The Position of Cysteine Relative to the Transmembrane Domain Is Critical for Palmitoylation of H1, the Major Subunit of the Human Asialoglycoprotein Receptor*

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The mammalian hepatic asialoglycoprotein receptor (ASGP-R) is an endocytic recycling receptor that mediates the internalization of desialylated glycoproteins and their delivery to lysosomes where they are degraded. The human ASGP-R is a hetero-oligomeric complex composed of two subunits designated H1 and H2. Both subunits are palmitoylated at the cytoplasmic Cys residues near their transmembrane domains (TMD). The cytoplasmic Cys36 in H1 is located at a position that is five amino acids from the transmembrane junction. Because the sequences of subunits in all mammalian ASGP-R species are highly conserved especially at the region near the palmitoylated Cys, we sought to identify a recognition signal for the palmitoylation of H1. Various types of H1 mutants were created by site-directed or deletion mutagenesis including alteration of the amino acids surrounding Cys36, replacing portions of the TMD with that of a different protein and partial deletion of the cytoplasmic domain as well as transposing the palmitoylated Cys to positions further away from the TMD. Mutant H1 cDNAs were transiently expressed in COS-7 cells, and the H1 proteins were analyzed after metabolic labeling with [3H]palmitate. The results indicate that neither the native amino acid sequence surrounding Cys36 nor the majority of the cytoplasmic domain sequence is critical for palmitoylation. Palmitoylation was also not dependent on the native TMD of H1. In contrast, the attachment of palmitate was abolished if the Cys residue was transposed to a position that was 30 amino acids away from the transmembrane border. We conclude that the spacing of a Cys residue relative to the TMD in the primary protein sequence of H1 is the major determinant for successful palmitoylation.

Palmitoylation is a post-translational modification in which the 16-carbon fatty acid palmitate is covalently attached to a protein, usually via a thioester linkage (1–3). The attachment of palmitate to integral membrane proteins occurs at Cys residues located in the cytoplasmic region near the membrane or within the transmembrane domain (TMD) itself. A wide variety of proteins are palmitoylated. Some of these are soluble proteins such as Ras (4) and G protein (5). Palmitoylation of these soluble proteins enhances their membrane binding ability and targets them to specific membrane domains (1).

Many palmitoylated proteins are also integral membrane proteins that are already anchored to the lipid bilayer through their membrane-spanning regions. These proteins include the β2-adrenergic receptor (6), the transferrin receptor (7), the cation-dependent mannose-6-phosphate receptor (8), and the asialoglycoprotein receptor (9, 10). It is probable that palmitoylation plays a role other than membrane anchoring in these transmembrane proteins. For example, because of its close proximity to Ser345 and Ser346, the palmitoylation of Cys341 inhibits phosphorylation of the β2-adrenergic receptor by restricting the access of these phosphorylation sites to the cAMP-dependent protein kinase (11). The endocytic rate of a palmitoylation-defective transferrin receptor increased ~2-fold compared with that of the wild-type receptor (7), indicating that palmitoylation might regulate transferrin receptor trafficking. Palmitoylation is also important for the normal trafficking and lysosomal enzyme-sorting functions of the cation-dependent mannose-6-phosphate receptor (8). Therefore, palmitate modifications can have important physiological functions in addition to serving as a membrane localization signal.

The human ASGP-R is an endocytic receptor found predominantly in hepatocytes (12–15). Although its function remains unknown and no endogenous ligands have been identified (16–18), the receptor mediates the endocytosis of a wide range of exogenous desialylated glycoproteins with terminal galactose or N-acetylgalactosamine residues in their carbohydrate chains (19). The human ASGP-R is a hetero-oligomer composed of two homologous subunits designated H1 and H2. H1 is a type II single-pass transmembrane protein with a 40-amino acid N-terminal cytoplasmic domain, an extracellular stalk, and an ~130-amino acid C-terminal carbohydrate recognition domain (12–15). H1 is palmitoylated at Cys36 in the cytoplasmic domain (10), whereas H2 is palmitoylated at the analogous cytoplasmic domain residue Cys54 as well as the juxtamembrane Cys58 (20). The two subunits in all mammalian species are highly homologous, especially at the region surrounding the cytoplasmic Cys. Therefore, this region could potentially contain a recognition motif for palmitoylation. To determine whether H1 contains a sequence motif for palmitoylation, we constructed a variety of cytoplasmic or TMD mutants of H1 and determined their ability to be metabolically labeled with [3H]palmitate.

