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Characterization and Identification of Early Proteins in *Chlamydia trachomatis* Serovar L2 by Two-Dimensional Gel Electrophoresis

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The synthesis of early proteins from *Chlamydia trachomatis* serovar L2 was analyzed by two-dimensional gel electrophoresis. By pulse-label experiments, the synthesis of seven proteins was observed at 2 to 8 h postinfection before the major outer membrane protein was detected at 8 to 10 h after infection. The early proteins were synthesized throughout the 30-h period investigated, but the synthesis of three proteins of 75, 62, and 45 kilodaltons decreased from 26 to 30 h postinfection. Pulse-chase analysis showed that the signals from the same three proteins declined 26 to 30 h after infection. Three of the early proteins were identified as the S1 ribosomal protein, the GroEL-like protein, and DnaK-like protein, respectively.

The genus *Chlamydia* consists of three species, *Chlamydia trachomatis*, *C. psittaci*, and *C. pneumoniae*, all causing a broad spectrum of well-known and well-characterized diseases. Chlamydia is an obligate intracellular organism with a unique life cycle alternating between two different forms, the elementary body (EB) and the reticulate body (RB). The nonreplicating EB is osmotically stable and metabolically inactive and represents the extracellular form which, upon infection, enters the eucaryotic host cell. During the first 6 to 8 h, the EB transforms into the noninfective RB (40). The RBs are metabolically active and divide within the inclusion. RBs are restricted to intracellular growth, because they lack the ability to produce high-energy compounds, making them dependent on ATP supply from the infected host cell (39). After 20 to 40 h of infection, the RBs reorganize to EBs, which after 48 to 72 h burst the host cell and liberate the EBs into the extracellular environment ready to infect new cells (24).

Although this biphasic life cycle is well described, the actual mechanisms that control and regulate the intracellular transformation between the two developmental forms are not known. However, several structural and functional differences between EB and RB have been characterized. Besides the divergence in infectivity, size, and metabolic activity, several differences at the protein level have been described. EBs contain several proteins in the outer membrane synthesized late in the developmental life cycle and therefore not seen in RBs (13, 15, 16, 25, 38). Furthermore, the proteins in the outer membrane of EBs are in contrast to those in RBs, cross-linked by disulfide bridges (14–16, 25, 26), and the relation between the major outer membrane protein (MOMP) and the lipopolysaccharide in the membrane is different in EBs and RBs (1). DNA-binding proteins of 58, 26, and 17 kilodaltons (kDa) are found in EBs but are not present in RBs (46), and also the ultrastructural appearance of the DNA-containing nucleoid differs in EBs and RBs (8). The RNA/DNA ratio is higher in RBs than in EBs (20, 40, 45). These differences reflect regulatory mechanisms responsible for coordinate regulation of gene products.

Several well-documented regulatory mechanisms have been described. Regulation of gene activity by temporary adaptations in response to changing environmental circumstances is described for *Bacillus subtilis*. Temporal programmed gene expression important for endospore formation in *B. subtilis* is in part controlled by regulatory sigma factors that modify the promotor recognition specificity of RNA polymerase (33).

To establish further knowledge of the intracellular transformation from EB to RB early in the developmental life cycle, we have characterized in this study the synthesis of early proteins of *C. trachomatis* L2 by pulse-labeling and pulse-chase experiments analyzed by two-dimensional gel electrophoresis. Seven early proteins synthesized before the MOMP were observed. With monoclonal antibodies in immunoblotting, three of these proteins were identified as the chlamydial S1 ribosomal protein, the chlamydial GroEL-like protein, and the chlamydial DnaK-like protein.

MATERIALS AND METHODS

Microorganisms and cultivation. *C. trachomatis* serovar L2 LGV-II/434/Bu (kindly provided by Per Anders Mårdh, Uppsala, Sweden) was cultivated in monolayers of McCoy cells (obtained from the American Type Culture Collection, Rockville, Md.) in RPMI 1640 medium as described earlier (1, 2, 34). The protein synthesis of McCoy cells was stopped with cycloheximide (10 mg/liter) after 2 h of infection. Some 50 to 80% of the McCoy cells were infected. For radioactive 2-h pulse-labeling of chlamydiae, 100 μCi of Trans35S-Label (ICN, Irvine, Calif.) per ml containing both [^{35}S]methionine and [^{35}S]cysteine was added to infected cultures in methionine- and cysteine-free RPMI as described earlier (2). For labeling EB from 2 to 30 and 2 to 44 h, 1 mg each of methionine and cysteine per liter was added.

Purification of *Chlamydia* EBs. EBs were purified from the infected McCoy cells by using density gradient centrifugation, as described earlier (1).

Production of monoclonal antibodies (MAbs). BALB/c mice were immunized with purified EBs as described earlier (1) except that immunization was performed intramuscularly instead of subcutaneously. Fusion and subcloning were done as described by Birkelund et al. (1).

SDS-polyacrylamide gel electrophoresis and immunoblotting. *Chlamydia* antigens were solubilized in sodium dodecyl sulfate (SDS) sample buffer (125 mM Tris, pH 6.8, 2.4% [wt/vol] SDS, 10% [wt/vol] glycerol) and separated by SDS-polyacrylamide gel electrophoresis followed by electropho-

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retic transfer to nitrocellulose membranes (BA85; Schleicher & Schuell, Dassel, Federal Republic of Germany). Immunodetection of antigens with MAbs was performed as described in reference 1.

