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 $H\text{-}2^b$ 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.

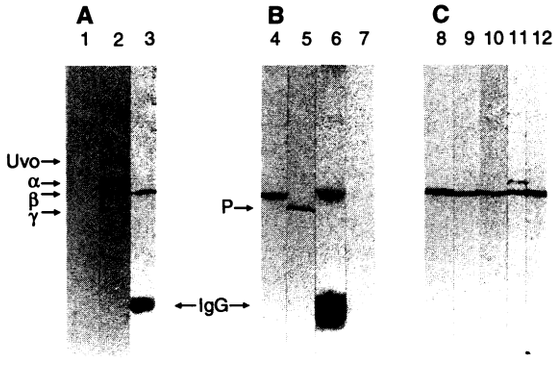

Fig. 2. Detection of β-catenin in uvomorulin-catenin complexes by antibodies to β-catenin and recognition of the homologous proteins in human, bovine, canine, chicken, and *Xenopus* cell lysates by SDS-PAGE analysis. (**A**) Uvomorulin-catenin complexes immunoprecipitated by antibodies to uvomorulin (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.