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Endoplasmic reticulum resident protein of 90 kilodaltons associates with the T- and B-cell antigen receptors and major histocompatibility complex antigens during their assembly

(molecular chaperone)

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ABSTRACT **In the endoplasmic reticulum (ER), newly synthesized subunits of the T-cell antigen receptor (TCR), membrane-bound immunoglobulin (mIg), and major histocompatibility complex (MHC) class I antigens must fold correctly and assemble completely into multimeric protein complexes prior to transport to the cell surface. Although folding and assembly may occur spontaneously, the concept that molecular chaperones facilitate these events is emerging. Here, an intracellular protein of 90-kDa apparent molecular mass, denoted IP90, was shown to be an ER resident protein that associated with partial complexes of the TCR, mIg, and MHC class I proteins but was absent from fully assembled complexes. We speculate that IP90 might participate in folding and assembly processes of these and other multisubunit protein complexes during their transit through the ER.**

During biosynthesis, transmembrane proteins are translocated into the endoplasmic reticulum (ER) lumen and become anchored in the membrane. Subsequently, they have to fold correctly, and in the case of multisubunit proteins, all components must assemble completely before transport via the Golgi complex to the cell surface can occur. Incorrectly folded proteins or partial protein complexes are retained in the ER, ensuring that only functional proteins become expressed on the cell surface. Increasing evidence is emerging that the folding of newly synthesized polypeptides and their assembly into multimeric complexes might be mediated by a class of proteins collectively called molecular chaperones or polypeptide chain-binding proteins (1–3).

The multimeric T-cell antigen receptor (TCR) complex consists of a clonally variable heterodimer (TCR-$\alpha\beta$ or TCR-$\gamma\delta$) that is associated with the invariant CD3 complex (CD3-γ, CD3-δ, and CD3-ε) and the ζ-ζ homodimer (4–6). To identify intracellular binding proteins that associate with the TCR complex during assembly, we performed intracellular radioiodinations of permeabilized T lymphocytes. Anti-TCR immunoprecipitates from these permeabilized radioiodinated cells (where intracellular proteins are labeled) were compared with those from cell-surface radioiodinated cells (where intracellular proteins are not labeled). This screening method allowed the identification of a new ER resident protein that associated not only with partial TCR complexes but also with a number of other multisubunit proteins, including the membrane-bound immunoglobulin (mIg) complex and major histocompatibility complex (MHC) class I proteins. We call this 90-kDa intracellular protein IP90.

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MATERIALS AND METHODS

Cell Lines. The following cell lines were used: 3A9, a murine T-cell hybridoma (7); A20, a murine B-cell lymphoma (8); human T-cell leukemia lines MOLT-4 (9), HPB-MLT, and DND41 (10); MCF7, a human breast carcinoma; and Daudi, a human Burkitt lymphoma (11).

Antibodies. Anti-TCR β chain monoclonal antibody (mAb) βF1 (IgG1) has been described (12). The following anti-human CD3-ε chain mAbs were gifts: mAb anti-Leu4 (IgG1) (13) from N. Warner (Becton Dickinson), mAb SPV-T3b (IgG2a) from H. Spits (DNAX), and mAb UCHT-1 from P. Beverley (University College and Middlesex School of Medicine, London). P. Anderson provided anti-TCR ζ chain mAb TIA-2 (IgG1) (14); H. Ploegh, anti-MHC class I heavy-chain mAb HC-10 (15); D. Toft (Mayo Medical School, Rochester), anti-90-kDa heat shock protein (HSP90) mAb AC88 (IgG1); D. Bole (University of Michigan), anti-BiP mAb; J. Hansen (Fred Hutchinson Cancer Research Center, Seattle), anti-CD28 mAb 9.3 (IgG2a); and M. Hemler (Dana–Farber Cancer Institute, Boston), anti-transferrin receptor mAb 5E9. Control mAb P3 (IgG1), anti-murine CD3-ε mAb 145-2C11 (16), anti-CD5 mAb OKT1 (IgG1), and anti-immunoglobulin κ chain (Ig_κ) mAb 187.1 were obtained from the American Type Culture Collection. Anti-GRP94/ERp99 (94-kDa glucose-regulated protein/99-kDa ER protein) antiserum HS-3 was a gift from Michael Green (St. Louis University School of Medicine).