Cloning of *C. trachomatis* DNA into pEX vector and detection of L2 antigen expressing *Escherichia coli* recombinants. L2 DNA purification and cloning of DNA fragments into pEX1-3 were performed as described previously (2) except that recombinants were cultivated on LB plates (10 g of tryptone [Difco Laboratories, Detroit, Mich.], 10 g of NaCl, 5 g of yeast extract [Difco], and 20 g of agar [Difco] per liter) with 50 mg of kanamycin and 100 mg of ampicillin per liter. Transfer of colonies, growth of colonies on nitrocellulose, lysis of colonies with SDS, and electroelution of SDS were done by the method of Stanly (43). Immunodetection of recombinants expressing chlamydial antigen was performed with MAbs as described for immunoblotting (1).

Preparation of plasmid DNA from the recombinant clones (pCtX1-88 and pCtX2-3), agarose gel electrophoresis, and Southern blotting. Plasmid DNA was extracted from recombinants by the alkaline lysis method or the large-scale method (35). Agarose gel electrophoresis, Southern transfer, and hybridization were done as described before (2, 35, 42) except that hybridization with [α-^{32}P]dATP-labeled probes was done in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) instead of 6× SSC for 16 h at 67°C.

Triton X-114 phase separation. A 175-cm^2 semiconfluent monolayer of McCoy cells infected with *C. trachomatis* L2 was subjected to Triton X-114 (Sigma Chemical Co., St. Louis, Mo.) phase separation as described previously (2) and by Bordier (4). The distribution of proteins in the Triton X-114 and aqueous phases was analyzed by immunoblotting with MAbs.

DNA sequence of pEX clones pCtX1-88 and pCtX2-3. The L2 insert of pCtX1-88 and pCtX2-3 was partly sequenced. Denaturation of the recombinant plasmid DNA was done by the method of Hattori and Sakaki (17). The denaturated DNA was sequenced by the dideoxy-chain termination method (36) by using T7 DNA polymerase (Pharmacia, Uppsala, Sweden). The attachment site of the primer 5'-CCCGTCACTATCGGCGG-3' was located 10 bases upstream from the cloning site (*Bam*HI). Computer analysis and comparison were carried out with GENEPRO (Riverside Scientific, Seattle, Wash.) and the GenBank data base.

Immunofluorescence. Semiconfluent McCoy cell monolayers on cover slips were infected with 1 inclusion-forming unit per 10 cells. At 20 or 48 h of infection, monolayers were incubated with MAbs diluted 1:2 in phosphate-buffered saline for 30 min at 37°C, washed in phosphate-buffered saline, and fixed in methanol. For methanol-fixed chlamydiae, the infected monolayer was washed in phosphate-buffered saline and fixed in methanol before the addition of primary antibodies. Secondary antibodies, mounting, and examination were done as described earlier (2).

Pulse-label and pulse-chase of chlamydial proteins. Semiconfluent monolayers were cultivated in 9-cm^2 petri dishes (Nunc, Roskilde, Denmark) infected with *C. trachomatis* L2 (50 to 80% of the McCoy cells were infected); after 2 h, cycloheximide (10 mg/liter) was added to stop host cell protein synthesis. For radioactive labeling, infected cultures were labeled (see above) from 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 14, 14 to 16, 16 to 18, 18 to 20, 20 to 22, 22 to 24, 24 to 26, 26 to 28, 28 to 30, 2 to 30, and 2 to 44 h and harvested immediately after the end of the labeling period. As negative controls, uninfected cultures were labeled from 2 to 4, 10 to 12, 28 to 30, 2 to 20, 2 to 30, and 2 to 44 h after infection. Pulse-chase experiments were performed with a 2-h pulse-labeling 10 to 12 h postinfection, the cells were washed in phosphate-buffered saline, and medium containing cold methionine and cysteine was added. Monolayers were harvested immediately after pulse (12 h) and at 14, 16, 18, 20, 22, 24, 26, 28, and 30 h postinfection. As negative controls, two cultures were pulsed from 10 to 12 h and harvested at 12 and 30 h, respectively. Samples from both sets of experiments were washed twice in phosphate-buffered saline, and the samples were dissolved in lysis buffer (9.5 M urea, 2% Nonidet P-40 [BDH, Dorset, United Kingdom], 2% ampholytes, pH 7 to 9 [LKB, Stockholm, Sweden], 5% mercaptoethanol) before two-dimensional gel electrophoresis. Each gel was loaded with radioactively labeled chlamydiae from 1 cm^2 of infected McCoy cells.