EXPERIMENTAL PROCEDURES

Materials—[9,10-3H]Palmitic acid (30–60 Ci/mmol) was purchased from PerkinElmer Life Sciences. Human orosomucoid was obtained from Sigma and desialylated to generate ASOR as described previously (21). ASOR-Sepharose 4B was prepared as described previously (9). Rabbit polyclonal anti-H1 antibody was made against a peptide corre-
sponding to the H1 C-terminal sequence as described previously (22). Buffer 1 contains 10 mM N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (Research Orgincs, Inc.), pH 7.4, 150 mM NaCl, and 6.7 mM KCl. Phosphate-buffered saline contains 137 mM NaCl, 8 mM potassium phosphate, 2.7 mM KCl, and 1.5 mM magnesium phosphate, pH 7.4.

Restriction enzymes were from Promega. Oligonucleotides were synthesized using cyanoethyl phosphoramidite chemistry by Midland Certified Reagent Company. All other chemicals were from Sigma unless otherwise noted.

**Plasmid Constructs**—H1 and H1(C36S) cDNAs were subcloned into pcDNA3.1 (+) (Invitrogen) as described previously (10). The cDNAs for mutants H1(L4–11), H1(D4–33A), H1(L12–33), and H1(V26–40) were generously provided by Dr. Martin Spiess (University of Basel, Basel, Switzerland) and subcloned into pcDNA3.1 (+) at HindIII and EcoRI restriction sites. Site-directed mutagenesis was performed using the QuickChange™ site-directed mutagenesis kit (Stratagene) according to the manufacturer’s instructions. The mutagenic oligonucleotides and H1-derived templates used to generate all constructs are shown in Table I. The mutant H1(5A) in which amino acid residues Gln36 to Ser37 were mutated to Ala was a generous gift from Dr. Robert Fallon (Indiana University). All mutations were verified by DNA sequencing of the complete open reading frame.

**Cell Culture and Transfections**—COS-7 cells (ATCC) were cultured in phenol red-free Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% (w/v) fetal bovine serum (Sigma), 2 mM l-glutamine, and 100 units/ml each penicillin and streptomycin (Sigma) in an atmosphere of 5% CO2 at 37°C.

**Palmitoylation of H1 Subunit Variants**—The normalized values for the palmitoylation of various H1 subunits were calculated based on the ratio of the densities of the bands in the parallel fluorography and Western analysis. The experiments shown in each figure were repeated 3 or 4 times. For each individual experiment, the 100% palmitoylation value was determined by its own internal wild-type control and the value for each mutant was then determined relative to that internal control value. Finally, the mean value ± S.E. (n = 3 or 4) for each mutant H1 is shown in the bar graphs. Because the wild-type values within each experiment are set at 100%, these are not shown in every experiment. However, to show the variation between experiments, Fig. 1 displays the mean value (set at 100%) ± S.E. for the palmitoylation of wild-type H1 calculated using the combined internal wild-type controls from all experiments. Some values apparently greater than 100% may reflect the error in determining the mean normalized palmitoylation value of the wild-type H1 as well as the comparative ratios.

**General**—Non-reducing SDS-PAGE was performed according to the method of Laemmli (23). Western blotting procedures were performed essentially as described by Burnette (24). DNA sequencing was performed using the dideoxynucleotide method of Sanger et al. (25) by the Department of Microbiology & Immunology Sequencing Facility (University of Oklahoma Health Sciences Center) using Applied Biosystems model 377 automated DNA sequencers.