Radiolabeling and Immunoprecipitation. For intracellular iodinations 4×10^7 cells were permeabilized in 8 μM digitonin for 10 min prior to radioiodination as described (14), whereas for cell-surface iodinations, 2×10^7 intact cells were used. After the lactoperoxidase-mediated iodinations, cells were solubilized in ≈8 mM digitonin (Aldrich). Immunoprecipitates with the indicated mAbs were analyzed on SDS/10% polyacrylamide gels under reducing conditions.

Generation of Anti-IP90 mAb. For immunogen preparation, 2×10^9 cells of the human T-cell leukemia line DND41 were solubilized overnight in 10 ml of 1% digitonin in Tris-buffered saline (TBS; 140 mM NaCl/50 mM Tris, pH 7.6). After a 20-min centrifugation, IP90-containing TCR complexes were isolated by using a mixture of 3 μl of anti-CD3ε mAb anti-Leu-4 ascites fluid, 350 μl of rat anti-mouse Ig_κ mAb

Abbreviations: ER, endoplasmic reticulum; TCR, T-cell antigen receptor; mIg, membrane-bound immunoglobulin; MHC, major histocompatibility complex; mAb, monoclonal antibody; GRP94, 94-kDa glucose-regulated protein; HSP90, 90-kDa heat shock protein; IP90, 90-kDa intracellular protein; Ig_H and Ig_L, immunoglobulin heavy and light chains; Ig_k, immunoglobulin κ light chain.

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187.1 supernatant, and 12.5 μl of formalin-fixed *Staphylococcus aureus* suspension (SAC I isolate). A 2-month-old BALB/c male mouse was immunized six times at 1-month intervals with the immunogen preparation suspended in phosphate-buffered saline and injected i.p. For the final booster, the immunogen was eluted from the SAC I isolate with a 50 mM diethylamine buffer (pH 11.5) neutralized with an acetate buffer and injected in the tail vein. Three days later the splenocytes were fused with the myeloma P3X63Ag8.653 as described (12). The hybridoma supernatants were screened for their ability to immunoprecipitate IP90 from a Triton X-100 cell lysate of intracellularly iodinated DND41 cells.

Amino-Terminal Sequencing. For IP90 protein purification, 25×10^9 MOLT-4 cells were solubilized in 150 ml of TBS containing 1% Triton X-100, 5 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride, and 0.1 unit of aprotinin per ml for 1 hr. After a 20-min centrifugation at 10,000 × *g*, the lysate was precleared over a DNase- and human immunoglobulin-conjugated Sepharose column. For the specific antibody column, AF8 mAb was cross-linked to protein A-agarose beads by using dimethyl suberimidate (Pierce). The column was incubated twice with the lysate and washed in TBS containing 0.5% Triton X-100, 0.05% SDS, and 0.5% deoxycholic acid. The IP90 protein was eluted from the column with 0.1 M glycine, pH 3/0.1% Triton X-100/140 mM NaCl and neutralized immediately with 1 M Tris (pH 8). The immunopurified IP90 was resolved on a SDS/7.5% polyacrylamide gel under nonreducing conditions and transferred to an Immobilon-P polyvinylidene difluoride membrane (Millipore). Staining of the membrane with Coomassie brilliant blue R-250 revealed the presence of one predominant band at 90 kDa that was cut out and subjected to amino-terminal sequence analysis with an Applied Biosystems 470A protein sequencer and a 120A on-line phenylthiohydantoin-conjugated amino acid analyzer.

Immunofluorescence and Immunogold Labeling. For the EM studies, cells and tissues were fixed with 2% paraformaldehyde/0.01% glutaraldehyde for 1 hr. Daudi and MCF7 cells were spun into a pellet, resuspended in 3% gelatin, and solidified on ice. Cells and tissue samples were cut into 1-mm^3 cubes; infused with 2.3 M sucrose overnight; and prepared, sectioned, and labeled as described (17). Primary antibody (5 μg/ml) was revealed with 5-nm goat anti-mouse–gold conjugates (Amersham). Observation was with Jeol 100CXII electron microscope. Fluorescence microscopy was performed on MCF7 cells that were fixed in 3% paraformaldehyde, permeabilized in methanol, and incubated with the anti-IP90 followed by rhodamine-conjugated goat anti-mouse reagents.