Two-dimensional gel electrophoresis. Two-dimensional gel electrophoresis was carried out as described by O'Farrell (27) and O'Farrell et al. (28) with the modifications described by Fey et al. (11). The first-dimension gels were cast in 21-cm-long tubes, and the actual gels were 17.5 cm long. The gel mixture (18.2 ml) for isoelectric focusing (IEF) consisted of 10 g of urea, 2.42 ml of sol D1 (28.38% acrylamide [Bio-Rad Laboratories, Richmond, Calif.], 1.62% bisacrylamide [Bio-Rad]), 2.73 ml of H_2O, 1.02 ml of ampholytes (pH 5 to 7), 1.25 ml of ampholytes (pH 3.5 to 10; LKB), 3.64 ml of 10% Nonidet P-40 (BDH), 15.4 μl of *N,N,N',N'*-tetramethylethylenediamine (TEMED; Bio-Rad), and 22 μl of 10% ammoniumpersulfate (Bio-Rad). The gel mixture (18.2 ml) for nonequilibrium pH gel electrophoresis consisted of 10 g of urea, 2.42 ml of sol D1, 3.08 ml of H_2O, 0.69 ml of ampholytes (pH 7 to 9; LKB), 0.69 ml of ampholytes (pH 8 to 9.5; LKB), 0.1 ml of ampholytes (pH 3.5 to 10; LKB), 3.64 ml of 10% Nonidet P-40 (BDH), 25.5 μl of TEMED (Bio-Rad), and 36.4 μl of 10% ammoniumpersulfate. A 30-μl portion of sample in lysis buffer or lysis buffer (only lysis buffer for prefocusing) was applied to the top of the gels, followed by 15 μl of overlay buffer (8 M urea, 0.8% ampholytes [pH 5 to 7; LKB], 0.2% ampholytes [pH 3.5 to 10; LKB]). For IEF, the tubes were filled with 0.02 M NaOH and prefocused at 2.45 mA until the limiting voltage at 1,200 V was reached followed by replacing the lysis buffer and overlay with sample and fresh overlay. The tubes were filled with 0.02 M NaOH, and electrophoresis was carried out for 18 h at 1,200 V and 2.45 mA. For nonequilibrium pH gel electrophoresis, the tubes were filled with 0.01 M H_3PO_4 and electrophoresis was run at 2.45 mA. Electrophoresis was completed 4 h after the voltage had reached the limiting value at 1,200 V. After gel electrophoresis, the gels were blown out and equilibrated in SDS sample buffer for 10 min and stored at −20°C. In the second dimension, first-dimension gels were embedded in 1% agarose (in SDS sample buffer) and placed on an SDS–15% polyacrylamide gel (acrylamide-bisacrylamide, 200:1). The gels were run overnight at 200 mA per h until the bromophenol blue line had reached the bottom of the gel. After electrophoresis, the gels were fixed in 45% methanol–7.5% acetic acid for 45 min followed by treatment with Amplify (Amersham Corp., Amersham, United Kingdom) for 45 min. The gels were then dried and exposed to X-ray film (3M R2 or Kodak X-Omat AR) for 5 to 120 days. To the samples labeled for 2 to 44 h, 30 μg of purified EB was added before electrophoresis. From these gels the proteins were transferred to polyvinyldifluoride membranes and immunostained with each of the MAbs as described previously (Z. Jie, S. J. Fey, H. Hager, P. Høllsberg, P. Ebbesen, and P. Mose Larsen, In Vitro Cell. Dev. Biol., in press). After immunoblotting, the polyvinyl-

TABLE 1. Characterization of MAbs to *C. trachomatis* L2

MAb	Immunoglobulin isotype	Size of antigen (kDa)	Immunofluorescence reaction[a]		Reactivity with antigen separated by Triton X-114 phase partition (phase)
			Unfixed	Fixed	
18.1	G2a	75	−	+	Aqueous
26.3	G2a	40	+	+	Hydrophobic
13.1	G1	62	−	+	Aqueous
124.4	ND[b]	74	−	+	Aqueous

[a] −, No reaction; +, positive reaction.
[b] ND, Not determined.

TABLE 2. Characterization of recombinants reacting with MAbs 13.1 and 124.4

MAb	Recombinant	Size of DNA insert (kilobase)[a]	Size of fusion protein (kDa)[b]
13.1	pCtX1-88	1	150
124.4	pCtX2-3	0.9	151

[a] *C. trachomatis* serovar L2 DNA fragment inserted into pEX.
[b] The vector part of the fusion protein (Cro-β-galactosidase) is 117 kDa.

difluoride membranes were allowed to dry and autoradiograms of the blots were obtained by exposing them to X-ray film for 10 and 40 days.

RESULTS

Characterization of MAbs. Characterization of the four MAbs used in this study is shown in Table 1. MAb 26.3 was specific for a surface-localized epitope of MOMP, and MAb 18.1 was specific for the early cytoplasmic 75-kDa DnaK-like protein, as described by Birkelund et al. (2, 3). MAb 13.1 reacted with a 62-kDa protein and MAb 124.4 reacted with a 74-kDa protein, determined by SDS-polyacrylamide gel electrophoresis and immunoblotting. In Triton X-114 phase partitioning, which separates integral membrane proteins from hydrophilic proteins (4), MAbs 13.1 and 124.4 reacted with antigens present only in the aqueous phase.

Immunofluorescence with MAbs 13.1 and 124.4 on methanol-fixed McCoy cells infected for 20 h showed various sized RBs as brightly fluorescent structures with evenly distributed fluorescence over the RB. MAbs 13.1 and 124.4 did not react with unfixed infected cultures. This immunofluorescence reaction pattern is similar to that of the 75-kDa protein described earlier (2), indicating together with the Triton X-114 separation that the location of the 62- and 74-kDa proteins probably is cytoplasmic. In contrast, immunofluorescence with MAb 26.3 against the surface-exposed MOMP showed ring-shaped structures in both methanol-fixed and unfixed cultures (2).

Cloning, characterization, and sequence analysis of the genes coding for the 62- and 74-kDa proteins. MAbs 13.1 and 124.4 were used to screen a *C. trachomatis* L2 DNA library prepared in the expression vector pEX1-3. Characterization of recombinants reacting with MAb 13.1 or 124.4 is shown in Table 2. Since the size of the vector part of the fusion protein (Cro-β-galactosidase) is 117 kDa, pCtX1-88 and pCtX2-3 were coding for about 33 and 34 kDa of the 62- and 74-kDa proteins, respectively. The first 334 bases of pCtX1-88, expressing the 33-kDa chlamydial part of the fusion protein, were sequenced. This sequence was shown by data base search to be 84% homologous to the DNA sequence of the *C. psittaci* GroEL-like protein (22) (Fig. 1A). The first 396 bases of pCtX2-3 were 82% homologous to the DNA sequence of the S1 ribosomal protein from *C. trachomatis* MoPn (37) (Fig. 1B).