### RESULTS

**The Native Amino Acid Sequence Surrounding Cys36** Is Not Critical for Palmitoylation—The regions immediately adjacent to the palmitoylated Cys are highly conserved in all mammalian ASGP-R subunits (e.g. the sequences among both subunits from mouse, human, and rat are all QRLCSG). Therefore, site-directed mutagenesis was performed to alter one or more of the amino acid residues in this region to identify a possible palmitoylation recognition motif. First, the three amino acids upstream or downstream of Cys36 in H1 were mutated individually to Ala (Fig. 1A, lanes 1–3 and 5–7). The wild-type and six-mutant H1 variants were expressed in COS-7 cells and tested for their ability to be palmitoylated. The results showed that all six of these single-point mutations in H1 had minimal or no effect on the palmitoylation of Cys36 (Fig. 1B, lanes 1–7). Next, we generated mutant H1(4A+ C) by substituting residues Gln36, Arg37, Leu37, and Ser37 of H1 with four Ala residues (Fig. 1A, lane 8). These multiple mutations in the region surrounding Cys36 reduced but did not prevent palmitoylation (Fig. 1B, lane 8). Taken together, these data indicate that the
native amino acid sequence immediately surrounding Cys 36 is not critical for palmitoylation of H1.

The H1 bands detected by Western analysis appeared at more than one position and were diffuse; however, this finding is consistent with observations made in HepG2 or HuH-7 cells, which are human hepatoma cell lines that express the native ASGP-R. These multiple or diffuse bands are caused by the heterogeneity of N-linked glycosylation. After deglycosylation with endoglycosidase F, the multiple H1 protein bands migrated as a single discrete band in SDS-PAGE with a Mr value matching the predicted size of the nascent peptide (data not shown).

**The Majority of the Cytoplasmic Domain of H1 Is Not Required for Palmitoylation**—We next determined whether the cytoplasmic region upstream of Cys 36 is required for palmitoylation. As shown in Fig. 2A, the H1 cytoplasmic residues from Glu 4 to Leu 11 (lane 1), Glu 4 to Gln 33 (lane 2), Glu 12 to Gln 33 (lane 3), or Pro 26 to Arg 40 (lane 4) were deleted. The mutant cDNAs were expressed in COS-7 cells, and the H1 subunits were analyzed for their ability to be labeled with [3H]palmitate as described above. All of the deletion mutants with the exception of the mutant in which the Cys was also deleted were labeled with [3H]palmitate (Fig. 2B, lanes 1–4). In fact, the palmitoylation of the H1 variant was enhanced (lane 3). These data demonstrate that the cytoplasmic region spanning the residues from Glu 4 to Gln 33 is not required for palmitoylation of H1.

**The Sequence within the TMD Is Not Important for H1 Palmitoylation**—We next determined whether the sequence within the H1 TMD contains a recognition signal for palmitoylation.
lation. Chimeric constructs of H1 were generated by replacing the H1 transmembrane sequence with that of the non-palmitoylated fusogenic spike protein (F protein) of Sendai virus. F protein is a non-palmitoylated protein (26), because it does not contain Cys in its transmembrane or cytoplasmic region. Poni-maskin and Schmidt (27) found that the introduction of Cys residues into the cytoplasmic domain of the F protein is also not sufficient to induce palmitoylation. Palmitate modification only occurs if part of the transmembrane sequence or cytoplasmic sequence of a palmitoylated protein is introduced along with a Cys residue into the cytoplasmic domain of the F protein is also not sufficient to induce palmitoylation. Palmitoylation of protein 1 occurs if part of the transmembrane sequence or cytoplasmic sequence of a palmitoylated protein is introduced along with a Cys residue into the cytoplasmic domain of the F protein. As shown in Fig. 3A, cDNA constructs in which one-third (lane 1), two-thirds (lane 2), or the whole TMD of H1 (lane 3) was replaced with that of the Sendai virus F protein were transfected into COS-7 cells and analyzed for their [3H]palmitate labeling. The H1 transmembrane mutant with the whole transmembrane region replaced with the corresponding F protein sequence (lane 3) was not expressed or was degraded completely before it reached maturity in transfected COS-7 cells, possibly because of incorrect folding or instability of H1 in the absence of its native TMD. As a result, the palmitoylation status of this mutant could not be assessed. However, the H1 mutants with one-third or two-thirds of the TMD replaced were still palmitoylated (Fig. 3B). These results indicate that the native sequence of at least the first 15 amino acids of the TMD is not important for palmitoylation of H1.