RESULTS AND DISCUSSION

For the cell-surface iodination, intact cells of the murine T-cell hybridoma 3A9 expressing the TCR-αβ–CD3–ζ-ζ complex were radioiodinated and solubilized in the mild detergent digitonin (≈8 mM). Immunoprecipitates with the anti-CD3-ε mAb 145-2C11 revealed the TCR-αβ subunits together with CD3 chains in SDS/PAGE analysis (Fig. 1*A*, lane 4). The ζ chain was not visualized under these conditions because it did not contain an extracellular tyrosine residue that could be labeled with radioiodine. To perform intracellular iodinations, we first permeabilized membranes of 3A9 cells with 8 μM digitonin and then performed a lactoperoxidase-mediated radioiodination as described (14). The anti-CD3-ε immunoprecipitate from these intracellularly iodinated cells visualized the presence of the 16-kDa ζ chain, which is known to be well radiolabeled in intracellular iodinations (Fig. 1*A*, lane 2). Importantly, comparison of this anti-CD3-ε immunoprecipitate from intracellularly iodinated cells with the analogous immunoprecipitate from cell surface-iodinated intact cells revealed the presence of a TCR-associated protein of 90-kDa apparent molecular mass that had not been observed

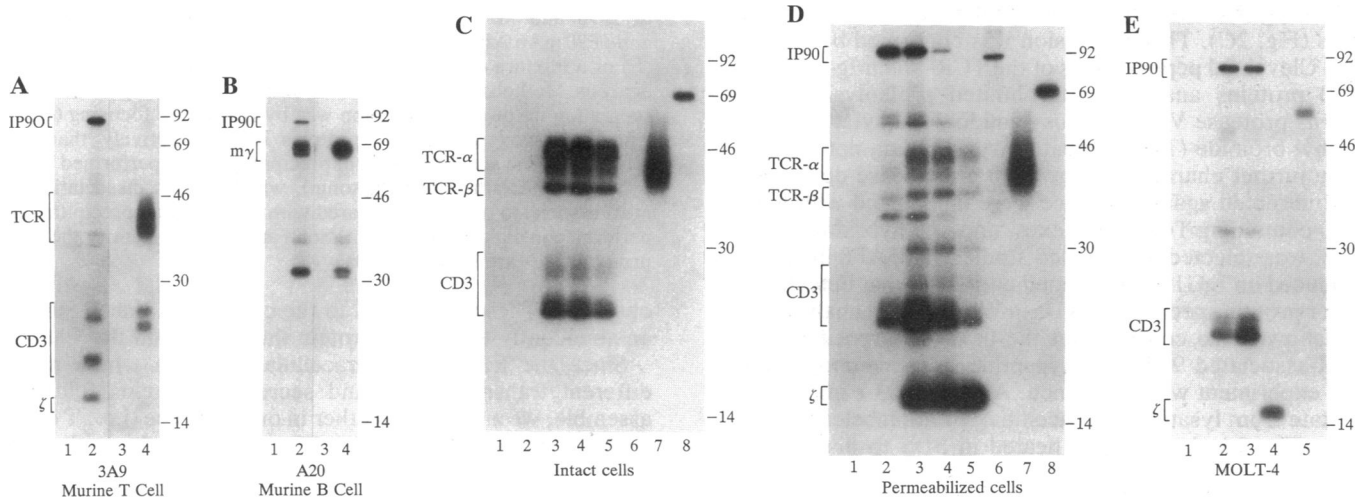


FIG. 1. A 90-kDa protein associated with the murine and human TCR- and B-cell receptor complexes is visualized in intracellular iodinations but not in cell-surface iodinations. Sizes are shown in kDa. (*A* and *B*) Intracellular radioiodinations (lanes 1 and 2) and cell-surface radioiodinations (lanes 3 and 4) were carried out on the murine T-cell hybridoma 3A9 (*A*) and the murine B-cell lymphoma A20 (*B*). Lysates were immunoprecipitated in *A* with hamster anti-mouse CD3-ε mAb 145-2C11 (lanes 2 and 4) or control mAb P3 (lanes 1 and 3) together with rabbit anti-hamster serum and protein A-conjugated Sepharose beads or in *B* with Affi-10 conjugated with anti-Ig_κ chain mAb 187.1 (lanes 2 and 4) or Affi-10 conjugated with control mAb SPV-T3b (lanes 1 and 3). (*C* and *D*) Cell-surface (*C*) and intracellular (*D*) radioiodinations were carried out on the human TCR-αβ-bearing leukemia cell line HPB-MLT. Lysates were immunoprecipitated with control mAb P3 (lanes 1), anti-TCR-β mAb βF1 (lanes 2), anti-CD3-ε mAbs anti-Leu4 (lanes 3) and SPV-T3b (lanes 4), anti-ζ chain mAb TIA-2 (lanes 5), anti-HSP90 mAb AC88 (lanes 6), anti-CD28 mAb 9.3 (lanes 7), or anti-CD5 mAb OKT1 (lanes 8) together with anti-Ig_κ mAb 187.1 and protein A-Sepharose beads. Surface radioiodinated T cells revealed TCR-α, TCR-β, and CD3 chains but not the associated ζ and IP90 chains. These latter proteins were visualized only in intracellular iodinations. Note in *D* the absence of IP90 from the anti-ζ chain immunoprecipitate (lane 5). (*E*) IP90 is associated with partial TCR complexes. Lysates of intracellularly radioiodinated MOLT-4 cells were immunoprecipitated with control mAb P3 (lane 1), anti-TCR-β mAb βF1 (lane 2), anti-CD3-ε mAb anti-Leu4 (lane 3), anti-ζ mAb TIA-2 (lane 4), and anti-CD5 mAb OKT1 (lane 5). MOLT-4 cells synthesize the CD3 chains and TCR-β, but lack TCR-α. Therefore, these cells only synthesize TCR-β/CD3 partial complexes that can be isolated with anti-TCR-β and anti-CD3 mAbs. The partial TCR complexes contain IP90 (but lack ζ chain).