Synthesis of early proteins in *C. trachomatis* L2 examined by two-dimensional gel electrophoresis. The synthesis of early proteins (proteins appearing before MOMP) was monitored by incorporation of [^{35}S]methionine and [^{35}S]cysteine into chlamydial proteins. Every 2 h the cells were harvested, and the proteins were separated by two-dimensional gel electrophoresis and exposed to X-ray film. The positions of the early proteins in IEF, radioactively labeled from 2 to 30 h, are shown in Fig. 2. Proteins of 39, 45, 48, 62, 74, 75, and 90 kDa in size were detected. We did not discover any early proteins in the low-molecular-weight area or in the nonequilibrium pH gel electrophoresis. Figure 3 shows the two-dimensional gel (IEF) of the radioactive pulse-labeling from 2 to 4 (panel A), 8 to 10 (panel B), 14 to 16 (panel C), and 28

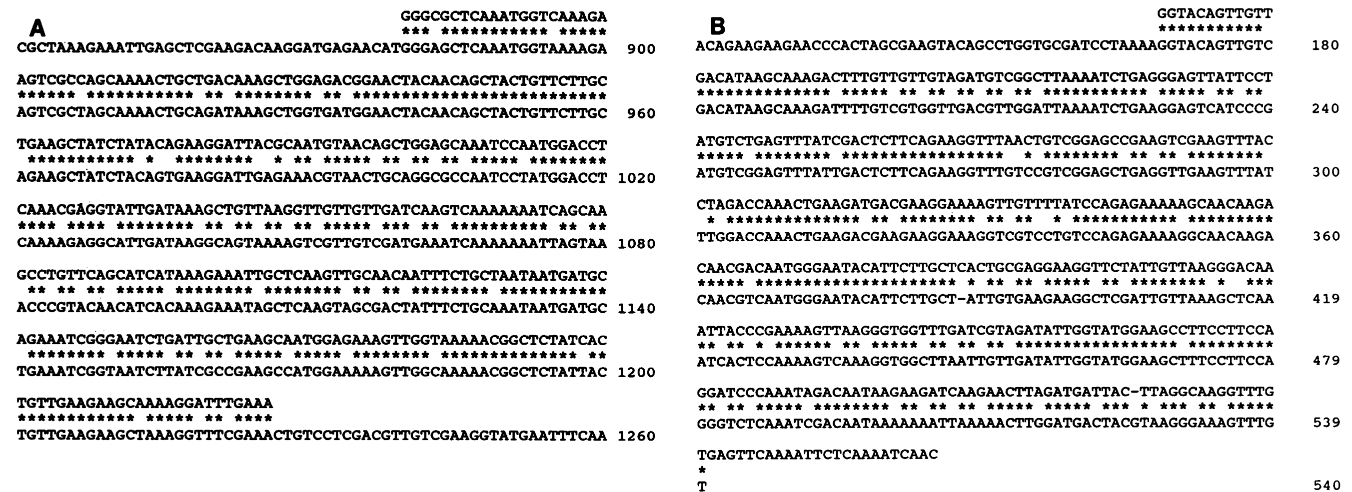

FIG. 1. DNA sequence of pCtX1-88 (334 bases) (A) and pCtX2-3 (396 bases) (B). (A) pCtX1-88 (upper line) is compared with the GroEL-like DNA sequence of *C. psittaci* (lower line). The homology is 84%. (B) pCtX2-3 (upper line) is compared with the DNA sequence of S1 ribosomal protein of *C. trachomatis* MoPn (lower line). The homology is 82%.

