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Human adenovirus type 9 is unique among oncogenic adenoviruses in generating estrogen-dependent mammary tumors in rats. The Ad9 E4 open reading frame 1 [9ORF1] oncogene is the major oncogenic determinant of this virus. 9ORF1 codes for a polypeptide related to the enzyme dUTP pyrophosphatase, although it lacks this enzymatic activity. The 9ORF1 protein also possesses a functional PDZ domain-binding motif at its C-terminus and disruption of this motif abolishes its transforming activity. PDZ domains are protein-protein interaction modules found in a novel class of cell signaling proteins that localize to specialized membrane sites in cells. Significantly, we have now identified two cellular PDZ-domain proteins (DLG and 9BP-1) that bind to the 9ORF1 oncoprotein. DLG is a mammalian homologue of the *Drosophila* discs-large tumor suppressor protein and complexes with the Adenomatous Polyposis Coli tumor suppressor protein in cells. 9BP-1 (9ORF1 Binding Protein-1) is a novel cellular factor containing thirteen PDZ domains. Our findings suggest that transformation by the 9ORF1 oncoprotein is, in part, mediated by its ability to bind to and perturb the activity of cellular PDZ-domain proteins. We hypothesize that these cellular factors are critical components of signaling pathways involved in the control of cellular proliferation and in oncogenesis.

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FOREWORD

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Ronald Jan 6/24/98

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INTRODUCTION

Adenovirus type 9 (Ad9) is unique in eliciting exclusively mammary tumors. Ad9 is a subgroup D human adenovirus (12) and, in people, several members of this subgroup cause epidemic outbreaks of keratoconjunctivitis, a painful and highly contagious eye infection that can lead to corneal opacities (16). Although subgroup D adenoviruses do not produce tumors in hamsters (47), Ad9 is oncogenic in Wistar-Furth (W-Fu) rats and distinct from prototype oncogenic adenoviruses by displaying a strict tropism for the mammary gland. Following subcutaneous injection of newborn rats, Ad9 elicits exclusively estrogen-dependent mammary tumors in female animals but is non-tumorigenic in males (2, 3, 20). The female rats normally develop multiple tumors involving several mammary glands (20). Like prototype oncogenic adenoviruses, Ad9 viral DNA is integrated into the chromosomes of tumor cells in a non-infectious state, and the viral genome copy number varies from less than one to multiple copies per cell (3, 20). The presence of unique viral-host DNA junction fragments in each Ad9-induced mammary tumor indicates that these neoplasms have a monoclonal origin (20). Ad9 also efficiently transforms established rat embryo fibroblast cell lines *in vitro* (7), yet transfection of Ad9 viral DNA into primary baby rat kidney cells or infection of primary rat embryo fibroblasts with Ad9 virus fails to produce transformed foci (19). Therefore, Ad9 differs from prototype oncogenic adenoviruses in generating mammary tumors in animals and failing to transform primary rat embryo fibroblast or kidney epithelial cells.

Ad9 E4 region open reading frame 1 (9ORF1) encodes a novel oncogenic determinant. In contrast to Ad9, most subgroup D adenoviruses, including Ad26, fail to generate tumors of any kind in rats (21). This observation suggested that an Ad9 gene required for mammary oncogenesis might be identified by constructing and analyzing Ad9-Ad26 recombinant viruses. From such studies, it was determined that, unlike prototype oncogenic adenoviruses in which only the viral E1 region is needed for tumorigenicity, Ad9 has a critical oncogenic determinant(s) within its E4 region (21). The E4 regions of human adenoviruses are approximately 3-kilobase transcription units containing six open reading frames (ORFs) (14, 15, 22) and coding for regulatory proteins involved in the control of gene expression (6, 13-15, 17, 34). E4 proteins are expressed from a complex group of mRNAs arising from alternative splicing of a primary transcript and sharing a common start site and polyadenylation signal. Sequences for the Ad9 and Ad26 E4 regions indicate that these two transcription units possess no major organizational differences and are 93% identical at the nucleotide (nt) level (22).

Significantly, besides its requirement for mammary tumorigenesis, the Ad9 E4 region alone also produces transformed foci on the rat embryo fibroblast cell line CREF (21), implying that this viral transcription unit codes for an oncoprotein. Prompted by this observation, we performed transformation assays with each Ad9 E4 ORF introduced individually into an expression plasmid. Among seven different Ad9 E4 ORFs, only 9ORF1 generated transformed foci on CREF cells (23). In these cells, 9ORF1 also produced morphological alterations, anchorage-independent growth, higher saturation densities, as well as dramatically elevated oncogenicity in animals (53). Additionally, in human TE85 cells, the E4 ORF1 genes from subgroup A, B, and C human adenoviruses exhibit cellular growth-transforming potentials similar to that of 9ORF1 (52). This suggests that E4 ORF1s represent a family of related viral transforming genes. The 9ORF1 gene codes for a 14-kD polypeptide that is detected in both Ad9-induced rat mammary tumors and 9ORF1-transformed CREF cells (23). The results of confocal laser scanning microscopy further indicate that this viral protein is located primarily within the cytoplasm of cells (53). Significantly, mutant viruses specifically unable to express the 9ORF1 protein also fail to generate mammary tumors in rats (23). Taken together, these findings indicate that 9ORF1 encodes a cytoplasmic oncoprotein necessary for the generation of Ad9-induced rat mammary tumors.

Transformation-defective 9ORF1 mutant genes define three protein regions important for transformation. To identify 9ORF1 protein regions important for transformation and oncogenesis, we generated 48 different mutant 9ORF1 genes (50). From this collection, we identified seven mutants (mut-IA, mut-IIA, mut-IIB, mut-IIIA, mut-IIIB, mut-IIIC, mut-IIID) that, in general, express approximately wild-type levels of 9ORF1 protein in CREF cells, yet are severely deficient for inducing focus formation and growth in soft agar, as well as for increasing the tumorigenic growth of these cells. The altered amino-acid residues of these mutant polypeptides localize to three separate 9ORF1 protein

regions designated Region I (residues 34-41), Region II (residues 89-92) and Region III (residues 122-125) at the extreme C-terminus of the polypeptide.

C-terminal Region III of the 9ORF1 protein mediates direct binding to multiple cellular polypeptides. We also demonstrated that the 9ORF1 protein associates directly with multiple cellular polypeptides (p220, p180, p160, p155, p145, p140/p130) *in vitro* and *in vivo* (51). These cellular factors, most of which are phosphoproteins, are implicated in transformation because all four 9ORF1 Region III transformation-defective mutants (mut-IIIA, mut-IIIB, mut-IIIC, mut-IIID) show reduced capacities to associate with these polypeptides. The correlation between 9ORF1 transforming potential and binding to these cellular factors is further strengthened by the facts that in CREF cells (i) mut-IIIA and mut-IIIB fail to interact detectably with any of the cellular proteins and are severely transformation defective whereas (ii) mut-IIIC and mut-IIID show reduced protein binding activity and retain weak, leaky transforming activity (51). Wild-type Ad5 and Ad12 E4 ORF1 transforming proteins also interact with most of the 9ORF1-associated cellular polypeptides, as do C-terminal Region III-containing 9ORF1 protein fragments, albeit at reduced levels. Collectively, these findings indicate that Region III is a primary determinant in mediating direct binding of the 9ORF1 protein to cellular polypeptides.