previously (Fig. 1*A*, compare lanes 2 and 4). This protein was denoted IP90 because it was labeled only in intracellular iodinations.

Like T cells, B cells express on the cell surface a membrane-bound multimeric receptor for foreign antigen called mIg. The mIg complex consists of two clonally variable transmembrane immunoglobulin heavy chains (Ig_H chains) that are disulfide-linked to two variable immunoglobulin light chains (Ig_L chains), and these are noncovalently associated with three invariant immunoglobulin chains called α, β, and γ (18, 19). To identify intracellular binding proteins that might associate with the mIg complex during assembly, we repeated the above comparative analysis with the murine B-cell lymphoma line A20, which expresses a mIgG2a complex. Anti-Ig_L chain immunoprecipitations from cell surface-iodinated B cells revealed the presence of the Ig_H chain together with the immunoglobulin 32- to 37-kDa α, β, and γ chains (the Ig_L chain was not labeled in radio-iodinations) (Fig. 1*B*, lane 4). When we compared this immunoprecipitate from intact cells with an immunoprecipitate from intracellularly iodinated A20 cells, a protein of 90-kDa apparent molecular mass was observed that was labeled in permeabilized cells but not in intact cells (Fig. 1*B*, compare the immunoprecipitation from intracellularly iodinated cells in lane 2 with that from surface-iodinated cells in lane 4). In conclusion, intracellular complexes of both the murine TCR and mIg contained IP90.

To determine whether the 90-kDa proteins associated with the TCR and the mIg complex were the same or distinct proteins, these coimmunoprecipitated proteins were analyzed in two-dimensional gel electrophoresis (nonequilibrium pH gradient electrophoresis followed by SDS/PAGE). Both the murine TCR- and mIg-associated 90-kDa proteins possessed acidic isoelectric points (see arrowheads in Fig. 2 *A* and *B*). Analysis of a mixture of both immunoprecipitates showed that these proteins displayed indistinguishable mobilities in pH-gradient electrophoresis and in SDS/PAGE, indicating that they were very similar, if not identical, proteins (Fig. 2*C*). This conclusion was confirmed by the identical Cleveland peptide maps of the TCR- and mIg-associated IP90 proteins analyzed after limited proteolysis with *S. aureus* protease V8 or L-1-tosylamido-2-phenylethyl chloromethyl bromide (TPCK)-treated trypsin (data not shown).

To further characterize the IP90 protein, we developed a mAb directed against it. Mice were immunized with human IP90-containing TCR complexes, and one hybridoma clone, AF8, was selected for detailed studies. The AF8 hybridoma produced an IgG1 mAb, termed anti-IP90, that detected one major protein species of 90 kDa in Western blot analysis (data not shown). To confirm that the mAb was specific for the TCR-associated 90-kDa polypeptide, a reimmunoprecipitation experiment was performed. An anti-CD3-ε immunoprecipitate from lysates of intracellularly radiolabeled MOLT-4 cells (Fig. 3*A*, lane 3) was heated in SDS to dissociate all subunits in the immunoprecipitate. Reimmunoprecipitation with the new mAb revealed the presence of the 90-kDa polypeptide (Fig. 3*A*, lane 5). This result confirmed that the anti-IP90 mAb recognized the 90-kDa polypeptide that is associated with the TCR complex.