Insertion of Ala between Cys36 and the TMD Does Not Prevent Palmitoylation—We next analyzed the importance of the spacing between Cys36 and the TMD for H1 palmitoylation. A series of mutant cDNAs was constructed by inserting one, two, or three Ala residues immediately after Pro39 in H1, thus presumably increasing the relative distance between Cys36 and the TMD (Fig. 4A, lanes 1–3). The results showed that insertion of up to three Ala residues between the palmitoylated Cys residue and the TMD had minimal effects on H1 palmitoylation in transiently transfected COS-7 cells.

The Spacing between a Cys Residue and the TMD Affects Palmitoylation of H1—To investigate further the relationship of palmitoylation and the spacing between the Cys and the TMD, we generated another series of H1 mutants. The data in Fig. 1 demonstrate that the native amino acid sequence surrounding Cys36 was not critical for palmitoylation. Therefore, instead of increasing the spacing between Cys and the TMD by inserting increasingly more Ala residues until palmitoylation might be inhibited, we replaced Cys36 with a Ser residue and then reintroduced a new Cys residue further upstream from its original position. This strategy effectively increased the spacing between the TMD and the target Cys but at the same time kept the length and most of the sequence of the cytoplasmic domain constant. As shown in Fig. 5A, a Cys residue was transposed to a position that was either 10 (lane 1), 20 (lane 2), or 30 (lane 3) residues away from the TMD. Results from [3H]palmitate-labeling experiments showed that H1 was still palmitoylated when the Cys was transposed to a position 10 residues away from the TMD (Fig. 5B, lane 1). However, palmitoylation no longer occurred when the Cys residue was relocated to a position 20 or 30 residues away from the TMD (Fig. 5B, lanes 2 and 3), indicating that H1 palmitoylation

![Fig. 2. The majority of the H1 cytoplasmic domain is not required for palmitoylation. A, the schematic diagram shows the regions of H1 deleted in mutants 1–4. B, COS-7 cells transfected with H1 deletion mutant cDNAs were labeled with [3H]palmitate and analyzed as described in Fig. 1. Results are the average ± S.E. of three separate experiments. There were no significant differences between these mutants and wild type (p > 0.05). WB, Western blot; FL, fluorography; WT, wild type.]
Fig. 3. The TMD of H1 is not essential for palmitoylation. A, the TMD (underlined) of H1 was systematically replaced in mutants 1–3 with all or a portion of the TMD of the non-palmitoylated Sendai virus F protein (shown in boldface). B, COS-7 cells transfected with H1 transmembrane mutant cDNAs were labeled with [3H]palmitate and analyzed as described in Fig. 1. Results are the average ± S.E. of three separate experiments. Mutant 2 was significantly different from wild type (p < 0.05). WB, Western blot; FL, fluorography; WT, wild type.

DISCUSSION

Our previous studies showed that the two subunits of the human ASGP-R, H1 and H2, are palmitoylated at their cytoplasmic Cys residues (10, 20). The amino acid sequence in the region surrounding the palmitoylated Cys residue is highly conserved between both ASGP-R subunits in all mammals. In this study, we attempted to determine a recognition signal in the H1 cytoplasmic domain or TMD for targeting the palmitoylation of Cys. Our results indicate that neither the native amino acid sequence surrounding the Cys nor those within the TMD are essential for palmitoylation of H1. In contrast, the relative distance between the palmitoylated Cys and the transmembrane border in the primary protein sequence is a major determinant for successful H1 palmitoylation.