C-terminal Region III of the 9ORF1 protein represents a functional PDZ domain-binding motif. We have now identified two cellular polypeptides that bind to the wild-type 9ORF1 protein but not to the mut-IIIA protein (28) (see *Body of Annual Report* below). Screening a λgt11 murine cDNA expression library with a 9ORF1 protein probe led to the isolation of a partial 9BP-1 (9ORF1-Binding Protein-1) cDNA which codes for the C-terminal 526 amino-acid residues of a novel cellular polypeptide. Although novel, the analysis of the partial sequence of 9BP-1 indicated that it possesses four PDZ domains (28). PDZ domains are found in proteins from a variety of organisms (37) and represent 80 to 100 residue modular units which, like SRC homology region 2 (SH2), SH3, and phosphotyrosine-binding (PTB) domains, mediate protein-protein interactions (24, 27, 45). While the term PDZ derives from the names of three proteins first recognized to contain these domains (Postsynaptic density protein [PSD-95], Discs large tumor suppressor [dlg], and Zonula occludens protein [ZO-1]), more than 50 PDZ-domain proteins are now known (8, 10, 11). In general, PDZ-domain proteins function in signal transduction by serving as adapter proteins that cluster membrane proteins and signaling molecules into multiprotein complexes at specialized membrane sites, such as adherens and tight junctions (40, 42, 43). In addition to forming specific homophilic associations with other PDZ domains (5), PDZ domains also bind to sequence motifs present at the free C-terminus of target polypeptides (45). With regard to the latter type of interaction, one well-known consensus C-terminal binding motif for certain PDZ domains is -(S/T)-X-(V/I)-COOH (where X denotes any amino acid) (24, 27, 45). Significantly, 9ORF1 and other adenovirus E4 ORF1 transforming proteins, as well as the HTLV-1 Tax and all high-risk HPV E6 oncoproteins, possess this consensus PDZ domain-binding motif at their C-termini (28). Moreover, the Region III mutations of transformation-defective 9ORF1 mutants mutIII-A, mutIII-B, mutIII-C, mutIII-D are contained within or adjacent to this motif (28, 51). For this type of PDZ domain-binding motif, mutation of the conserved residue at position 0 or position -2 from the C-terminus destroys binding activity (24). Similarly, for the 9ORF1 protein, substitution of alanine for valine at position 0 or aspartic acid for threonine at position -2 abolishes binding to cellular proteins, as well as transforming activity (unpublished results) (28). From these results, we conclude that 9ORF1 Region III is a functional PDZ domain-binding motif. This finding also argues that the detected 9ORF1-associated factors are cellular PDZ-domain proteins.

The 9ORF1 protein binds to the cellular PDZ-domain protein DLG, a putative tumor suppressor. Because 9ORF1 is an oncogene, the findings presented above prompted investigations into whether the 9ORF1 protein interacts with a known PDZ-domain protein having an established or suspected role in neoplasia. This survey revealed that DLG is a mammalian homologue of the *Drosophila* discs large tumor suppressor protein dlg (29, 33). These related proteins are members of the membrane-associated guanylate kinase (MAGUK) family of proteins which contain, in addition to three PDZ domains, an SH3 domain and a region with homology to guanylate kinases (Fig. 1) (26). In *Drosophila* imaginal disc epithelia, dlg localizes to septate junctions, the equivalent of tight junctions in mammalian cells, and homozygous *dlg* mutations lead to disruption of cell junctions, shape, and polarity, as well as to neoplastic growth (54). Mammalian DLG is functionally homologous to dlg because expression of DLG in *dlg* null mutant *Drosophila* rescues their defects (46). In addition, DLG PDZ domains bind to proteins having the C-terminal consensus sequence -(S/T)-X-(V/I)-COOH (45) and, interestingly, one such cellular factor is the

tumor suppressor protein APC (31). We also suspected that DLG might be 9ORF1-associated protein p140/p130 because DLG migrates as a group of bands at approximately 130-140 kD in protein gels (31, 33).

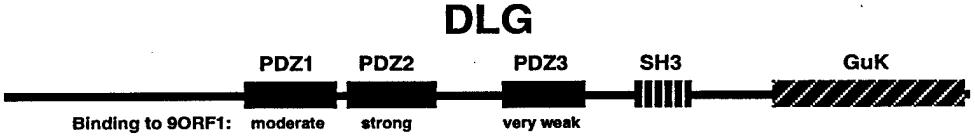

Fig. 1. Domain organization of DLG. Relative binding preferences of the 9ORF1 protein to PDZ domains were determined using protein blotting assays (28). SH3, Src homology 3 domain; GuK, Guanylate kinase-like domain.

Urged by these observations, we tested whether DLG binds to the 9ORF1 protein. We found that DLG and wild-type 9ORF1 protein, but not mut-IIIA protein, co-immunoprecipitate from CREF cell lysates and, in addition, that DLG also complexes with the Ad12 and Ad5 E4 ORF1 transforming proteins, as well as the HTLV-1 Tax and HPV type 18 E6 oncoproteins (28). That the 9ORF1-associated protein bands p140/p130 represent DLG was suggested by the facts that protein bands comprising each of these factors (i) co-migrate in protein gels, (ii) show identical species-specific mobility, and (iii) bind to mut-IIIC but not to mut-IIIA, mut-IIIB, or mut-IIID 9ORF1 protein (28). Consistent with the hypothesis that the 9ORF1 protein may require binding to several different 9ORF1-associated cellular factors to retain full transforming activity, leaky mutant mut-IIIC displays wild-type binding to DLG but binds weakly or not at all to other 9ORF1-associated cellular factors. The results of protein blotting assays further indicated that the 9ORF1 protein interacts directly with DLG, binding preferentially to DLG PDZ2, less strongly to PDZ1, and only very weakly with PDZ3 (28). These findings suggest that the 9ORF1 protein binds directly to the cellular factor DLG and that this interaction may be important for both 9ORF1 transforming activity and mammary oncogenesis by Ad9.