Using this mAb, we determined the intracellular localization of IP90 by immunofluorescence staining and immunogold EM. Immunofluorescence staining of cells of the breast carcinoma MCF7 with the anti-IP90 mAb showed a reticular pattern of staining that was characteristic for the ER (Fig. 4). EM using immunogold staining confirmed abundant labeling of the nuclear envelope and the rough ER in the human Burkitt lymphoma cell line Daudi (Fig. 5 *A–C*), MCF7 cells (Fig. 5*D*), and in bronchial epithelium (Fig. 5*E*). However, labeling of the Golgi cisternae (Fig. 5*F*), other cellular organelles, or the plasma membrane (Fig. 5*G*) was not

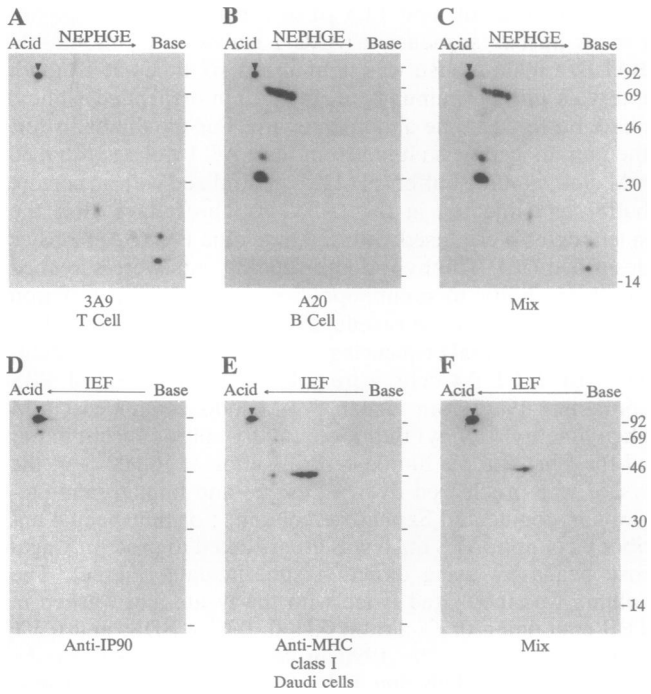
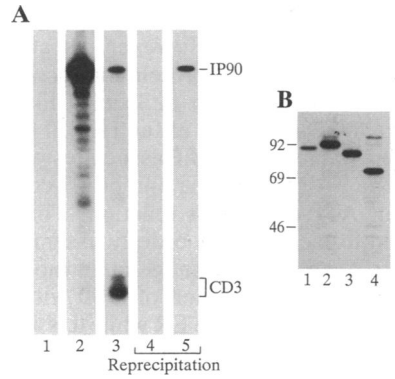


FIG. 2. (*A–C*) The TCR- and mIg-associated 90-kDa proteins appear identical. Immunoprecipitates with the anti-CD3-ε mAb 145-2C11 from intracellularly iodinated 3A9 T cells (*A*) or with protein A from intracellularly iodinated A20 B cells (*B*) were analyzed by nonequilibrium pH-gradient gel electrophoresis (NEPHGE) followed by resolution in a SDS/10% polyacrylamide gel. Note that both the TCR- and mIg-associated IP90 proteins migrate at acidic positions (see arrowheads). (*C*) Analysis of a mixture of the immunoprecipitates from *A* and *B* shows that the TCR- and mIg-associated IP90 proteins migrate at identical positions in the nonequilibrium pH gradient and in the SDS/10% polyacrylamide gel, forming a single radiolabeled species in this two-dimensional gel. (*D–F*) Association of IP90 and MHC class I proteins. Immunoprecipitations with anti-IP90 mAb AF8 (*D*) or anti-MHC class I heavy chain mAb HC-10 (*E*) or a mixture of both (*F*) were resolved by two-dimensional gel analysis. Methods were similar to those in *A–C* except that the charge separation in the first dimension was by isoelectric focusing (IEF), which resolved the class I proteins more effectively than did NEPHGE. The anti-IP90 immunoprecipitation was performed in 1% Triton X-100 (rather than digitonin), which causes dissociation of most associated proteins compared with IP90 immunoprecipitations performed in digitonin detergent. Solid arrowheads identify the IP90 protein; sizes are shown in kDa.

observed. These results led to the conclusion that IP90 was an abundantly expressed protein that resides in the ER.

