinoma cells PCC4 and probed with radioactively labeled, full-length cDNA for human plakoglobin (*4*) under intermediate conditions (1× standard sodium citrate; 0.1% sodium dodecyl sulfate at 65°C). The inserts from plaques with strong and weak signals were sized, the bacteriophages with the largest insert

in each class, designated λ W6 and λ H8, were cloned into pBluescript I SK+ vector, and both strands were sequenced (Sequenase Version 2.0, protocol U.S. Biochemicals, Cleveland, OH). The 3.4 kb fragment of λ H8 represents the full-length cDNA of mouse β-catenin, whereas the 2.8 kb fragment of λ W6 encodes part of mouse plakoglobin, beginning at amino acid 124. The deduced amino acid sequence of mouse β-catenin has a calculated molecular size of 85.5 kD, which agrees with 88 kD, the molecular size of uvomorulin-complexed β-catenin. Protein sequence alignment was done with a Genetics Computer Group program for the VAX. The GenBank accession number for mouse β-catenin is M90364 and for mouse plakoglobin, M90365.

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7. A peptide containing the amino acid sequence overlined in Fig. 1, which represents the carboxyl terminus of β-catenin, was coupled by glutaraldehyde to keyhole limpet hemocyanin (Sigma, St. Louis, MO). After three subcutaneous immunizations at 3-week intervals, specific antibodies were isolated on a peptide-ε-aminohexanoyl (EAH)–Sepharose (Pharmacia, Fairfield, NJ), column (5 mg of peptide coupled to 1 ml of EAH-Sepharose 4B, as described by the manufacturer.
8. Immunoprecipitation and immunoblot experiments were performed as described in (*1*). Immunocomplexes were collected from lysates of 4 × 10^6 cells with 4 μg of antibodies to uvomorulin and Protein A–Sepharose (Pharmacia). Proteins were separated by 8% SDS-PAGE under reducing conditions. Antibodies to β-catenin (5 μg per milliliter) and to plakoglobin (1 μg per milliliter) were diluted in phosphate-buffered saline in immunoblots. Mouse monoclonal antibody to bovine plakoglobin was purchased from Progen (Heidelberg, Federal Republic of Germany).
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11. We thank B. M. Gumbiner for providing unpublished sequences and W. W. Franke for providing full-length cDNA of human plakoglobin. Comments on the manuscript by D. Solter, K. Schughart, S. Wood, and R. Cassada are gratefully acknowledged. We thank V. Person and U. Birsner for synthesis and purification of oligonucleotides and peptides, L. Lay for the preparation of photographs, and R. Schneider for typing the manuscript. Supported by the Deutsche Forschungsgemeinschaft.

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Response: The work presented by Butz *et al.* appears to be in agreement with the principal conclusion of our earlier work (*1*), which demonstrated that β-catenin is highly homologous to the *Drosophila* gene product *armadillo* and to human plakoglobin. At that time, we erred in favoring the interpretation that plakoglobin and β-catenin were the same protein because we did not notice the small difference in their gel mobilities and did not yet have antibodies to β-catenin (or to *armadillo*) that would have made that difference more obvious. Nonetheless, the greater degree of sequence conservation maintained over evolutionary time between *Xenopus* β-catenin and *Drosophila armadillo*, compared with that between *Xenopus* β-catenin and human plakoglobin (both from vertebrates), led us to discuss the possibility that β-catenin and plakoglobin might be distinct members of a gene family.

Butz *et al.* present excellent and interesting evidence that β-catenin and plakoglobin are distinct, although closely related, proteins within the same cell. We are in complete agreement concerning this issue; on the basis of recent immunological evidence from experiments with MDCK cells, we have, in a collaborative effort, reached the same conclusion (*2*).

Our findings in (*2*) and that of Butz *et al.* differ on one point. We find that plakoglobin is a component of the E-cadherin–catenin complex. Although it is more weakly associated with the complex than is β-catenin [as determined by the ease with which it is removed from the complex with detergents and other washes (*3*)], it specifically coimmunoprecipitates with E-cadherin from MDCK cells (*1*, *2*). As Butz *et al.* point out, it is conceivable (we think likely) that differences in the composition of the solutions used for cell extraction, immunoprecipitate washing, and so forth may explain why we more readily observe plakoglobin in E-cadherin immunoprecipitates. It remains to be determined whether plakoglobin is the γ-catenin polypeptide present in E-cadherin immunoprecipitates.

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