Although the function of DLG in mammalian cells is not yet known, the protein domains found in this cellular factor suggest an involvement in cell signaling. Because related *Drosophila* dlg is a tumor suppressor, it seems possible that both dlg and DLG transmit growth-inhibitory signals from sites of cell-cell contact to downstream effectors. Additionally, it may be important that, like the 9ORF1 protein, the tumor suppressor protein APC also complexes with DLG in mammalian cells and that this interaction similarly involves a C-terminal PDZ domain-binding motif and DLG PDZ2 (31). Further considering that APC sustains C-terminal truncations in most sporadic and familial colon cancers (36), it is conceivable that DLG:APC protein complexes participate in negatively regulating cell cycle progression. If so, one interesting possibility is that, by binding to DLG, the 9ORF1 protein blocks the formation or activity of DLG:APC complexes and, thereby, permits unregulated cellular proliferation. Clearly, more work is necessary to determine the functional consequences of the interaction between DLG and the 9ORF1 oncoprotein.

The 9ORF1 oncoprotein is related to dUTPase enzymes, yet lacks this enzymatic activity. We have also shown that human adenovirus E4 ORF1 polypeptides display sequence similarity with a variety of cellular and viral dUTPase enzymes (52). Moreover, avian adenovirus CELO codes for a dUTPase gene in a genomic location analogous to that of the human adenovirus E4 ORF1 genes (52), suggesting that these proteins may be evolutionarily related. A high cellular dTTP:dUTP ratio is essential to avoid uracil incorporation into DNA, and dUTPases function to hydrolyze dUTP to dUMP and pyrophosphate (38). This enzymatic activity also provides dUMP for dTTP biosynthesis. Despite their sequence similarity, however, the five conserved protein motifs found in dUTPase enzymes, including a nucleotide phosphate-binding p-loop domain, are not preserved in E4 ORF1 polypeptides. Consistent with this observation, E4 ORF1 proteins do not exhibit detectable dUTPase enzymatic activity (52), although it remains possible that these viral proteins possess an enzymatic activity related to but different from that of dUTPases. Nevertheless, in addition to their sequence similarity, E4 ORF1 and dUTPase proteins are also predicted to be structurally related (52). In support of this idea, we presented data in the application for this grant award suggesting that, in cells, the 9ORF1 protein forms homo-trimers, similar to dUTPase enzymes. The observed sequence similarity of the 9ORF1 protein with dUTPases is likely to provide important insights into the function of this viral oncoprotein.

BODY OF ANNUAL REPORT

OBJECTIVE 1: Identify the 9ORF1-associated cellular proteins.

The 9ORF1 oncoprotein forms complexes with DLG in Ad9-induced mammary tumors cells and may mislocalize this cellular factor in cells. We previously showed that 9ORF1 and the putative tumor suppressor protein DLG form a complex in 9ORF1-transformed CREF cells (28). We were next interested in assessing whether similar complexes were also formed in Ad9-induced mammary tumor cells. To examine this possibility, we immunoprecipitated 9ORF1 protein from a cell line derived from an Ad9-induced mammary tumor and immunoblotted immunoprecipitates with DLG antiserum. Significantly, as for 9ORF1-transformed CREF cells, DLG co-immunoprecipitated with 9ORF1 protein in this experiment (Fig. 2), indicating that 9ORF1 and DLG are also complexed within Ad9-induced rat mammary tumor cells.

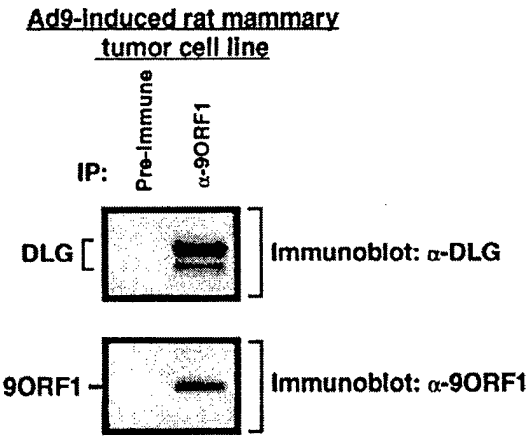

Fig. 2. DLG co-immunoprecipitates with 9ORF1 protein expressed in an Ad9-induced rat mammary tumor cell line. RIPA-buffer cell extracts containing 4 mg of protein were immunoprecipitated with 9ORF1 antiserum or the matched pre-immune serum. Recovered proteins were separated by SDS-PAGE and transferred to a membrane. Relevant portions of the membrane were immunoblotted with either DLG or 9ORF1 antiserum as described previously (28).

Like the 9ORF1 protein, the neuronal membrane potassium channel protein Kv1.4 utilizes a C-terminal PDZ domain-binding motif to bind directly to DLG PDZ(1+2) (24, 25). When either DLG or Kv1.4 is overexpressed alone in COS7 cells, each exhibits diffuse cellular staining by indirect immunofluorescence. When co-expressed, however, these proteins redistribute into plaque-like clusters (25). This redistribution is thought to reflect the ability of DLG to aggregate specific protein targets to specialized sites of cell-cell contact. Therefore, we wanted to determine whether the 9ORF1 protein would also cluster DLG in cells. Significantly, when co-expressed with wild-type 9ORF1 protein in COS7 cells, DLG redistributed into clusters within the cytoplasm of these cells (Fig. 3). These results were specific because, when expressed alone or co-expressed with mut-IIIA 9ORF1 protein, DLG remained diffusely distributed in cells. These findings confirm that the 9ORF1 and DLG proteins physically associate within cells and, more important, hint that the 9ORF1 oncoprotein may cause DLG to become mislocalized in cells. Future experiments will examine this interesting possibility.

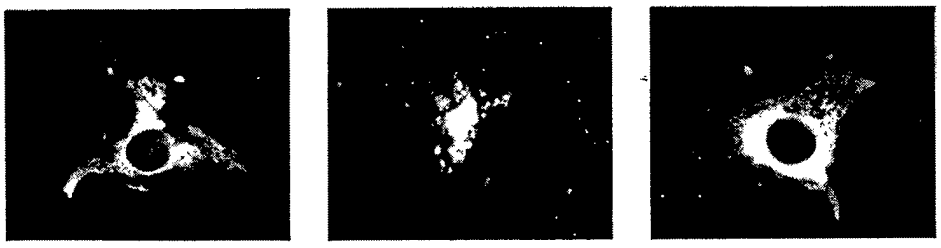

Fig. 3. Wild-type but not mut-IIIA 9ORF1 protein expression causes specific clustering of DLG in COS cells. COS7 cells were lipofected with 2.5 μg of expression plasmid GW1-CMV rat SAP97 (DLG) alone or with 2.5 μg of either GW1-CMV wild-type 9ORF1 (DLG + wild-type 9ORF1) or GW1-CMV mut-IIIA 9ORF1 (DLG + 9ORF1 mut-IIIA). 48h post-transfection, cells were fixed in methanol and analyzed by indirect immunofluorescence using a primary DLG antiserum (provided by Dr. Kyung-Ok Cho, Department of Cell Biology, Baylor College of Medicine) and secondary goat anti-rabbit fluorescein isothiocyanate (FITC)-conjugated antiserum. A single representative cell from each experiment is shown. Separate analyses indicated that wild-type and mut-IIIA 9ORF1 proteins were expressed at similar levels in the cells (data not shown).

