Receptor-associated Protein in an Oviparous Species Is Correlated with the Expression of a Receptor Variant*

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The biosynthesis of proteins containing cysteine-rich domains requires chaperones for their correct folding. For instance, the 39-kDa receptor-associated protein (RAP) aids in the cell-surface targeting of newly synthesized members of the mammalian low density lipoprotein receptor (LDLR) gene family, which contains tandemly arranged clusters of hexacysteine repeats. In the chicken, an LDLR relative with eight such repeats is expressed as two different splice variant forms in cell type-specific fashion (Bujo, H., Lindstedt, K. A., Hermann, M., Mola Dalmau, L., Nimpf, J., and Schneider, W. J. (1995) J. Biol. Chem. 270, 23546–23551). To learn more about evolutionary aspects of RAP, its role in escorting of these different receptor splice variants, and other potential functions, we have extended our studies on the avian LDLR family to RAP. cDNA cloning, determination of tissue expression at both the transcript and the protein level, stable expression in COS cells, and binding studies with chicken RAP revealed that mammalian RAPs have retained many features of the non-amniotic proteins. However, structural details, e.g. the well defined internal triplicate repeats in the chicken protein, have been somewhat diluted during evolution. Interestingly, chicken RAP was found to correlate positively with the expression levels in somatic cells of the larger splice variant of the eight-cysteine repeat receptor, but not with those of the smaller variant, expressed only in germ cells. This is compatible with the possibility that RAP may play a role in receptor biology that could be complementing its function in assisting folding. Chicken RAP in crude extracts of the stable expression COS cells is able to bind to LDLR relatives in ligand blots without requirement for prior purification of the ligand. Thus, in conjunction with the avian model of massive lipid transport to germ cells, these cells provide a novel comparative system amenable to investigation of the biological functions of RAP.

Low density lipoprotein receptor (LDLR)† gene family mem-

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1 The abbreviations used are: LDLR, low density lipoprotein receptor; RAP, receptor-associated protein; chRAP, chicken RAP; LR8, LDLR relative with eight binding repeats; ER, endoplasmic reticulum; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; bp, base pair(s); PCR, polymerase chain reaction; BSA, bovine serum albumin.
Isolation of Chicken RAP cDNA—A 290-bp fragment of human RAP cDNA (pSA39) was produced by PCR using two synthetic oligonucleotides: 5′-GAGCAGCTGCGTGAACAA (A) and 5′-TGAACCTTTCTCTTTTGATG (B). The obtained fragment was 32P-labeled using the Megaprime DNA labeling kit (Amersham) and used as probe to screen a chicken genomic library (CLONTECH). Hybridization conditions were as follows: 42°C, 5× SSC, 1 tablet Denhardt’s solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% BSA), 1% SDS, and 100 µg/ml salmon sperm DNA for 20 h at 42°C. The membranes were washed 2× 10 min in 2× SSC, 0.1% SDS at room temperature and 2× 30 min in 0.1× SSC, 0.1% SDS at 42°C. Positive clones were amplified by PCR using Agt11-specific primers, subcloned into the pGEM-T vector (CLONTECH), and sequenced on the pGEM-T vector (CLONTECH) with the primers 5′-GGGGCCTGGCTGACGCCAAGCGGC (C) and 5′-GTGAGAGCTCT-TCTTCTCGT (D) as follows: 5× NET (0.5 M NaCl, 75 mM Tris, 20 mM EDTA), 5× Denhardt’s solution, 0.2% SDS, and 100 µg/ml salmon sperm DNA for 20 h at 65°C. The membranes were washed 2× 30 min in 2× NET, 0.2% SDS at 65°C.