Since the ER is the intracellular location where many different transmembrane and secretory proteins fold and assemble, we analyzed whether in one cell type (i.e., T cells) IP90 associated only with the TCR complex or also with other protein species. For this purpose cells of the T-cell leukemia line MOLT-4 were permeabilized, radio-iodinated, and solubilized in the mild detergent digitonin. When approximately equal amounts of MOLT-4 lysates were immunoprecipitated with the anti-IP90 mAb or an anti-CD3-ε mAb (Fig. 3*A*, lanes 2 and 3), it became clear that the vast majority of IP90 molecules were not associated with TCR complexes in these cells. Instead, IP90 appeared to be associated with a number of other proteins (Fig. 3*A*, lane 2). Two-dimensional gel analysis resolved at least 50 individual species (of undetermined identity) that were specifically coimmunoprecipitated by the anti-IP90 mAb from biosynthetically labeled cells (data not shown). On the other hand, IP90 did not appear to bind to intracellular chains of all transmembrane protein species, since it was not coimmunoprecipitated with the CD5 monomer, the CD28 homodimer, or several other proteins tested



C

POSITION:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
RESIDUE:	H	D	G	H	D	D	D	V	I	D	I	E	D	D	L	-	[D]	V	I	E	[E]	[E]

FIG. 3. (*A*) Characterization of an IP90-specific mAb. Immunoprecipitations were performed with control mAb MPC11 (lane 1), anti-IP90 mAb AF8 (lanes 2 and 5), anti-CD3-ε mAb UCHT-1 (lane 3), and anti-transferrin receptor mAb 5E9 (lane 4). Intracellularly iodinated MOLT-4 cells were solubilized in digitonin. Approximately 5% of the lysate was used for each immunoprecipitation in lanes 1, 2, and 3. The remainder of the lysate was immunoprecipitated with anti-CD3 (as in lane 3) and then boiled in 50 μl of 1% SDS and diluted to 2 ml with 2% (vol/vol) Triton X-100 detergent. This material was divided into two aliquots and reprecipitated with the anti-IP90 mAb (lane 5) or with anti-transferrin receptor mAb 5E9 as a control (lane 4). (*B*) Molecular size comparisons of IP90, HSP90, GRP94, and BiP. Immunoprecipitations of GRP94 with anti-GRP94/ERp99 peptide antiserum (lane 2), HSP90 with mAb AC88 (lane 3), and BiP with an anti-BiP mAb (lane 4) were carried out from biosynthetically labeled murine 3A9 cells. IP90 was visualized with the intracellular iodination technique and coimmunoprecipitation with anti-CD3-ε mAb 145-2C11 (lane 1). All immunoprecipitates were visualized side-by-side in the same 2,5-diphenyloxazole-impregnated SDS/polyacrylamide gel. For the biosynthetic labeling 20×10^6 3A9 cells were incubated for 6 hr with 1 mCi (1 Ci = 37 GBq) of [^{35}S]methionine and 1 mCi of [^{35}S]cysteine and solubilized in a 2% Triton X-100 buffer. Sizes are shown in kDa to the left. (*C*) N-terminal amino acid sequence of IP90. Using the anti-IP90 mAb, we purified ≈50 pmol of IP90 protein from MOLT-4 cells, which was subjected to amino acid sequence analysis. The sequence was distinct from known HSP90 proteins. The residues determined with low confidence are indicated in brackets, while the residue at position 16 could not be determined.

from intracellularly iodinated cells (Fig. 1*D*, lanes 7 and 8; also data not shown).

We ruled out that IP90 was a known member of the HSP90 family (20, 21). Namely, IP90 displayed a higher apparent molecular mass than did the HSP90 proteins (Fig. 3*B*, compare lanes 1 and 3) and a lower relative molecular mass than GRP94 (Fig. 3*B*, compare lanes 1 and 2). In fact, after immunoaffinity column purification of the IP90 polypeptide, the N-terminal amino acid sequence was determined and found to be unique (Fig. 3*C*).