9BP-1 is 9ORF1-associated protein p220. Like DLG, the novel cellular PDZ-domain protein 9BP-1 may also represent a previously observed 9ORF1-associated protein. To test this idea, we generated rabbit polyclonal antisera to a histidine-tagged fusion protein containing the 9BP-1 C-terminal 526 amino-acid residues. In cell lysates from mouse, rat, and human cell lines, immunoblot analyses with 9BP-1 antiserum, but not with matched pre-immune serum (data not shown), revealed one prominent band having the same species-specific gel mobility as 9ORF1-associated protein p220 (Fig. 4) (51). To determine whether 9BP-1 in cell lysates binds to the 9ORF1 protein *in vitro*, we performed GST pulldown assays with wild-type or mutant 9ORF1 proteins, and immunoblotted recovered cellular proteins with 9BP-1 antiserum. We found that, like 9ORF1-associated protein p220 (51), 9BP-1 binds to wild-type and mut-IIID 9ORF1 proteins, but not to mut-IIIA, mut-IIIB, or mut-IIIC protein (Fig. 5). Additionally, 9BP-1 also bound to the related 5ORF1 and 12ORF1 transforming proteins. These results suggest that 9BP-1 is 9ORF1-associated protein p220. The fact that 9BP-1 binds mut-IIID but not mut-IIIC whereas, conversely, DLG binds mut-IIIC but not mut-IIID (28) may indicate that binding to both 9BP-1 and DLG is necessary for full transforming activity of the 9ORF1 protein.

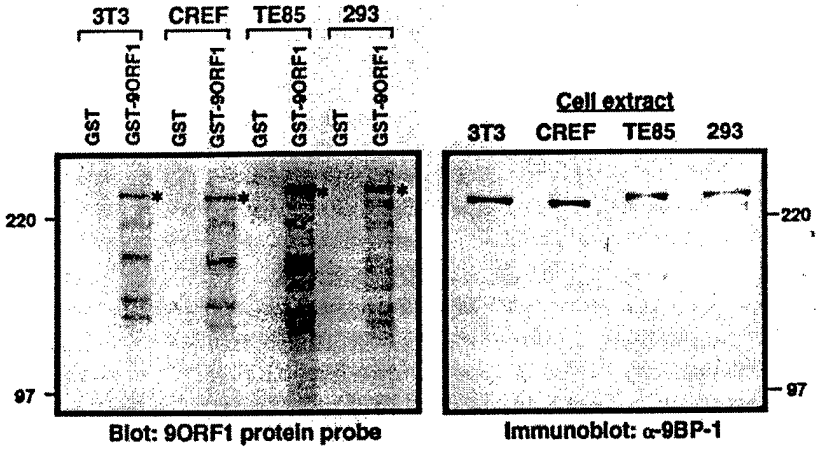

Fig. 4. 9BP-1 and 9ORF1-associated protein p220 co-migrate and show the same species-specific gel mobility. *Left panel*, Mouse 3T3, rat CREF, human TE85, or human 293 cells were lysed in RIPA buffer, and 2 mg of total cell proteins was incubated with 5 μg of GST or GST-9ORF1 fusion proteins on beads. Recovered cellular proteins were separated by 7.5% SDS-PAGE, transferred to a membrane, and protein blotted with a radiolabelled GST-9ORF1 protein probe as described previously (51). 9ORF1-associated protein p220 is indicated with an asterisk. *Right panel*, In parallel with the experiment described above, 20 μg of total cell proteins from the different cell lines was separated by 7.5% SDS-PAGE, transferred to a membrane, and immunoblotted with 9BP-1 rabbit polyclonal antiserum.

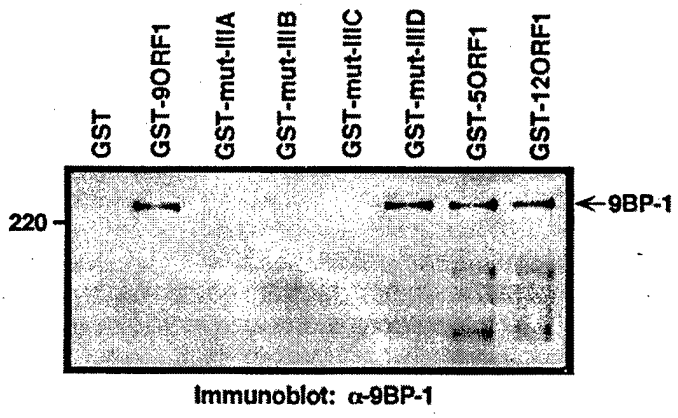

Fig. 5. 9BP-1 and 9ORF1-associated protein p220 show the same reactivity toward 9ORF1 mutant proteins. Total cell proteins (200 μg) from CREF cells were incubated with GST or the indicated GST fusion protein (5 μg) on beads. Recovered cellular proteins were separated by 7.5% SDS-PAGE, transferred to a membrane, and immunoblotted with 9BP-1 rabbit polyclonal antiserum.

9BP-1 co-immunoprecipitates with the 9ORF1 protein expressed in CREF cells and Ad9-induced rat mammary tumor cells. To determine whether the 9ORF1 protein and 9BP-1 may form complexes *in vivo*, we performed co-immunoprecipitation analyses. We found that immunoprecipitation with 9ORF1 antiserum but not with the matched pre-immune serum co-precipitated 9BP-1 from lysates of 9ORF1-expressing CREF cells (Fig. 6). This result was not due to 9ORF1 antiserum reacting directly with 9BP-1 because an immunoprecipitation performed with 9ORF1 antiserum and lysates of normal CREF cells failed to precipitate this protein. Moreover, in reciprocal experiments, 9BP-1 antiserum but not the matched pre-immune serum also co-precipitated 9ORF1 protein from lysates of 9ORF1-expressing CREF cells (Fig. 7). Significantly, the 9ORF1 protein similarly

co-precipitated with 9BP-1 from lysates of an Ad9-induced rat mammary tumor cell line (Fig. 7). These findings suggest that, like DLG, the novel cellular PDZ-domain protein 9BP-1 forms a complex with the 9ORF1 oncoprotein in cells.