Antibody Production and Immunofluorescence—Antiserum against chicken RAP was generated against a synthetic peptide corresponding to 16 residues (101–116) of the deduced amino acid sequence of the cloned cDNA for chicken RAP. The peptide was coupled to keyhole limpet hemocyanin (28) and used for immunization of female New Zealand White rabbits as described (29). Antiserum against LR8 was prepared against purified recombinant receptor as described (30). Isolated granulosa cell sheets (31) were attached to adhesion slides (Bio-Rad) and fixed in methanol:acetone (4:1) for 10 min at −20°C. Alternatively, fixation was performed in 4% formaldehyde in PBS for 20 min at 23°C, followed by incubation in 50 mM NH4Cl for 15 min and 0.1% Triton X-100 for 5 min. Samples were then blocked in PBS containing 0.2% gelatine for 30 min at room temperature and incubated with a 1:100 dilution of antisera in PBS containing 0.2% gelatine for 2 h at room temperature. After several washes in PBS, samples were incubated with goat anti-rabbit IgG conjugated to BODIPY FL (Molecular Probes, Leiden, Netherlands) for 30 min at 20°C. The sections were hybridized overnight at 45°C with prehybridization solution containing 10% dextran sulfate and 300 ng/ml of the digoxigenin-labeled antisense or sense RNA probes. The RNA probes were prepared as follows: A 269-bp PCR fragment (27–295) prepared from the chicken RAP cDNA by PCR amplification with the primers 5′ (C) and 3′ (D) was subcloned into the pGEM-T vector (Promega). The purified plasmid was linearized, and the RNA probe was prepared and labeled with digoxigenin-UTP by in vitro transcription with SP6 and T7 RNA polymerase (DIG RNA labeling kit (SP6/T7)) according to the manufacturer’s recommendations (Boehringer Mannheim). The slides were then washed 3× 30 min with 0.2× SSC and 2× 10 min with 0.1× SSC at 50°C. After washing, the slides were preincubated with 100 µl of blocking buffer (2% bovine serum albumin, 0.1% Tween (washing buffer) and then incubated in blocking buffer with our anti-chicken RAP antisera (1:500). After washing for 3× 20 min, the primary antibody was detected with protein A-horseradish peroxidase (1:5000) and the chemiluminescence detection method as described above.

Northern Blot Analysis—For Northern blotting, total RNA (20 µg) prepared from various tissues of female and male chickens was denatured using glyoxal and dimethyl sulfoxide, separated by electrophoresis on a 1.25% agarose gel, and blotted onto Hybond N nylon membrane (Amersham) using standard methods (35). The above described 269-bp chicken RAP cDNA fragment was labeled with 32P using the Megaprime DNA labeling kit and used as probe. The membrane was hybridized at 65°C in 10 mg/ml BSA, 70 mg/ml SDS, 0.5 µm sodium phosphate buffer (pH 6.8), 1 mM EDTA (pH 8), and the 32P-labeled DNA probe. Washing was performed at 65°C in 5 mg/ml BSA, 50 mg/ml SDS, 40 mM sodium phosphate buffer (pH 6.8), and 1 mM EDTA and then in 10 mg/ml SDS, 40 mM sodium phosphate buffer (pH 6.8), and 1 mM EDTA (washing buffer) for 2 h at 80°C.

In Situ Hybridization—Tissue sections from testes (>24-week-old rooster) and follicles (adult hens) were prepared for in situ hybridization as described earlier (18, 26). The sections were hybridized overnight at 45°C with prehybridization solution containing 10% dextran sulfate and −300 µg/ml of the digoxigenin-labeled antisense or sense RNA probes. The RNA probes were prepared as follows: A 269-bp PCR fragment (27–295) prepared from the chicken RAP cDNA by PCR amplification with the primers 5′ (C) and 3′ (D) was subcloned into the pGEM-T vector (Promega). The purified plasmid was linearized, and the RNA probe was prepared and labeled with digoxigenin-UTP by in vitro transcription with SP6 and T7 RNA polymerase (DIG RNA labeling kit (SP6/T7)) according to the manufacturer’s recommendations (Boehringer Mannheim). The slides were then washed 3× 30 min with 0.2× SSC and 2× 10 min with 0.1× SSC at 50°C. After washing, the slides were preincubated with 100 µl of blocking buffer (2% bovine serum albumin, 0.1% Tween (washing buffer) and then incubated in blocking buffer with our anti-chicken RAP antisera (1:500). After washing for 3× 20 min, the primary antibody was detected with protein A-horseradish peroxidase (1:5000) and the chemiluminescence detection method as described above.