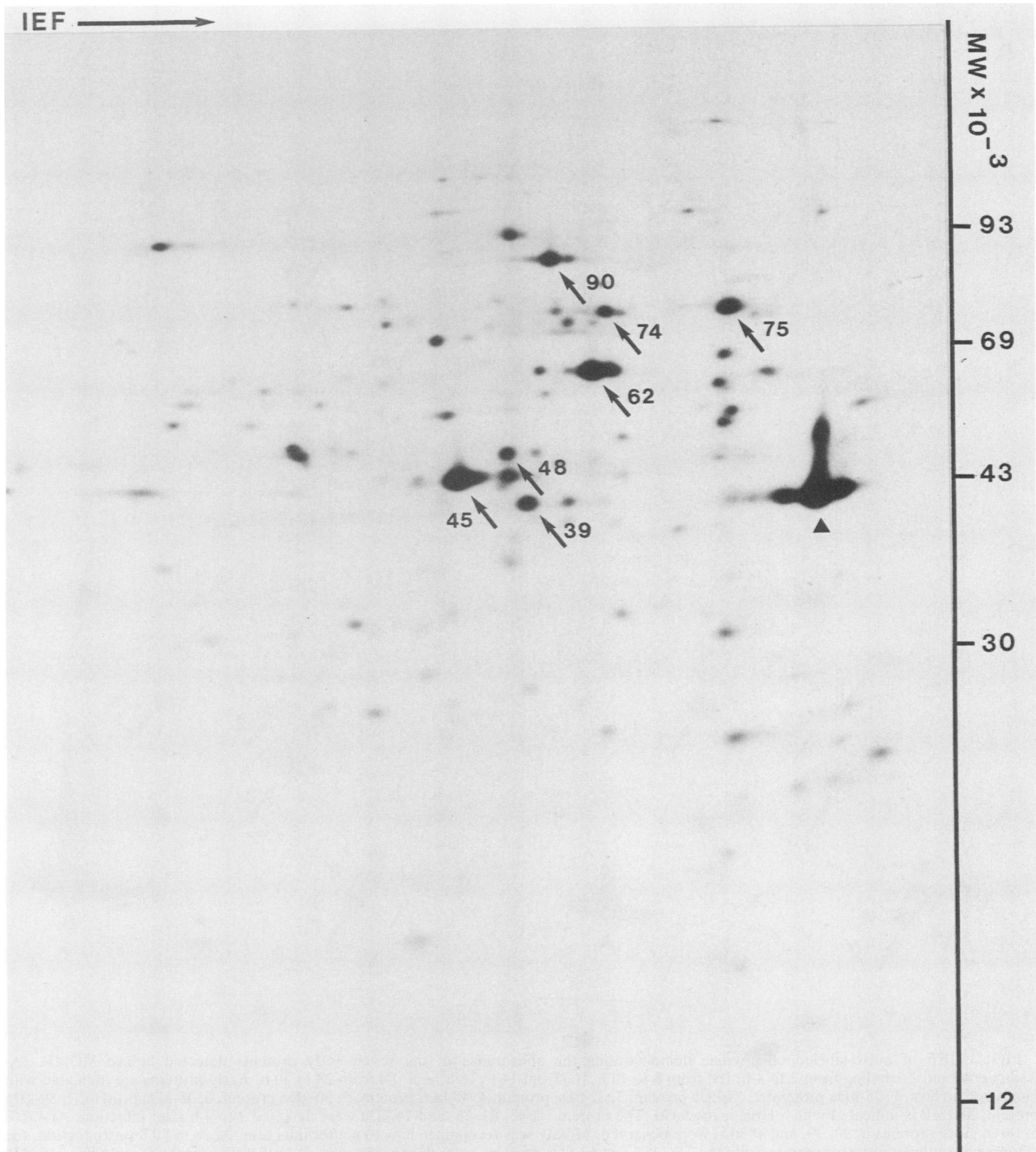

FIG. 2. IEF of 2- to 30-h radioactively labeled chlamydiae showing the accurate positions of the early proteins. The number representing each protein is the size in kilodaltons. MOMP is indicated with a large arrowhead. The protein at 75 kDa is the DnaK-like protein, the 74-kDa protein is the S1 ribosomal protein, and the 62-kDa protein is the GroEL-like protein.

to 30 (panel D) h. Four proteins of 45, 62, 75, and 74 kDa were detected 2 to 4 h after infection (Fig. 3A); one protein of 90 kDa appeared at 4 to 6 h postinfection. At 6 to 8 h, two proteins of 39 and 48 kDa were detected. MOMP was recognized 8 to 10 h after infection (Fig. 3B). The rate of incorporation of radioactivity into the 45-, 62-, and 75-kDa proteins was higher than for the other proteins. The total amount of incorporation into 74-, 90-, 39-, and 48-kDa proteins never reached the level of incorporation into 45-, 62-, and 75-kDa proteins in the period investigated. Not until 14 to 16 h did the amount of MOMP exceed that of the earlier proteins (Fig. 3C). The rate of incorporation into MOMP