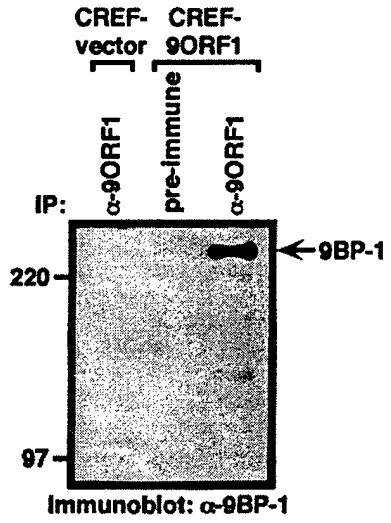

Fig. 6. 9BP-1 co-immunoprecipitates with the 9ORF1 protein. Total cell proteins (2.5 mg) from pools of CREF cells selected for expression plasmid pJ4Ω (CREF-vector) or pJ4Ω-9ORF1 plasmid (CREF-9ORF1) were immunoprecipitated with either 9ORF1 antiserum or the matched pre-immune serum. Recovered proteins were separated by 7.5% SDS-PAGE, transferred to a membrane, and immunoblotted with 9BP-1 rabbit polyclonal antiserum.

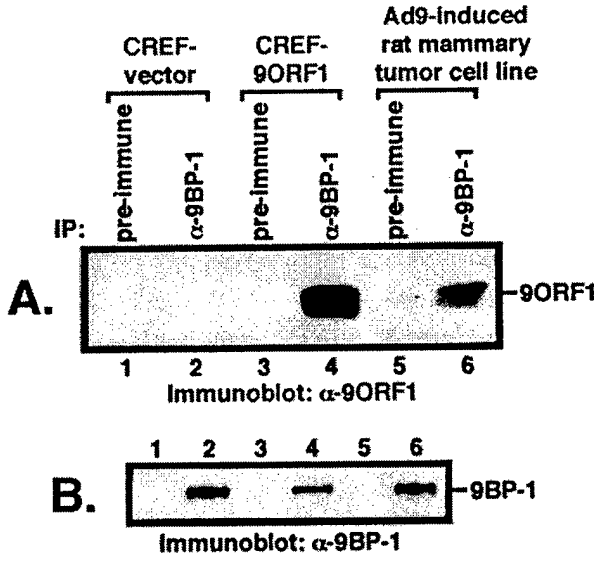

Fig. 7. The 9ORF1 protein co-immunoprecipitates with 9BP-1. Total cell proteins (3 mg, CREF-vector and CREF-9ORF1;'6 mg, Ad9-induced mammary tumor cell line) were immunoprecipitated with either 9BP-1 antiserum or the matched pre-immune serum. Recovered proteins were separated by 7.5% SDS-PAGE, transferred to a membrane, and the relevant portion of the membrane was immunoblotted with either (A) 9ORF1 or (B) 9BP-1 rabbit polyclonal antiserum.

Murine 9BP-1 is the same protein as the rat multi-PDZ domain protein MUPP1. By rescreening phage libraries with a 9BP-1 DNA probe, we have now obtained additional 9BP-1 cDNA sequences coding for a total of 1,042 amino-acid residues. During the course of our experiments, a 2,054-residue multi-PDZ domain protein called MUPP1 was reported (49). The C-terminus of the rat MUPP1 polypeptide exhibits approximately 95% sequence identity with the C-terminal 1,042-residue portion of murine 9BP-1 sequence (data not shown), indicating that these proteins are the same. Therefore, 9BP-1 (MUPP1) has thirteen PDZ domains and no other recognizable domains (Fig. 8). Results obtained by immobilizing fusion proteins containing each 9BP-1 PDZ domain on a membrane and blotting with a radiolabelled 9ORF1 protein probe further show that the 9ORF1 protein selectively binds to PDZ10 of this polypeptide (data not shown; see Fig. 8).

9BP-1 may function as an adapter protein. BLAST searches reveal that 9BP-1/MUPP1 is most closely related to human INADL (35), a 1,524 amino-acid residue PDZ-domain protein related to the *Drosophila* INAD protein (9, 18, 44), and to an approximately 2,100 amino-acid residue *C. elegans* PDZ-domain protein of unknown function (see Fig. 8). The sequence similarity with INADL is potentially significant because it may hint to a possible function for 9BP-1. The *Drosophila* INAD protein consists of five PDZ domains and functions as a scaffold to assemble different components of the phototransduction cascade (48). In a specialized subcellular compartment of *Drosophila* photoreceptor neurons called the rhabdomere, light-activated rhodopsin initiates a signaling cascade by activating the G_q protein α-subunit, which activates phospholipase C-β (PLC-β). PLC-β catalyzes hydrolysis of phosphatidylinositol-4,5-

bisphosphate to the intracellular second messengers inositol triphosphate and diacylglycerol, which then induce opening of the principle light-activated calcium channels (TRP). Finally, through a calcium-dependent process that includes activation of protein kinase C (PKC), the light response is deactivated. Each INAD PDZ domain interacts with a specific component of this phototransduction cascade, with PLC-β binding to PDZ5, TRP binding to PDZ3, and PKC binding to PDZ4. Besides having significant deficiencies in phototransduction, *INAD* null mutants display dramatic defects in the subcellular distributions of PLC-β, TRP, and PKC (48). These findings indicate that INAD organizes components of the *Drosophila* phototransduction cascade both architecturally and spatially into microdomains of signaling. In so doing, INAD may prevent cross-talk between related signaling pathways and enhance signaling response times and specificity. In summary, the mechanisms of INAD may serve as an important paradigm for related multivalent PDZ-domain proteins like 9BP-1 and, perhaps, DLG as well.

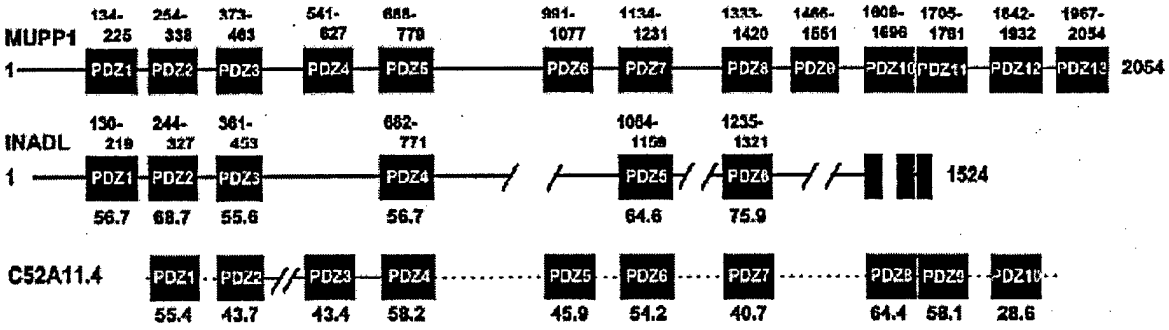

Fig. 8. 9BP-1 is the murine homologue of the rat multi-PDZ domain protein MUPP1. This figure shows the common domain organization of three different multi-PDZ domain proteins: rat MUPP1, human INADL, and yeast protein C52A11.4. Rat MUPP1 has 2,054 amino-acid residues and thirteen PDZ domains, and exhibits approximately 95% sequence identity with murine 9BP-1 (see text), indicating that these proteins are the same. The numbers below the PDZ domains of INADL and C52A11.4 denote their sequence identity with the respective PDZ domains of 9BP-1/MUPP1. (*Adapted from Ullmer et al., FEBS Letters 424:63-68, 1998*).