Other investigators have attempted to determine a structural requirement for palmitoylation of various integral membrane proteins. However, the comparison of the cytoplasmic tails of 12 different palmitoylated transmembrane proteins failed to reveal a consensus sequence for palmitate modification (27). Hansen et al. (29) and Grosenbach et al. (30) identified a loosely conserved palmitoylation motif (TMDX(x12)AAC[C]A) among 20 different integral membrane proteins where TMD represents the transmembrane domain, “X” represents a stretch of any amino acids up to 12 residues in length, and “A” represents aliphatic amino acids. However, numerous other palmitoylated transmembrane proteins including the mutant H1(L21C,P26–29A) generated here have Cys residues that are >14 residues away from the TMD or contain positively charged residues immediately adjacent to the Cys residue. Therefore, this putative palmitoylation motif cannot be applied to all transmembrane proteins that are palmitoylated.

Schweizer et al. (8) investigated the structural requirement for palmitoylation of an endoplasmic reticulum resident transmembrane protein, the ASGP-R major subunit H1. Our findings are consistent with their study in that the transmembrane domain is not an essential determinate for palmitoylation of H1. This conclusion is further supported by the results obtained by Shu et al. (28) in which the transmembrane domain of a C2C12 cell line stably transfected with a retrovirally generated H1 cDNA was shown to be sufficient for the palmitoylation of H1.

occurs only if the position of Cys is within a certain distance from the transmembrane junction. As a control to show that palmitoylation is independent of the amino acid sequence surrounding the target Cys residue, the amino acids from Pro26 to Arg40 of mutant H1(L21C) (Fig. 5A, lane 2) were deleted to generate mutant H1(L21C,Δ26–40) (Fig. 5A, lane 4) so that the position of Cys was restored to within five residues from the TMD as in the wild-type H1. The palmitoylation defect was rescued in this deletion mutant (Fig. 5B, lane 4), indicating that the spacing of Cys relative to the TMD rather than the native amino acid sequence near the target Cys is the major determinant for palmitoylation.

In the non-palmitoylated mutants H1(L21C) and H1(L11C) (Fig. 5A, lanes 2 and 3) in which the Cys was transposed to a position 20 or 30 residues from the TMD border, the target Cys residue was now upstream of the four consecutive prolines (Pro26–29). Because the proximity of a Cys to the membrane surface may be important for its palmitoylation (28), the proline string in H1 thus could introduce structural constraints that may prevent an upstream Cys residue from reaching a position close enough to the membrane bilayer. To test this hypothesis, residues from Pro26 to Pro29 in H1(L21C) and H1(L11C) were substituted with four Ala residues, resulting in four Pro residues with four Ala residues, indicating that these Pro residues were inhibiting palmitoylation. However, the palmitoylation defect in the H1(L11C) mutant (Fig. 5B, lane 3) was not rescued by the same replacement of Pro4 with Ala4 (Fig. 5B, lane 6).
membrane protein, p63, and concluded that the majority of the cytoplasmic domain and the native amino acid sequence surrounding the palmitoylated Cys100 as well as that in the TMD were not critical for p63 palmitoylation. ten Brinke et al. (28) also found that the native amino acid sequence near the palmitoylated Cys residue or within the TMD was not critical for palmitoylation of the pulmonary surfactant protein C precursor. These above findings are in agreement with our present results on the sequence requirements for H1 palmitoylation. On the other hand, the palmitoylation of p63 is drastically reduced if the spacing between the Cys residue and the TMD is either increased or decreased by only five residues (8). The same is true for the palmitoylation of surfactant protein C precursor, which is reduced 3-fold if eight Ala residues are inserted between the Cys and the TMD. These results are in contrast to our finding that Cys transposed to a position 20 residues away from the TMD can still be palmitoylated to roughly the same extent (Fig. 5, lane 5) compared with the Cys residue in wild-type H1, which is five residues removed from the TMD.