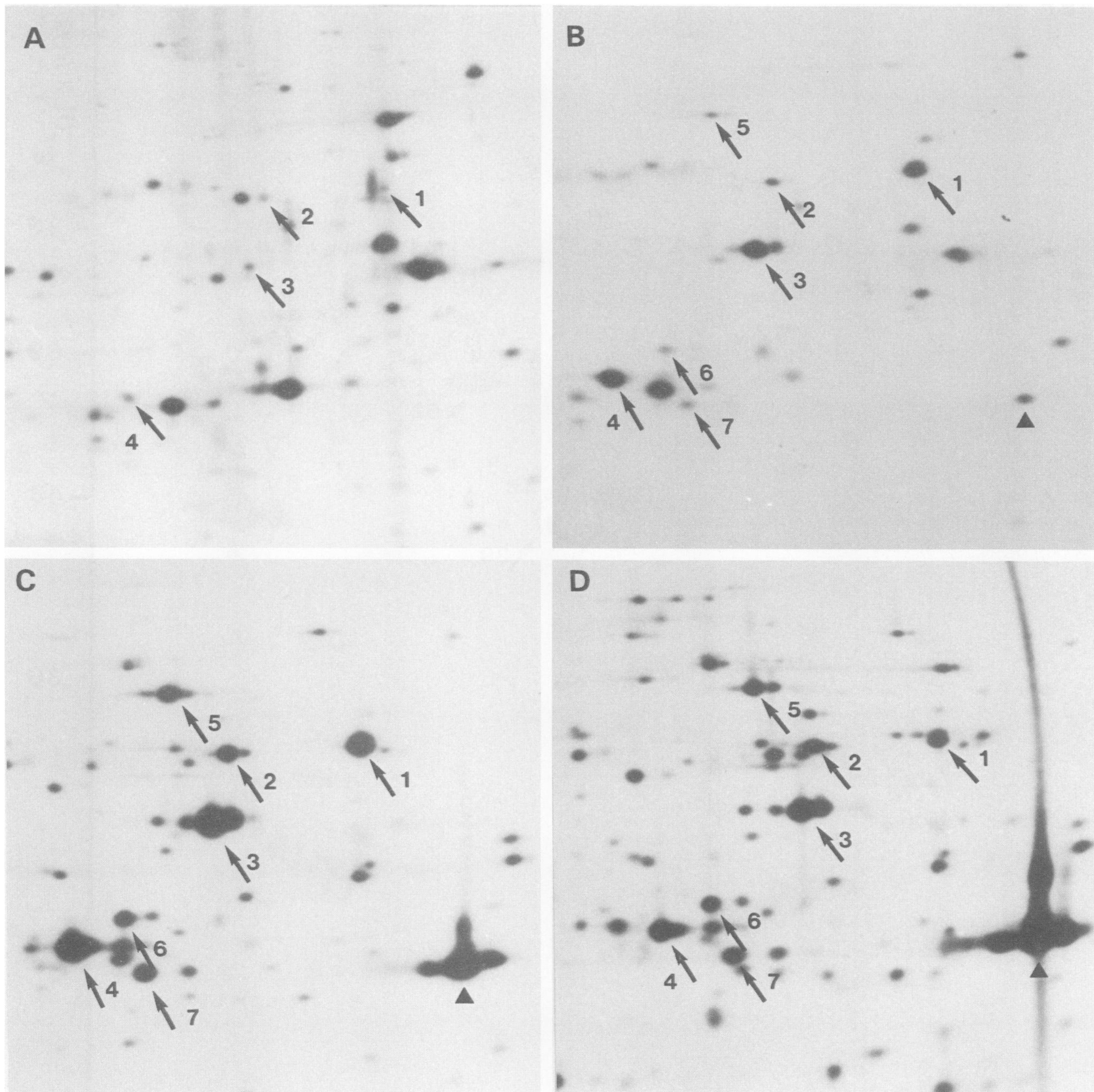

FIG. 3. IEF of pulse-labeled chlamydiae demonstrating the appearance of the seven early proteins detected before MOMP. (A) Radioactive pulse-labeling from 2 to 4 h, (B) from 8 to 10 h, (C) from 14 to 16 h, and (D) from 28 to 30 h. Early proteins are indicated with numbered arrows. 1, 75-kDa protein; 2, 74-kDa protein; 3, 62-kDa protein; 4, 45-kDa protein; 5, 90-kDa protein; 6, 48-kDa protein; 7, 39-kDa protein. MOMP is indicated with a large arrowhead. The proteins at 45, 62, 75, and 74 kDa were detected 2 to 4 h after infection. At 4 to 8 h, three more proteins at 90, 39, and 48 kDa were observed. MOMP was recognized 8 to 10 h after infection. At 28 to 30 h postinfection, the amounts of radioactivity incorporated into the 75-, 45-, and 62-kDa proteins were decreasing relative to the other proteins including MOMP. Panel A was exposed to X-ray film for 40 days; panels B, C, and D were exposed for 10 days. The darker background spots seen in panel A versus panels B, C, and D are related to the fourfold-longer exposure of panel A.

increased throughout the rest of the period investigated. At 14 to 26 h of infection, incorporation of radioactivity into the early proteins was similar for each of the 2-h intervals. At 26 to 28 h, the period when RB had begun transformation into EB, the amount of radioactivity incorporated into the 75-kDa protein was decreasing, and from 28 to 30 h a similar decrease was observed for the 45- and 62-kDa proteins (Fig. 3D). Identification of the early 75-, 62-, and 74-kDa proteins was determined by immunostaining the chlamydial proteins transferred from two-dimensional gels to polyvinyldifluoride membranes reacted with MAb 18.1, 13.1, or 124.4. Figure 4 shows a membrane immunostained with a mixture of MAbs 13.1, 18.1, 124.4, and 26.3. After staining, the membranes were autoradiographed and the spots were compared with

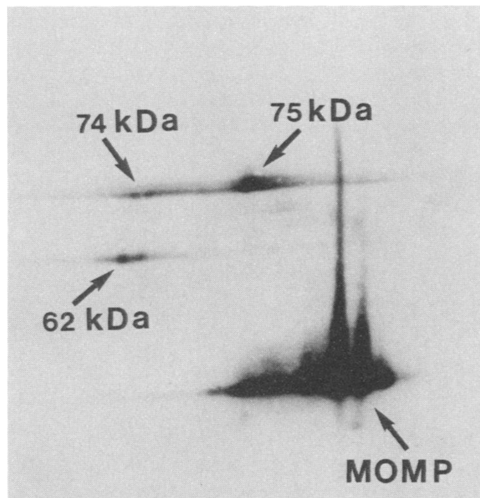

FIG. 4. Immunoblotting of the two-dimensional gel (only IEF) transferred to a polyvinyldifluoride membrane and reached with a combination of MAbs 13.1, 124.4, 18.1 and 26.2, visualizing the 62-, 74-, and 75-kDa proteins and MOMP, respectively. The protein at 75 kDa is the DnaK-like protein, the 74-kDa protein is the S1 ribosomal protein, and the 62-kDa protein is the GroEL-like protein.

the autoradiogram of the two-dimensional gel showing the synthesis of the early proteins (Fig. 2). It was seen that the early proteins of 75, 62, and 74 kDa were identical to the DnaK-like protein, the GroEL-like protein, and the S1 ribosomal protein, respectively. Several proteins appeared in nonequilibrium pH gel electrophoresis and in IEF along with MOMP at 10 h postinfection, and from 10 h postinfection an increasing number of new proteins could be detected.

Pulse-chase of labeled early *Chlamydia* proteins examined by two-dimensional gel electrophoresis. To investigate whether some of the early proteins were exposed to degradation during the first 30 h of infection, a pulse-chase experiment was performed. *Chlamydia*-infected McCoy cells were radioactively labeled from 10 to 12 h postinfection, the medium was changed, and the incorporated activity was followed by harvesting the infected McCoy cells every 2 h from 12 to 30 h after infection. The results are shown in Fig. 5. No changes were observed from 12 to 26 h for any of the early proteins or for MOMP (Fig. 5A and B), indicating that none of these proteins was degraded. At 28 h postinfection, the size of the signal from the 75-kDa protein started to decrease (arrow 1, Fig. 5C) and this continued for up to 30 h (Fig. 5D). At 28 to 30 h, the 45- and 62-kDa protein signals also decreased, but not to the same extent as seen for the 75-kDa protein (Fig. 5C and D). No other proteins seen in IEF or nonequilibrium pH gel electrophoresis disappeared during the pulse-chase experiment.