OBJECTIVE 2: Determine the functional significance of 9ORF1 protein oligomerization.

Problems recovering soluble 9ORF1 protein. For this objective, we planned to determine the molecular-weights of purified 9ORF1 and mut-IIIA homo-oligomers. This was to be accomplished by expressing the proteins in *E. coli* as GST fusions, purifying them, removing the GST moiety by cleavage with thrombin, and determining their size by gel filtration and glycerol gradient sedimentation analyses. Although we succeeded in expressing and purifying these proteins as GST fusions, 9ORF1 and mut-IIIA became completely insoluble following thrombin cleavage. We also attempted to obtain soluble 9ORF1 and mut-IIIA protein by expressing them as 6xHis fusion proteins. Again, although expressed at high levels in *E. coli*, the proteins were completely insoluble, even after addition of 0.2% SDS or 1% sarkosyl. Therefore, due to these unanticipated problems, we are presently unable to complete the proposed oligomerization studies with purified wild-type and mutant 9ORF1 proteins. As an alternate approach, we plan to express 9ORF1 protein in insect cells using 9ORF1-expressing recombinant baculoviruses. If this approach fails, however, we will refocus these efforts toward determining whether the 9ORF1 protein possesses an enzymatic activity related to but different from that of dUTPases. Such an activity may be suggested by recent observations (see below).

9ORF1 Region I and Region II mutations correspond to dUTPase residues present within or near the catalytic site. The crystal structure of human dUTPase complexed with dUTP has recently been solved (32). Two amino-acid residues, glycine 87 and valine 89, are present in the active site, and their main-chain atoms interact with the uracil base. These residues lie within a conserved region of eukaryotic dUTPases and, interestingly, 9ORF1 Region II mutations are contained within an analogous highly-conserved region of adenovirus E4 ORF1 proteins (52). dUTPase valine 89 is located in an

equivalent position as 9ORF1 leucine 89, which is changed to glutamine in mut-IIA and, two residues away, phenylalanine 91 is changed to serine in mut-IIB (50). Thus, 9ORF1 Region II mutations correspond to dUTPase residues in or near the catalytic site. Furthermore, using the three-dimensional structure of human dUTPase as a model, we predict that 9ORF1 Region I mutations of mut-IA (F34I, H39Q, V41A) are located on β-strand 3 near Region II residues on β-strand 6 (32, 50). These intriguing observations hint that 9ORF1 transforming activity may depend on an enzymatic activity related to dUTPase or, alternatively, an ability to bind to nucleic acid.

Considering that the possibility that the 9ORF1 protein may possess an undetermined enzymatic activity, it seems pertinent that DLG has been shown to bind ATP *in vitro* and that this binding is mediated by PDZ(1+2) (30). Specifically, fusion proteins containing full-length DLG, DLG PDZ(1+2+3), and DLG PDZ(1+2), but not DLG PDZ3, display high-affinity binding to ATP *in vitro* (30). This binding is specific because no binding was detected with GTP and, in addition, binding with radiolabelled ATP was blocked with a large excess of cold ATP but not GTP. The biological significance of ATP binding to DLG is not known but possible implications include the induction of conformational changes in DLG by ATP hydrolysis similar to those seen in nucleotide-bound forms of Ras (1), indirect effects on the functions of DLG-associated proteins, or modulation of DLG PDZ domain protein-protein interactions. Although the protein sequences that mediate ATP binding were not determined, it may be relevant that DLG PDZ2 contains a "reverse" nucleotide-binding motif KGXXGXG (X = any amino acid) (1, 41). This observation may be significant in light of the fact that, among the three DLG PDZ domains, DLG PDZ2 binds most strongly to the 9ORF1 protein (28). In addition, crystal structures of DLG and PSD-95 PDZ domains (8, 10) suggest that residues for these putative nucleotide-binding motifs would be located within and near the binding site responsible for interacting with C-terminal peptide sequences. Therefore, we propose to determine whether the 9ORF1 protein hydrolyzes ATP molecules bound to DLG.

For this purpose, the plasmid encoding the GST-DLG PDZ(1+2+3) fusion protein used by Marfatia, *et al.* in their ATP binding study (30) has been obtained from Dr. Athar Chishti. Using this plasmid to obtain purified protein, we will first confirm that, as opposed to GST, the GST-DLG PDZ(1+2+3) protein has a high capacity for binding specifically to ATP. Next, while attached to glutathione sepharose beads, the purified DLG PDZ(1+2+3) protein will be bound to [γ-^{32}P]ATP or [α-^{32}P]ATP as described by Marfatia *et al.* (30), washed to remove free ATP, and incubated with a binding buffer containing saturating amounts of purified GST-9ORF1 protein. Aliquots of binding buffer will be monitored for the release of radioactivity. To measure possible hydrolysis of the radiolabelled ATP directly by the GST-9ORF1 protein, we will assay these aliquots for the presence of free $^{32}PO_4$ by thin layer chromatography (TLC) on polyethyleneimine-cellulose plates (4). Different radiolabelled nucleotide and phosphate standards will be included in the TLC assays because it is possible that pyrophosphate may be a hydrolysis product or intact ATP may be displaced from the DLG protein. As negative controls, the DLG protein bound to radiolabelled ATP will also be incubated with binding buffer lacking GST-9ORF1 protein or containing GST protein. If we observe specific ATP hydrolysis by GST-9ORF1 protein, then we will perform similar assays with GST proteins containing available 9ORF1 mutants and, in addition, determine whether GST-9ORF1 protein can hydrolyze ATP or other nucleotides free in solution. We will also mutate the nucleotide-binding motif in DLG PDZ2 to determine whether binding to ATP is abolished. These experiments are expected to allow us to determine whether the 9ORF1 protein can hydrolyze ATP molecules bound to the PDZ domains of DLG and, if so, whether this activity is linked to transformation by the 9ORF1 protein.

dUTPases and PDZ domains share a similar protein fold. For known C-terminal PDZ domain-binding motifs, full binding activity is mediated by as few as three C-terminal amino-acid residues (39). In contrast, the C-terminal 17 or 65 amino-acid residues of the 9ORF1 protein show significantly impaired PDZ-domain protein binding activity (51), suggesting that additional unknown interactions contribute to stable complex formation. In this regard, besides binding to the C-termini of target proteins, PDZ domains also form homotypic dimers with other PDZ domains (5). Interestingly, the crystal structure of a PDZ domain has been solved, and it is reported to share a similar β-clip fold with dUTPases (8). This intriguing observation suggests that 9ORF1 and related adenovirus E4 ORF1 transforming proteins also have a β-clip fold which, conceivably, could permit these viral proteins to use both C-terminal peptide and homotypic-like interactions to bind PDZ domains. Therefore, 9ORF1 may have arisen from a dUTPase precursor because such a structural framework may provide the most effective means to prevent PDZ domains from binding to their normal targets in cells.