RESULTS

A full-length chRAP cDNA was isolated from a brain Agt11 cDNA library by homology screening. The 1497-bp cDNA specifies an open reading frame of 1044 bp and a 3′-untranslated region of 453 bp (Fig. 1). The 348-residue protein deduced from the cDNA contained a putative signal sequence of 21 residues spanning from the initiator methionine to the start of the coding region. The mature protein consists of 327 amino acids spanning from the initiator methionine to the cleavage site, and the 348-residue protein deduced from the cDNA by PCR amplification with the primers 5′ (C) and 3′ (D) was subcloned into the pGEM-T vector (Promega). The purified plasmid was linearized, and the RNA probe was prepared and labeled with digoxigenin-UTP by in vitro transcription with SP6 and T7 RNA polymerase (DIG RNA labeling kit (SP6/T7)) according to the manufacturer’s recommendations (Boehringer Mannheim). The slides were then washed 3× 30 min with 0.2× SSC and 2× 10 min with 0.1× SSC at 50°C. After washing, the slides were preincubated with 100 µl of blocking buffer (2% bovine serum albumin, 0.1% Tween (washing buffer) and then incubated in blocking buffer with our anti-chicken RAP antisera (1:500). After washing for 3× 20 min, the primary antibody was detected with protein A-horseradish peroxidase (1:5000) and the chemiluminescence detection method as described above.

Avian Receptor-associated Protein

A full-length chRAP cDNA was isolated from a brain Agt11 cDNA library by homology screening. The 1497-bp cDNA specifies an open reading frame of 1044 bp and a 3′-untranslated region of 453 bp (Fig. 1). The 348-residue protein deduced from the cDNA contained a putative signal sequence of 21 residues spanning from the initiator methionine to the start of the coding region, which was assigned according to von Heijne (39) to Ala/Ser (see Fig. 1). The mature protein consists of 327 amino acids with a calculated Mr of 38,654. Comparison of the avian protein sequence with those of RAPs from man, mouse, and rat (Fig. 3) revealed identities of 65, 63, and 58%, respectively. Analysis of the primary sequence showed that chRAP contains a single potential site for N-glycosylation, as well as the highly conserved carbonyl-terminal tetrapeptide, His-Asn-Glu-Leu (HNEL, Fig. 1), previously shown to serve as an ER retention signal for human RAP (1). Indeed, determination of the subcellular distribution of chRAP in ovarian granulosa cells by immunofluorescence (Fig. 2) revealed a typical ER staining pattern with highest levels in the perinuclear region (panels A and...
The granulosa cell "sheets" were obtained \textit{ex vivo} from ovarian follicles (32) and displayed the characteristic coherent epitheloid phenotype. For comparison, we also analyzed the distribution of the cell surface receptor LR8 (Ref. 18 and discussed below), a member of the LDL receptor gene family, in these cells (Fig. 2C); highest steady state levels of the receptor are found in the cell periphery.

In chRAP, the region immediately preceding the retention signal tetrapeptide HNEL contains an additional residue, Gln, and is different from those in the known mammalian homologues, in all of which it is Arg-Ala-Arg. The degree of identity of the avian RAP with its mammalian homologues appears lower in the central repeat domain (Fig. 3). Upon close inspection of the chicken RAP sequence, we found nine tetrapeptides consisting of three or four basic residues and zero or one hydrophobic amino acid. Interestingly, only four of the nine tri- or tetrabasic peptides in chicken RAP are conserved in the mammalian homologues (Fig. 3). RAP has been suggested to contain an internal triplication of approximately 100 residues (1, 2). Such triplication is clearly identifiable in chRAP, in that each repeat not only contains three of the nine basic tetrapeptides but also the sequences Lys-Asp-Glu-Leu (KDEL, residues 56–64).

**Fig. 1.** Nucleotide and deduced amino acid sequence of the cDNA for chicken RAP. The putative signal sequence is boxed, and the numbering of amino acids starts from the predicted signal sequence cleavage site. A potential N-linked glycosylation site is indicated by dashed underlining, and a presumptive ER retention signal at the C terminus (HNEL) is underlined. Nine basic tetrapeptides (see text) are underlined with thin lines.

**Fig. 2.** Immunofluorescence of chRAP in granulosa cell sheets. chRAP was detected in isolated granulosa cell sheets as described under "Experimental Procedures" using immunofluorescence microscopy. The antibody against chRAP shows an ER-typical perinuclear staining (panel A) compared with the antibody against LR8, which is found exclusively on the cell surface (panel C). Panel B shows the propidium iodide-stained nuclei of the cells in panel A. Bar, 10 \textmu m.