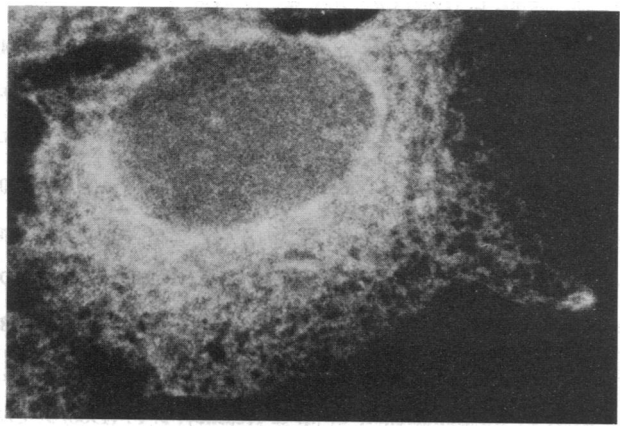


FIG. 4. Fluorescence microscopic visualization of anti-IP90-stained MCF7 cells.

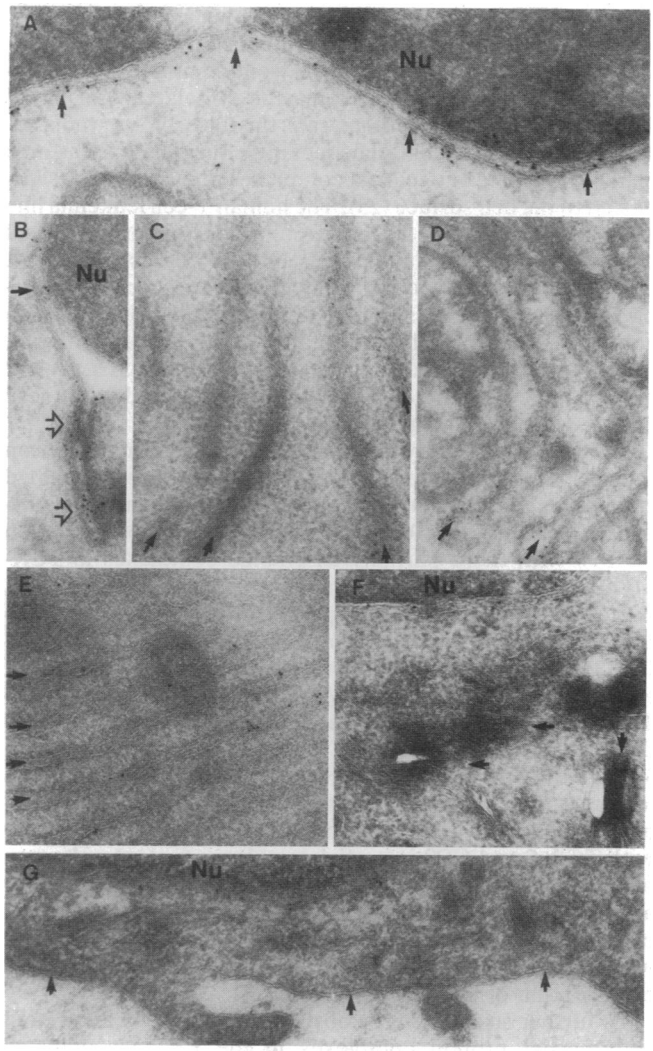


FIG. 5. Subcellular localization of IP90 with a specific mAb. Immunogold-labeled ultrathin cryosections show the distribution of IP90 in Daudi cells (*A*, *B*, *C*, *F*, and *G*), MCF7 cells (*D*), and human bronchial epithelium (*E*). In all cases labeling was most intense between the nuclear membranes (Nu). This labeling extended into the rough ER both when this membrane system was observed in association with the nuclear membrane (arrows in *B*) and when seen as cytoplasmic membrane stacks (arrows in *C*, *D*, and *E* indicate rough ER). Labeling did not extend beyond the rough ER, as the Golgi cisternae (arrows in *F*) and cell surface (arrows in *G*) were unlabeled. (*A*, ×71,800; *B*, ×59,850; and *C*–*G*, ×41,000.)

The above findings that IP90 was an ER resident protein that bound to a number of different protein species led us to hypothesize that IP90 might play a role during the assembly process of these protein complexes. To investigate this possibility we assessed whether IP90 was associated with partially assembled or with completely assembled TCR complexes. First, complete complexes were analyzed for associated IP90. The ζ chain was used as a marker of complete complexes, since the ζ-ζ dimer only associated with intermediary TCR complexes that contain the TCR α and β chains and the CD3 subunits, thereby forming the complete TCR multimer (22). The IP90 protein was not coimmunoprecipitated with the anti-ζ mAb TIA-2 from permeabilized human HPB-MLT cells (Fig. 1*D*, lane 5), suggesting that IP90 was not associated with complete TCR complexes. This was

consistent with the EM studies showing that IP90 was localized to the ER and not to the plasma membrane.