The hydrophobic tail of the fatty acid in a palmitoylated protein is probably intercalated into the membrane bilayer. The study of the palmitoylation of surfactant protein C precursor (28) as well as the present results on the palmitoylation of the pulmonary surfactant, protein C precursor, suggest that for Cys palmitoylation to occur, a Cys residue has to be physically in close proximity to the inner membrane surface. However, palmitoylation may still be possible even when there are a large number of spacer residues between the Cys and the TMD if the region encoded by the spacer sequence is folded into a favorable conformation that allows the Cys to be situated in the vicinity of the lipid bilayer. Several palmitoylated transmembrane proteins such as the transforming growth factor-α (31) and the cation-dependent mannose-6-phosphate receptor (8) contain Cys residues that are located at a position >30 residues distal to the transmembrane border. Presumably, the region between the Cys and the transmembrane junction in these proteins could form a looplike structure to bring the Cys residue closer toward the plasma membrane (8).

The H1 cytoplasmic domain contains a region in which 5 of 6 residues are Pro (Pro26–28 and Pro30). Palmitoylation of H1 mutants appeared to be inhibited when the original Cys was transposed to positions upstream of this proline stretch (Fig. 5, lanes 2 and 3), which could introduce a structural constraint in the H1 cytoplasmic domain that prevents the transposed Cys residue from approaching the membrane bilayer. This hypothesis was supported by the observation that the palmitoylation defect was rescued by replacing residues Pro26–Pro29 with four Ala residues in the H1(L21C) mutant (Fig. 5, lanes 2 and 5). However, this same strategy of replacing the proline stretch failed to revert the palmitoylation defect in the H1(L11C) mutant (Fig. 5, lanes 3 and 6). A possible explanation for this latter result is that the region to which the Cys is transposed in the H1(L11C,P26–29)A mutant may be in a conformation such that the Cys residue is buried within the tertiary structure and is inaccessible for palmitoylation or is not in a favorable position near the membrane bilayer.

Another possible scenario is that the region to which the Cys was transposed may interact with other proteins or other H1 subunits, thus blocking the interaction between the Cys residue and a palmitoyltransferase. For example, the location of the transposed Cys residue in mutant H1[L11C,P26–29]A is...
relatively close to the consensus signaling sequence for endocytosis YQDL, which interacts with adaptin to target receptor internalization through clathrin-coated pits (32). Furthermore, the position of the Cys residue in this mutant is also near the phosphorylated Tyr and Ser residues (33, 34). Therefore, this region of the cytoplasmic domain is probably in association with adaptin or protein kinase(s), which may compete for the binding of the palmitoyltransferase. The presence of nearby phosphorylated residues might also interfere with the recognition of a Cys residue by a palmitoyltransferase, as is the case with CD4 (35). The C terminus of CD4 is located in the cytoplasm and contains several Cys residues; however, only Cys394 and Cys397, which are near the border of the TMD, are palmitoylated. Mutating both of these Cys residues to Ser abolishes palmitoylation of CD4, although Cys420 and Cys422, which are located further downstream from the TMD, are still available as potential palmitoylation acceptor sites. In fact, Cys420 and Cys422 of CD4 are part of an 8-amino acid motif that is involved in binding a cytoplasmic tyrosine kinase p56lck (36, 37). Therefore, the binding of p56lck to this motif or its phosphorylation could limit the accessibility of Cys420 and Cys422 to the palmitoyltransferase, and this situation could be analogous to that of the transposed Cys residue in the non-palmitoylated mutant H1[L11C,P26–29A].