**Fig. 3.** Comparison of the protein sequences of chicken, human, mouse, and rat RAP. Alignment was performed by the GeneWorks computer program (IntelliGenetics Inc.), and identical residues in all four species are boxed. Chicken RAP shows the highest degree of identity to human RAP (65%).
59; in repeat 1), His-Arg-Glu-Leu (HREL, residues 190–193; repeat 2), and the above-mentioned HNEL at the carboxyl terminus (i.e., the end of repeat 3). Finally, Pietromonaco et al. (37) noticed that the primary sequences surrounding each of the five tryptophanes in mammalian RAPs show conservation; this is true also for the five Trp residues (residues 31, 61, 124, 142, and 230) in chicken RAP.

In light of RAP’s proposed function as chaperone for members of the LDLR family (1, 8, 15), it was of interest to determine the sites of RAP expression in the chicken. The laying hen expresses several members of the LDLR gene family, as well as splice variants thereof (17, 18), in cell-specific fashion (17, 18, 26). As revealed by Northern blot analysis (Fig. 4), RAP expression was ubiquitous, although the levels varied widely between tissues (equal mRNA loading has previously been shown by hybridization for glyceraldehyde-3-phosphate dehydrogenase, see Fig. 2 in Novak et al. (19)). Of the tissues investigated, lung and kidney contained the highest levels, followed by testes, brain, uropygial gland, adrenals, granulosa cells (obtained from ovarian follicles as “granulosa sheet,” “Gs” in Fig. 4; see also Fig. 2), ovary (different stages of follicles), and liver. The expression of RAP in cultured chicken embryo fibroblasts was higher when they were maintained in complete serum supplemented with 4 μg/ml OH-cholesterol (fibroblast/fetal calf serum) than when they had been exposed to lipoprotein-depleted serum. A condition that leads to induction of LDLR, to which RAP binds with low affinity (38). In any case, RAP levels in cultured fibroblasts were low compared with levels found in tissues, with the possible exception of the liver.

The levels of RAP protein, determined by Western blotting with a polyclonal rabbit anti-chicken RAP peptide antibody (see “Experimental Procedures”) in several tissues confirmed its ubiquitous expression (Fig. 5A). Agreement between the transcript and protein data was generally good, with protein levels possibly lower and higher than expected in lung and liver, respectively. An interesting finding relates to the expression of RAP in the gonads of the chicken (Fig. 5, B and C). We have previously shown that the expression of a splice variant of the chicken LDLR homologue termed LR8+ (18) decreases during testicular maturation (26) and increases in granulosa cells during oocyte growth (cf. Fig. 2). LR8+ is undetectable in ejaculated sperm (26), in oocytes following ovulation, and in eggs. Here, we found that the levels of RAP parallel that of LR8+, in that RAP levels were much higher in immature testes than in mature animals (Fig. 5). Also, levels of RAP in ovarian follicles that had not entered the rapid growth phase yet (“large white,” diameter 4–5 mm, in Fig. 5C) were much lower than those in the second largest follicle (“follicle2”; diameter, 2 cm). Furthermore, RAP was undetectable in sperm and egg.

The expression of the smaller receptor splice variant, LR8−, which lacks a serine/threonine-rich domain, the so-called “O-linked sugar domain,” is specific for the germ cells of the chicken (17, 26) and increases during spermatogenesis (26) and oocyte growth, respectively. Thus, in testes, changes in RAP expression levels are inverse to those of LR8−, but at first sight did not appear to do so in the ovary (i.e., higher levels of RAP in larger follicles, Fig. 5C). To address this point in more detail, we determined the cellular sites of RAP expression in ovarian follicles and testis by in situ hybridization analysis on ovarian follicles and testis (Fig. 6, A and B) and testicular (Fig. 6, C and D) sections. In follicles the granulosa cells, and in testes the Sertoli cells contained by far the highest levels of RAP. While in the female gonads, the clear-cut cellular architecture allows unambiguous identification of the granulosa cells, i.e., the cell layer juxtaposed to the oocyte, within the seminiferous tubules of the testis, it is more difficult to distinguish somatic cells and maturing spermatocytes. Nevertheless, close inspection reveals that in both the male and female gonads, expression of RAP, which binds to both LR8+ and LR8− (18, 26), in the germ cell-supporting somatic cells (which express LR8+ and LR8−) prevails over that in the germ cells (which express LR8−).