DISCUSSION

In this study, we describe seven early proteins all synthesized at the time when EBs transform into RBs. Four proteins of 45, 62, 75, and 74 kDa were detected at 2 to 4 h postinfection; further, one protein of 90 kDa was detected at 4 to 6 h, and finally at 6 to 8 h after infection two proteins of 39 and 45 kDa were observed. Three of the early proteins (74, 62, and 75 kDa) were identified as the S1 ribosomal protein, the GroEL-like protein, and the DnaK-like protein, respectively. These three proteins are all very conserved proteins. The N-terminal part of the *C. trachomatis* MoPn S1 ribosomal protein was shown to be 43% homologous to the *E. coli* S1 ribosomal protein (37), the GroEL-like protein from *C. psittaci* was shown to be 60% homologous to the *E. coli* GroEL protein (22), and the DnaK-like protein from *C. trachomatis* serovar L2 was shown to be 57% homologous to *E. coli* DnaK (3). The DNA homology between *C. trachomatis* serovars A and L3 and *C. trachomatis* MoPn is 30 to 60%, but it is only 10% between *C. trachomatis* and *C. psittaci* (39). The high DNA homology of S1 ribosomal and GroEL-like proteins between the *Chlamydia* species (82 and 84%, respectively) emphasizes that these proteins are highly conserved in all *Chlamydia* species.

Some proteins contain only very few or no residues of methionine and cysteine and are therefore not detectable in the system we used. Thus, some early proteins may have been overlooked. The GroES-like protein at 20 kDa is cotranscribed along with the GroEL-like protein in *C. psittaci* (22). We did not observe any early 20-kDa protein, probably because GroES-like protein in *C. psittaci* only contains 2 residues of methionine, whereas GroEL contains 13 methionine and 5 cysteine residues (22).

Plaunt and Hatch (31) have described the synthesis of early proteins in *C. psittaci* 6BC. The incorporation of [^{35}S]methionine into early proteins of *C. psittaci* within host cells (in vivo) and in a host-free system was characterized by SDS-polyacrylamide gel electrophoresis. They were able to detect seven proteins already by 1 h postinfection in the host-free system. Because of the great number of proteins synthesized later, they were not able to tell whether the synthesis of early proteins was continued during the life cycle. However, some of the proteins were synthesized in larger amounts during the first hours, and two proteins were no longer synthesized at 9 h postinfection. The actual size of the proteins was not given, but three proteins correspond in size to our 45-, 62-, and 75-kDa (or 74-kDa) proteins.

The proteins synthesized early in the life cycle must serve several functions. Proteins are required for protein synthesis, including regulatory proteins and ribosomal proteins. Proteins might also be needed to protect the chlamydiae against the hostile host cell environment, and other proteins may play a direct role in the morphological transformation of EB to RB. Of the seven early proteins described in this study, one was a ribosomal protein needed for normal protein synthesis, whereas the functions of the DnaK-like and GroEL-like proteins and of the four other proteins are still unknown.

In *E. coli* the S1 ribosomal protein binds to the 30S ribosomal subunits and to mRNA and is connected with protein biosynthesis (44). However, the protein is easily detached from the ribosome and may serve other functions (44). Expression of the S1 ribosomal protein from *C. trachomatis* MoPn has been characterized by Sardinia et al. (37). The transcript of this 70-kDa protein was first detectable at 9 h postinfection, became maximal at 12 to 18 h, and then was detectable at all subsequent time points. In the present study, this protein was already observed 2 to 4 h after infection. This divergence is probably due to the different technique used by Sardinia et al. in which, perhaps, the amount of mRNA was insufficient for detection until 9 h postinfection.

The GroEL and DnaK proteins are both stress proteins (heat shock proteins). They are highly conserved proteins in both procaryotes and eucaryotes (19). The exact roles of these proteins are not known, but evidence suggests a role in unfolding, stabilization, and assembly of proteins (5, 6, 10,