Other types of post-translational modifications such as phosphorylation, glycosylation, or N-myristoylation have well defined consensus sequences or motifs for targeting the specific modification. Many scientists were surprised that a well characterized recognition motif common among palmitoylated integral membrane proteins could not be established. In fact, there is still debate regarding whether protein palmitoylation occurs enzymatically, catalyzed by palmitoyltransferases, or non-enzymatically by the reaction of compounds containing “high energy” thioacyl bonds with free thiols of proteins (38). However, based on the cloning and characterization of two protein thioesterases (39, 40), which catalyze the removal of palmitate from proteins, and the preliminary report of a protein palmitoyltransferase cloned from yeast (41), we believe that most protein palmitoylation reactions are enzyme-mediated.

In summary, we find no evidence for a recognition motif for palmitoylation in the cytoplasmic domain or TMD of the ASGP-R major subunit H1. In contrast, palmitoylation of H1 is abolished if the Cys residue is transposed to a position that is 30 residues away from the TMD. This could be attributed to the masking of the region near the transposed Cys by other proteins, thus denying the accessibility of the palmitoyltransferase, or the result of the positioning of the transposed Cys in an unfavorable tertiary structure that does not allow membrane proximity.

We have recently shown (20, 42) that palmitoylation of the human ASGP-R is required for efficient endocytosis of ligand. Palmitoylation-defective ASGP-Rs bind ASOR with the same affinity and show the same sensitivity in vitro for dissociation of ASOR/ASGP-R complexes at low pH as wild-type receptor. Despite these normal properties, ASGP-Rs that contain any combination of palmitoylation-defective H1 and H2 subunits are unable to uncouple efficiently from ligand so that receptor-mediated endocytosis, ligand delivery to lysosomes, and degradation are substantially decreased. Regardless of how the ASGP-R palmitoylation process occurs in vivo, it is critical for the normal function of the receptor.

Acknowledgments—We thank Dr. Robert Fallon for the kind gift of the H1[5A] plasmid, Dr. Martin Spiess for the generous gift of plasmids.
containing various H1 mutant cDNAs, and Dr. Fu-Yue Zeng for the subcloning of these H1 cDNAs.