To obtain functional chicken RAP and to initiate studies on the possible role of RAP in the biosynthesis of the two chicken LR8 splice variant forms (17, 18), we have generated a mam-

**Fig. 4. Expression of RAP in chicken tissues and cells.** Total RNA (15 μg/lane) was isolated from the various indicated chicken tissues and cells (Br, brain; Li, liver; Gs, granulosa sheet; Ov, ovary; Fi, fibroblasts; Ad, adrenal; Ki, kidney; Lu, lung; Ug, uropygial gland; Te, testis), denatured, and separated by electrophoresis on a 1.5% agarose gel, blotted onto nitrocellulose filters, and probed with a32P-labeled 269-nucleotide (nucleotides 27–295) fragment. **Fig. 5. Western blotting of RAP in different chicken tissues.** Crude Triton X-100 extract (20 μg of protein) from different chicken tissues (A), testes of different age (14–48 weeks) (B), and different stages of follicle growth (C) (large white, 4–5 mm in diameter; follicle2, 2 cm in diameter; and a laid egg) were subjected to electrophoresis on a 4–18% SDS-PAGE gel, blotted onto nitrocellulose filters, and subjected to Western blotting with rabbit antiserum (1:500) against chicken RAP as described under “Experimental Procedures.” Numbers on the left correspond to the molecular mass (kDa) of marker proteins.

2 J. Nimpf, W. J. Schneider, and H. Bujo, unpublished observation.
malian cell line that stably overexpresses chicken RAP. From the results in Fig. 7, it is obvious that these cells express high levels of chicken RAP, identified as a 39-kDa protein by Western blotting in the transformed cells, but not in control cells, with antibodies raised against a synthetic peptide derived from the sequence of the cloned cDNA. Under these conditions, endogenous simian RAP did not cross-react with our anti-peptide antibody. However, the antibody recognized a COS-7 cell membrane protein doublet of about 100–105 kDa, which may represent endogenous simian LDLR homologues (27).

The high level of chicken RAP expression in these cells allowed us to directly, i.e. without prior purification, test for biological activity of the recombinant protein (Fig. 8). SDS-PAGE-separated membrane proteins of chicken ovarian extracts were transferred to nitrocellulose membranes, which were subsequently incubated with extracts prepared from control or chicken RAP-expressing COS-7 cells. Chicken RAP bound to proteins in the ovarian extract was then detected by Western blotting with our rabbit anti-chicken RAP antibody. As Fig. 8 demonstrates, chicken RAP in extracts of expressor COS-7 cells is an active protein that can bind to the 95-kDa LR8− (lane 1). Incubation with extracts of control cells (lane 2) reveal a much weaker 95-kDa signal, which is possibly due to the cross-reactivity of anti-RAP with endogenous receptor(s) (Ref. 27; cf. Fig. 8, lane 3, and Fig. 7). Lane 4 of Fig. 8 shows an immunoblot with an antibody directed against the carboxyl-terminal 14 residues of LR8 (17) for comparison. Taken together, the results from this cell extract indirect ligand blotting procedure (EXLBlot) demonstrate that chicken RAP expressed in simian cells is an active in vitro ligand and that the chicken RAP/COS cell system will be useful to delineate the possible role of RAP in differential intracellular receptor splice variant targeting (17, 18).

DISCUSSION

The structural conservation of RAP in a nonamniote is compatible with important functions of the 39-kDa protein in eukaryotic cells. While a role of RAP as chaperone for members of the LDLR family seems established, other functions seem plausible, particularly in the light of our current and recent findings (20) as well as those of others (14, 39), as discussed below. Collectively, the data suggest that RAP interacts with nascent cysteine-rich polypeptide chains at a point in the biosynthetic pathway of receptors where interaction with endogeneous ligands may otherwise block the correct onward processing (2, 9, 15). This notion is based on the known competitive displacement of ligands of the LDLR family by intracellular RAP, believed to bind to the so-called LDLR binding repeats, which are six cysteine-containing subdomains of ~40 amino acids each, clusters of which constitute the ligand binding domains of LDLR family members (40). In addition or alternatively, RAP plays an important role in receptor folding itself, as shown, e.g. by the reduction of intracellular aggregation of soluble minireceptor forms of LDLR-related protein (8). In fact, recently, Obermoeller et al. (2) reported that the triplicate repeats in RAP can perform differential functions, with only the carboxy-terminal (third) repeat able to promote folding and secretion of...
soluble LDLR-related protein minireceptors (2).