The apparent absence of IP90 from complete TCR complexes indicated that instead it may be associated with partial TCR complexes. To demonstrate this clearly, we took advantage of the fact that cells lacking either the TCR α or β chain synthesize only partial complexes that cannot be transported to the cell surface (23). The human T-cell leukemia line MOLT-4, which lacks TCR-α expression, synthesizes partial complexes consisting of TCR-β and the CD3 subunits (9). Importantly, IP90 was associated with these partial complexes, because it was coimmunoprecipitated both with an anti-TCR-β mAb, βF1, and an anti-CD3-ε mAb, anti-Leu4, from intracellularly iodinated MOLT-4 cells (Fig. 1*E*, lanes 2 and 3). Note that the ζ chain was not associated with these partial complexes (Fig. 1*E*, lane 4).

Normal T cells expressing the complete TCR multimer on the cell surface contain a fraction of partially assembled complexes intracellularly because several subunits are produced in excess (24). We tried to demonstrate the association of IP90 with these partially assembled complexes in TCR-$\alpha\beta$-expressing HPB-MLT cells. To isolate partial complexes, we took advantage of the properties of the anti-TCR-β mAb βF1 that recognizes partial complexes containing TCR-β and CD3 chains but does not react with TCR-β chains as part of the complete TCR-$\alpha\beta$–CD3-ζ-ζ complex. Indeed, the anti-TCR-β mAb βF1 did not stain the surface of T cells (12) and did not recognize complete TCR complexes from cell surface-iodinated HPB-MLT cells (Fig. 1*C*, lane 2). Importantly, immunoprecipitates with mAb βF1 from intracellularly iodinated HPB-MLT cells showed the presence of IP90 together with TCR-β and CD3 in partial complexes (note the absence of the ζ chain) (Fig. 1*D*, lane 2). The anti-CD3-ε mAb anti-Leu4 reacted both with complete TCR structures (exemplified by the coimmunoprecipitation of the ζ chain) and with partial TCR complexes (exemplified by the coimmunoprecipitation of IP90) (Fig. 1*D*, lane 3).

To further strengthen the observation that IP90 associated with partial protein complexes in the ER, we analyzed another multimeric protein complex, namely the MHC class I molecule. MHC class I molecules consist of a polymorphic heavy chain and a light chain (β_2-microglobulin) (25), which bind small peptide antigens and carry them to the cell surface for presentation to T lymphocytes. In Daudi cells, however, the heavy chains cannot be transported to the cell surface because β_2-microglobulin is not available for assembly (11). We examined these free heavy chains for association with IP90. Immunoprecipitations with a mAb against MHC class I *HLA-B* locus heavy chains, HC-10, from intracellularly iodinated Daudi cells revealed the presence of a 90-kDa polypeptide that displayed an isoelectric point and relative molecular mass indistinguishable from the IP90 polypeptide (Fig. 2 *D–F*; note immunoprecipitations of the heavy chain–IP90 complex and the two-dimensional resolution of a mixture of anti-IP90 and anti-MHC class I immunoprecipitates). In this experiment the anti-IP90 immunoprecipitation was performed in 1% Triton X-100 (rather than digitonin), which caused dissociation of most associated proteins. IP90 might be similar to the recently reported 88-kDa protein that could be chemically cross-linked to murine MHC class I proteins (26). The association of IP90 with MHC class I proteins in the ER may be of special significance, since the ER appears to be the location where MHC class I molecules bind peptide antigens.

In summary, IP90 interacts with immature complexes of several different multisubunit proteins in the ER but remains at this site and is not part of the completed protein complex expressed on the cell surface. The exact effect IP90 exerts on partial complexes remains to be determined. It is possible that IP90 plays a role in the degradation pathway of partial complexes, whereby IP90 binding might target partial complexes for ER degradation. However, as an ER resident molecule involved in protein–protein interactions, IP90 also shares certain properties with molecules like the binding protein BiP, a 78-kDa member of the HSP70 family (27–29). Prior studies have shown that BiP associates with the soluble Ig_H chain in the ER of myeloma and pre-B-cells and can be released in an ATP-dependent reaction. This process has been proposed to facilitate the orderly assembly of Ig_H and Ig_L chains into complete immunoglobulin molecules. Based on our finding of the association of IP90 with a number of partial protein complexes in the ER while it is absent from the completed complexes on the cell surface, we propose that the IP90 molecule may function as a molecular chaperone and play a role in the folding and/or assembly of newly synthesized polypeptide chains.

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