CONCLUSIONS

We have discovered that several different human virus oncoproteins (adenovirus E4 ORF1, HTLV-1 Tax, and high-risk HPV E6) contain PDZ domain-binding motifs. In addition, these viral oncoproteins also share the ability to complex with the cellular PDZ-domain protein DLG, which is a putative tumor suppressor protein that binds to the tumor suppressor protein APC in cells. Moreover, both the adenovirus 9ORF1 and HPV-16 E6 oncoproteins are known to require their functional PDZ-domain-binding motifs to transform cells. In addition to DLG, the 9ORF1 protein has also been found to interact with the novel PDZ-domain protein 9BP-1, as well as several other putative cellular PDZ-domain proteins. This finding implicates a select group of cellular PDZ-domain proteins in transformation by 9ORF1. Because PDZ-domain proteins normally function in cell signaling, the 9ORF1 protein likely perturbs cell signaling pathways and, in so doing, potentiates abnormal cellular proliferation and neoplasia. Therefore, ascertaining the mechanisms of transformation by the 9ORF1 oncoprotein is expected to reveal important new cell signaling proteins and pathways involved in the development of cancer. It is hoped that the knowledge gained by these studies can be used to develop new therapeutic strategies for preventing and treating human malignancies.

REFERENCES

1. **Alberts, B., and R. Miake-Lye.** 1992. Unscrambling the puzzle of biological machines: the importance of details. Cell **68:**415-420.
2. **Ankerst, J., and N. Jonsson.** 1989. Adenovirus type 9-induced tumorigenesis in the rat mammary gland related to sex hormonal state. J. Natl. Cancer Inst. **81:**294-8.
3. **Ankerst, J., N. Jonsson, L. Kjellen, E. Norrby, and H. O. Sjogren.** 1974. Induction of mammary fibroadenomas in rats by adenovirus type 9. Int. J. Cancer **13:**286-290.
4. **Bradley, M. K.** 1990. Activation of ATPase activity of the simian virus 40 large T antigen by covalent affinity analog of ATP, fluorosulfonylbenzoyl 5'-adenosine. J. Virol. **64:**4939-4947.
5. **Brenman, J. E., D. S. Chao, S. H. Gee, A. W. McGee, S. E. Craven, D. R. Santillano, Z. Wu, F. Huang, H. Xia, M. F. Peters, S. C. Froehner, and D. S. Bredt.** 1996. Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains. Cell **84:**757-67.
6. **Bridge, E., and G. Ketner.** 1989. Redundant control of adenovirus late gene expression by early region 4. J. Virol. **63:**631-638.
7. **Brusca, J. S., R. Jannun, and G. Chinnadurai.** 1984. Efficient transformation of rat 3Y1 cells by human adenovirus type 9. Virology **136:**328-337.
8. **Cabral, J. H., C. Petosa, M. J. Sutcliffe, S. Raza, O. Byron, F. Poy, S. M. Marfatia, A. H. Chishti, and R. C. Liddington.** 1996. Crystal structure of a PDZ domain. Nature **382:**649-52.
9. **Chevesich, J., A. J. Kreuz, and C. Montell.** 1997. Requirement for the PDZ domain protein, INAD, for localization of the TRP store-operated channel to a signaling complex. Neuron **18:**95-105.
10. **Doyle, D. A., A. Lee, J. Lewis, E. Kim, M. Sheng, and R. MacKinnon.** 1996. Crystal structures of a complexed and peptide-free membrane protein-binding domain: Molecular basis of paptide recognition by PDZ. Cell **85:**1067-1076.
11. **Fanning, A. S., and J. M. Anderson.** 1996. Protein-protein interactions: PDZ domain networks. Current Biology **6:**1385-8.
12. **Green, M., J. K. Mackey, W. S. M. Wold, and P. Rigden.** 1979. Thirty-one human adenovirus serotypes (Ad1-Ad31) form five groups (A-E) based upon DNA genome homologies. Virology **93:**481-492.

13. **Halbert, D. N., J. R. Cutt, and T. Shenk.** 1985. Adenovirus early region 4 encodes functions required for efficient DNA replication, late gene expression, and host cell shutoff. J Virol. **56:**250-7.
14. **Herisse, J., M. Rigolet, S. D. de Dinechin, and F. Galibert.** 1981. Nucleotide sequence of adenovirus 2 DNA fragment encoding for the carboxylic region of fiber protein and the entire E4 region. Nucleic Acids Res. **9:**4023-4042.
15. **Hogenkamp, T., and H. Esche.** 1990. Nucleotide sequence of the right 10% of adenovirus type 12 DNA encoding the entire region E4. Nucleic Acids Res. **18:**3065-6.
16. **Horwitz, M. S.** 1996. Adenoviruses, p. 2149-2171. *In* B. N. Fields and D. M. Knipe and P. M. Howley (ed.), Fields Virology, vol. 2. Lippincott-Raven Publishers, Philadelphia.
17. **Huang, M. M., and P. Hearing.** 1989. Adenovirus early region 4 encodes two gene products with redundant effects in lytic infection. J. Virol. **63:**2605-15.
18. **Huber, A., P. Sander, A. Gobert, M. Bahner, R. Hermann, and R. Paulsen.** 1996. The transient receptor potential protein (Trp), a putative store- operated Ca2+ channel essential for phosphoinositide-mediated photoreception, forms a signaling complex with NorpA, InaC and InaD. EMBO J. **15:**7036-45.
19. **Jannun, R., and G. Chinnadurai.** 1987. Functional relatedness between the E1a and E1b regions of group C and group D human adenoviruses. Virus Res. **7:**33-48.
20. **Javier, R., K. Raska Jr., G. J. Macdonald, and T. Shenk.** 1991. Human adenovirus type 9-induced rat mammary tumors. J. Virol. **65:**3192-202.
21. **Javier, R., K. Raska Jr., and T. Shenk.** 1992. Requirement for the adenovirus type 9 E4 region in production of mammary tumors. Science **257:**1267-71.
22. **Javier, R., and T. Shenk.** 1996. Mammary tumors induced by human adenovirus type 9: a role for the viral early region 4 gene. Breast Cancer Res Treat. **39:**57-67.
23. **Javier, R. T.** 1994. Adenovirus type 9 E4 open reading frame 1 encodes a transforming protein required for the production of mammary tumors in rats. J. Virol. **68:**3917-24.
24. **Kim, E., M. Niethammer, A. J. Rothschild, Y. N. , and M. Sheng.** 1995. Clustering of Shaker-type K^+ channels by interaction with a family of membrane-associated guanylate kinases. Nature **378:**85-88.
25. **Kim, E., and M. Sheng.** 1996. Differential K+ channel clustering activity of PSD-95 abd SAP97, two related membrane-associated putative guanylate kinases. Neuropharmacology **35:**993-1000.
26. **Kim, S. K.** 1995. Tight junctions, membrane-associated guanylate kinases and cell signaling. Curr. Opin. Cell Biol. **7:**641-649.
27. **Kornau, H. C., L. T. Schenker, M. B. Kennedy, and P. H. Seeburg.** 1995. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science **269:**1737-40.
28. **Lee, S. S., R. S. Weiss, and R. T. Javier.** 1997. Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of the Drosophila discs large tumor suppressor protein. Proc. Nat. Acad. Sci. USA **94:**6670-5.
29. **Lue, R. A., S. M. Marfatia, D. Branton, and A. H. Chishti.** 1994. Cloning and charcterization of hdlg: the human homologue of the Drosophila discs large tumor suppressor binds to protein 4.1. Proc. Natl. Acad. Sci. USA **91:**9818-9822.
30. **Marfatia, S. M., J. H. Cabral, L. Lin, C. Hough, P. J. Bryant, L. Stolz, and A. H. Chishti.** 1996. Modular organization of the PDZ domains in the human discs-large protein suggests a mechanism for coupling PDZ domain-binding proteins to ATP and the membrane cytoskeleton. J. Cell Biol. **135:**753-66.
31. **Matsumine, A., A. Ogai, T. Senda, N. Okumura, K. satoh, G. H. Baeg, T. Kawahara, S. Kobayashi, M. Okada, K. Toyoshima, and T. Akiyama.** 1996. Binding of APC to the human homolog of the *Drosophila* Discs Large tumor suppressor protein. Science **272:**1020-1023.