Our present data and the finding that a non-LDLR family member, gp95/sortilin (39), and a receptor containing cysteine-rich repeats other than LDLR ligand binding repeats (20, 41, 42) can bind RAP are compatible with a role beyond its proposed functions as chaperone in the processing of LDLR family members and as inhibitor of premature ligand interaction with the receptors. Notably, the receptor termed LR11 by us (20, 42) and gp95/sortilin (39) contain domains that show sequence homology to segments of yeast Vps10p, the sorting receptor for soluble vacuolar carboxypeptidase Y (43). Both LR11 and gp95/sortilin have been purified from brain extracts by affinity chromatography on immobilized RAP (39, 42). Inasmuch as gp95/sortilin does not contain LDLR ligand binding repeats (six cysteines each) but Vps10p domains, which are characterized by 10 totally conserved cysteines (20, 39, 41, 42), it is reasonable to assume that the interaction between RAP and these proteins occurs via the cysteine-rich Vps10p domain(s). Thus, at least in vitro, interaction of receptors with RAP does not depend solely on the presence of six-cysteine repeats. In our chRAP-expressing COS cells, we have obtained preliminary evidence in [35S]cysteine labeling experiments for increased secretion of cysteine-containing proteins when compared with control cells (data not shown). Thus, RAP may have a more general role in escorting proteins with domains containing (even numbered) cysteines requiring correct pairing, in particular when the cells co-synthesize ligands(s).

Another point to consider is the expression of splice variant forms of the avian very low density lipoprotein receptor homologue, LR8 (17, 18, 26). As shown here, levels of RAP correlate positively with those of the variant expressed in somatic cells, LR8+, which contains the so-called O-linked sugar domain (18). Somatic cells, such as the hepatoma cell line LMH-2A and likely the granulosa cells, where high levels of RAP are found (Figs. 2, 4, and 6), produce apolipoproteins and other potential ligands of the LDL receptor family (44). Thus, RAP may well be required for efficient expression of LR8+ on the surface of these cells. On the other hand, the plasma membrane expression of LR8−, the germ cell-specific form lacking the O-linked sugar domain, does not seem to depend on RAP, possibly related to the fact that oocytes are not known to synthesize apolipoproteins. We have previously proposed that translocation of intracellular LR8− to the plasma membrane at onset of oocyte growth may be triggered by a specific signaling protein that interacts specifically with LR8− (17); the current data suggest that such signal is unlikely to involve RAP.

The molecular characterization of the first nonmammalian RAP revealed extensive similarity to amniotic RAPs. However, in reviewing the features of chicken RAP and published reports, we noted a discrepancy between the putative assignment of amino termini of different RAPs and the criteria of von Heijne (36). In the case of human RAP (5), amino-terminal sequencing of tryptic peptides, but not of the intact mature protein, resulted in the identification of Tyr as the amino terminus. However, this Tyr is preceded by Gly-Lys in the amino terminus of mature human RAP is Gly rather than Tyr and that of chicken RAP is Ser (Fig. 1). Another significant finding may be the fact that the three internal repeats in RAP are particularly distinct in the avian homologue, where they are characterized by triplication of potential ER retention signals and three times three tri-or tetrabasic sequences. This indicates that mammalian RAPs may have lost some of the ancestral structural features still discernible in the avian gene. We have obtained a COS-7 cell line expressing high levels of chicken RAP. Inasmuch as chicken RAP has all the structural hallmarks of hitherto known homologues, these cells should prove useful for receptor expression and processing studies. The high level of RAP in these cells has already allowed us to use crude cell extracts in a novel reverse ligand blotting procedure termed EXLBlot (Fig. 8), in which we have also obtained supporting evidence for our previous observation that RAP and certain members of the LDLR gene family share common epitope(s) (27). Here, the antibody was prepared against a synthetic peptide corresponding to residues 101–116 of chicken RAP, suggesting that receptor cross-reactive epitopes may not be limited to the region(s) of RAP previously identified in the mammalian system (27). Shared epitope recognition is, at least in part, responsible for the development in rats of the autoimmune disease, passive Heymann nephritis (27). While this immunopathological effect seems to be confined to the rat, clinical consequences of extracellular appearance of RAP in other animals cannot be excluded. At the very least, RAP bound to surface receptors would inhibit the binding and uptake of physiological ligands. This has been demonstrated in mice, which show delayed clearance of certain lipoprotein ligands as a consequence of overexpression of RAP (9, 15). We are now in a position to investigate the effects of intravenously administered homologous RAP on yolk precursor uptake into chicken oocytes, which is possibly the most dramatic LDLR family-mediated transport process.

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