REFERENCES
1. Resh, M. D. (1999) Biochim. Biophys. Acta 1451, 1–16
2. Morello, J. P., and Bouvier, M. (1996) Biochim. Cell Biol. 74, 449–457
3. James, G., and Olsen, E. N. (1990) Biochemistry 29, 2923–2934
4. Cadwallader, K. A., Paterson, H., MacDonald, S. G., and Hancock, J. F. (1994) Mol. Cell. Biol. 7, 4722–4730
5. Wedegaertner, P. B., Wilson, P. T., and Bourne, H. R. (1995) J. Biol. Chem. 270, 503–506
6. O'Dowd, B. F., Hnatowich, M., Caron, M. G., Letkowitz, R. J., and Bouvier, M. (1989) J. Biol. Chem. 264, 7564–7569
7. Alvarez, E., Girones, N., and Davis, R. J. (1990) J. Biol. Chem. 265, 16644–16655
8. Schweizer, A., Kornfeld, S., and Rohrer, J. (1996) J. Cell Biol. 132, 577–584
9. Zeng, F.-Y., and Weigel, P. H. (1996) J. Biol. Chem. 271, 32454–32460
10. Moffett, S., Adam, L., Bonia, H., Loisel, T. P., Bouvier, M., and Mouillac, B. (1996) J. Biol. Chem. 271, 21490–21497
11. Stockert, R. J. (1995) Physiol. Rev. 75, 591–609
12. Geffen, I., and Spiess, M. (1992) Int. Rev. Cytol. 137 B, 181–219
13. Weigel, P. H. (1993) Subcell. Biochem. 19, 125–161
14. Weigel, P. H., and Yik, J. H. N. (2002) Biochim. Biophys. Acta 1572, 341–363
15. Weigel, P. H. (1994) Bioessays 16, 519–524
16. Tozawa, R., Ishibashi, S., Osuga, J., Yamamoto, K, Yagyu, H., Ohashi, K., Tamura, Y., Yahagi, N., Iizuka, Y., Okazaki, H., Harada, K., Todaka, T., Shimano, H., Kimura, S., Nagai, R., and Yamada N. (2001) J. Biol. Chem. 276, 12624–12628
17. Ishibashi, S., Hammer, R. E., and Herz, J. (1994) J. Biol. Chem. 269, 27803–27806
18. Ashwell, G., and Harford, J. (1982) Annu. Rev. Biochem. 51, 531–554
19. Yik, J. H. N., Saxena, A., Weigel, J. A., and Weigel, P. H. (2002) J. Biol. Chem. 277, 40844–40852
20. Weigel, P. H., and Oka, J. A. (1982) J. Biol. Chem. 258, 5089–5094
21. Yik, J. H. N., Saxena, A., and Weigel, P. H. (2002) J. Biol. Chem. 277, 23076–23083
22. Zeng, F. Y., and Weigel, P. H. (1996) J. Biol. Chem. 271, 12624–12628
23. Laemmli, U. K. (1970) Nature 227, 680–685
24. Burnette, W. N. (1981) Anal. Biochem. 112, 195–203
25. Sanger, F., Nidder, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
26. Veit, M., Schmidt, M. F. G., and Rott, R. (1989) Virology 168, 173–176
27. Penin, S., and Schmidt, M. F. G. (1990) Virology 269, 325–335
28. Ten Brinke, A. Van der Grond, A. B., Haagsman, H. P., Rijter, A. N., van Golde, L. M., and Batenburg, J. J. (2002) J. Biol. Chem. 276, 663–671
29. Hansen, S. G., Grosenbach, D. W., and Hruby, D. E. (1999) Virology 254, 124–137
30. Grosenbach, D. W., Ulaeto, D. O., and Hruby, D. E. (1997) J. Biol. Chem. 272, 1956–1964
31. Shum, L., Turk, C. W., and Derynck, R. (1996) J. Biol. Chem. 271, 28502–28508
32. Geffen, I., Fuhrer, C., Leitinger, B., Weiss, M., Huggel, K., Griffiths, G., and Spiess, M. (1993) J. Biol. Chem. 268, 20772–20777
33. Geffen, I., Fuhrer, C., and Spiess, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8425–8429
34. Haynes, P. A., Oka, J. A., and Weigel, P. H. (1994) J. Biol. Chem. 269, 33146–33151
35. Crise, B., and Rose, J. K. (1992) J. Biol. Chem. 267, 13593–13597
36. Shaw, A. S., Chalupny, J., Whitney, J. A., Hammond, C., Amrein, K. E., Kavathas, P., Sefton, B. M., and Rose, J. K. (1990) Mol. Cell. Biol. 10, 1853–1862
37. Turner, J. M., Brodsky, M. H., Irving, B. A., Levin, S. D., Perlmuter, R. M., and Littman, D. R. (1990) Cell 60, 755–765
38. Veit, M., Sachs, K., Heckelmann, M., Maretzki, D., Hofmann, K. P., and Schmidt, M. F. G. (1998) Biochim. Biophys. Acta 1394, 50–58
39. Duncan, J. A., and Gilman, A. G. (1998) J. Biol. Chem. 273, 13580–13587
40. Camp, L. A., Verkruysse, L. A., Afendis, S. J., Slaughter, C. A., and Hofmann, S. L. (1994) J. Biol. Chem. 269, 23212–23219
41. Lobo, S., and Deschenes, R. J. (2001) Mol. Biol. Cell 12, (Suppl.) 6a
42. Yik, J. H. N., Saxena, A., Weigel, J. A., and Weigel, P. H. (2002) Biochem. Biophys. Res. Commun. 297, 980–986
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J. Biol. Chem. 2002, 277:47305-47312.
doi: 10.1074/jbc.M208751200 originally published online October 4, 2002

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