32. **Mol, C. D., J. M. Harris, E. M. McIntosh, and J. A. Tainer.** 1996. Human dUTP pyrophosphatase: uracil recognition by a beta hairpin and active sites formed by three separate subunits. Structure **4:**1077-92.

33. **Muller, B. M., U. Kistner, R. W. Veh, C. Cases-Langhoff, B. Becker, E. D. Gundelfinger, and C. C. Garner.** 1995. Molecular characterization and spatial distribution of SAP97, a novel presynaptic protein homologous to SAP90 and the Drosophila discs-large tumor suppressor protein. J Neurosci. **15:**2354-66.

34. **Muller, U., T. Kleinberger, and T. Shenk.** 1992. Adenovirus E4orf4 protein reduces phosphorylation of c-Fos and E1A proteins while simultaneously reducing the level of AP-1. J. Virol. **66:**5867-78.

35. **Philipp, S., and V. Flockerzi.** 1997. Molecular characterization of a novel human PDZ domain protein with homology to INAD form *Drosophila melanogaster*. FEBS letter **413:**243-248.

36. **Polakis, P.** 1995. Mutations in the APC gene and their implications for protein structure and function. Curr. Opin. Genet. Dev. **5:**66-71.

37. **Ponting, C. P.** 1997. Evidence for PDZ domains in bacteria, yeast, and plants. Protein Sci. **6:**464-8.

38. **Richards, R. G., L. C. Sowers, J. Laszlo, and W. D. Sedwick.** 1984. The occurrence and consequences of deoxyuridine in DNA. Adv. Enzyme Regul. **22:**157-185.

39. **Saras, J., U. Engstrom, L. J. Gonez, and C. H. Heldin.** 1997. Characterization of the interactions between PDZ domains of the protein- tyrosine phosphatase PTPL1 and the carboxyl-terminal tail of Fas. J. Biol. Chem. **272:**20979-81.

40. **Saras, J., and C. H. Heldin.** 1996. PDZ domains bind carboxy-terminal sequences of target proteins. Trends Biochem. Sci. **21:**455-8.

41. **Saraste, M., P. R. Sibbald, and A. Wittinghofer.** 1990. The P-loop - a common motif in ATP- and GTP-binding proteins. Trends Biochem. Sci. **15:**430-434.

42. **Sheng, M.** 1996. PDZs and receptor/channel clustering: Rounding up the latest suspects. Neuron **17:**575-578.

43. **Sheng, M., and E. Kim.** 1996. Ion channel associated proteins. Curr. Opin. Neurobiol. **6:**602-608.

44. **Shieh, B. H., and M. Y. Zhu.** 1996. Regulation of the TRP Ca2+ channel by INAD in Drosophila photoreceptors. Neuron **16:**991-8.

45. **Songyang, Z., A. S. Fanning, C. Fu, J. Xu, S. M. Marfatia, A. H. Chishti, A. Crompton, A. C. Chan, J. M. Anderson, and L. C. Cantley.** 1997. Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. Science **275:**73-7.

46. **Thomas, U., B. Phannavong, B. Muller, C. C. Garner, and E. D. Gundelfinger.** 1997. Functional expression of rat synapse-associated proteins SAP97 and SAP102 in Drosophila dlg-1 mutants: effects on tumor suppression and synaptic bouton structure. Mech. Dev. **62:**161-74.

47. **Trentin, J. J., G. L. Van Hoosier, and L. Samper.** 1968. The oncogenicity of human adenoviruses in hamsters. Proc. Soc.Exp. Biol. Med. **127:**683-689.

48. **Tsunoda, S., J. Sierralta, Y. Sun, R. Bodner, E. Suzuki, A. Becker, M. Socolich, and C. S. Zuker.** 1997. A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. Nature **388:**243-9.

49. **Ullmer, C., K. Schmuck, A. Figge, and H. Lubbert.** 1998. Cloning and characterization of MUPP1, a novel PDZ domain protein. FEBS Letter **424:**63-8.

50. **Weiss, R. S., M. O. Gold, H. Vogel, and R. T. Javier.** 1997. Mutant adenovirus type 9 E4 ORF1 genes define three protein regions required for transformation of CREF cells. J. Virol. **71:**4385-94.

51. **Weiss, R. S., and R. T. Javier.** 1997. A carboxy-terminal region required by the adenovirus type 9 E4 ORF1 oncoprotein for transformation mediates direct binding to cellular polypeptides. J. Virol. **71:**7873-7880.

52. **Weiss, R. S., S. S. Lee, B. V. V. Prasad, and R. T. Javier.** 1997. Human adenovirus early region 4 open reading frame 1 genes encode growth-transforming proteins that may be distantly related to dUTP pyrophosphatase enzymes. J. Virol. **71:**1857-1870.

53. **Weiss, R. S., M. J. McArthur, and R. T. Javier.** 1996. Human adenovirus type 9 E4 open reading frame 1 encodes a cytoplasmic transforming protein capable of increasing the oncogenicity of CREF cells. J. Virol. **70:**862-872.

54. **Woods, D. F., C. Hough, D. Peel, G. Callaini, and P. J. Bryant.** 1996. Dlg protein is required for junction structure, cell polarity, and proliferation control in Drosophila epithelia. J. Cell Biol. **134:**1469-82.