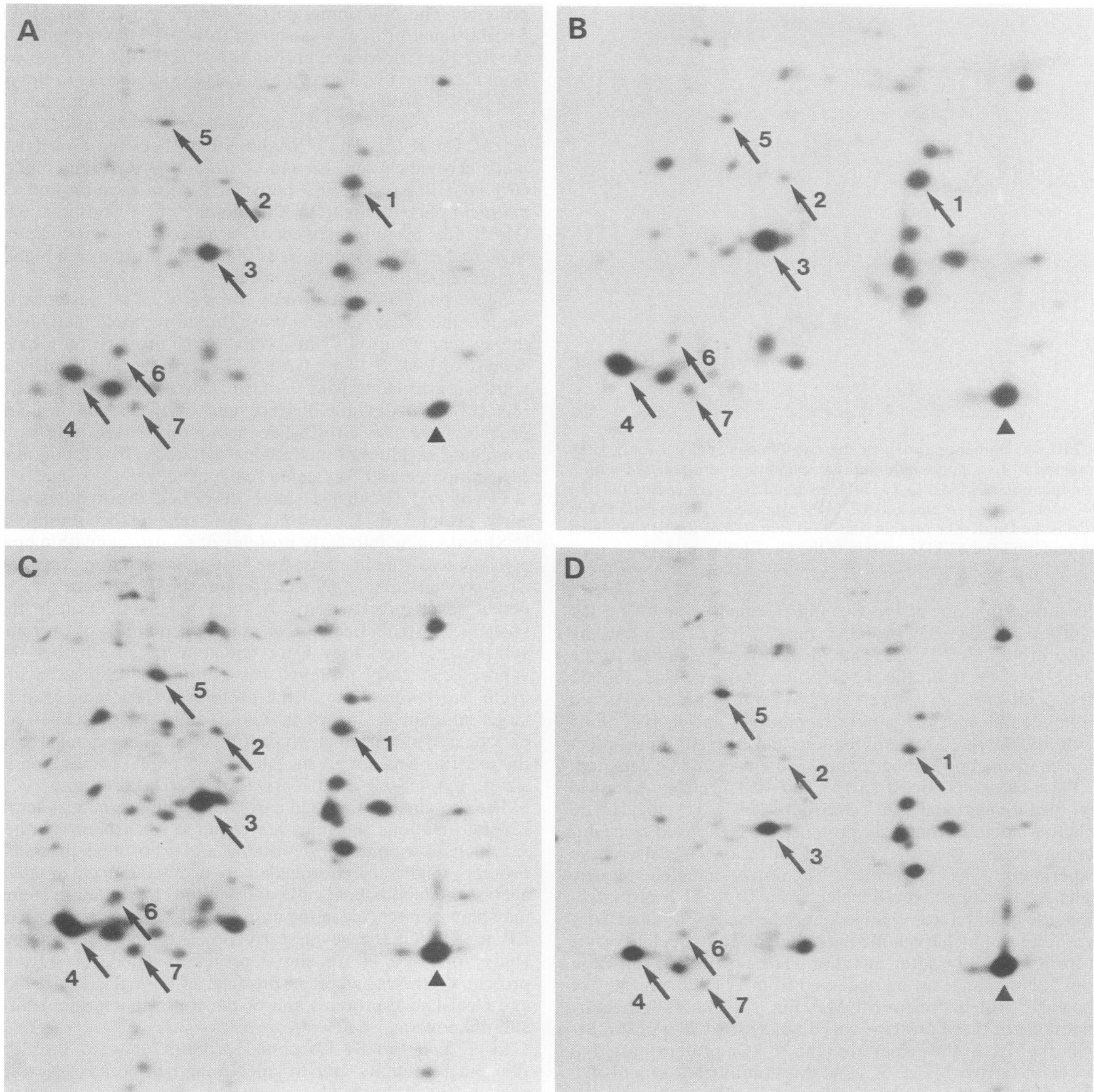

FIG. 5. IEF of the pulse-chase experiment. (A) Pulse-chase at 12 h after infection (just after the 2-h pulse), (B) after 26 h, (C) after 28 h, and (D) after 30 h postinfection. The seven early proteins are indicated with numbered arrows as in the legend to Fig. 3. At 26 h postinfection (B), the size of the signal from the 75-kDa protein (arrow 1) started to decrease and this continued at 28 (C) and 30 (D) h. At 28 and 30 h, the 45 (arrow 4)- and 62 (arrow 3)-kDa protein signals also decreased, but not to the same extent as seen for the 75-kDa protein.

12, 29, 30, 32). GroEL- and DnaK-like proteins are, in *Chlamydia* spp. (2, 3, 9, 22) as in other microorganisms (47), very immunogenic and they are both major antigens. The GroEL-like protein in chlamydiae is a protein that mediates a deleterious immunological response in which repeated stimulation causes a delayed hypersensitivity reaction (22, 23). Antibodies against the variable parts of the stress proteins might be protective (47), while the conserved regions either are not capable of producing an adequate response (self-like) or might, because of repeated stimulation, mediate a pathological autoimmune response (47). Since synthesis of stress proteins occurs early in the developmental cycle, their synthesis could be a reflection of chlamydiae existing in an unnatural and stressed environment produced by the cell culture system used in this study. However, chlamydial GroEL as well as DnaK proteins are recognized by antibody response during infection (2, 22, 23). These proteins must therefore also be produced under normal human infectious conditions.

EB undergoes reorganization to RB within 6 to 8 h after

infection at the same time the seven early proteins are synthesized. RNA synthesis is observed earlier than DNA synthesis and is seen before RB replication begins (40). RBs multiply by binary fission for 20 to 24 h before some RBs start the transformation into EBs (39). RNA and DNA syntheses peak 25 to 30 h postinfection and protein synthesis peaks at 30 to 35 h after infection (41). It is therefore surprising that the signal from three of the early proteins decreases 26 to 30 h postinfection. At 26 to 30 h, when RB has begun transformation into EB, the amount of radioactivity incorporated into the 45-, 75 (DnaK-like)-, and 62 (GroEL-like)-kDa proteins decreased, and in the pulse-chase experiment the signals from these three proteins were reduced. These results indicate that some early proteins, including the DnaK- and GroEL-like stress proteins, might be of importance for EB-to-RB transformation and for replication of RB but might not be necessary late in the developmental life cycle when the transformation of RB to EB and EB liberation from the host cell take place. It is known that these proteins are produced by intracellular bacteria such as *Salmonella* and *Mycobacterium* spp. (7, 21, 47). One of the functions of stress proteins could thus be to protect the chlamydiae from the hostile environment inside the host cell. The ability to produce stress proteins early and in large amounts could be important for the survival of bacteria within phagocytic host cells. The early rather than late synthesis of the chlamydial stress proteins during the developmental cycle could suggest that, as chlamydiae grow, the host cell becomes altered so that the environment is less stressful. In this way, both pathogen and host could be viewed as active members of the relationship that has evolved.

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