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Host cell responses to *Listeria monocytogenes* infection include differential transcription of host stress genes involved in signal transduction

(macrophages/bacterial invasion)

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ABSTRACT **We examined the effect of *Listeria monocytogenes* infection of J774 macrophage-like mouse cells on induction of several stress genes, including genes for heat shock proteins (HSPs) and a protein-tyrosine phosphatase (PTP), to understand the host response in various steps of the bacterial invasion process. Exposure to wild-type *L. monocytogenes* strain EGD elicited an early induction of HSP70 mRNA with a corresponding early appearance of HSP70 protein. Cytochalasin D pretreatment prevented the induction of HSP70 mRNA in *L. monocytogenes*-infected macrophages. After a 2-hr infection with *L. monocytogenes*, PTP and to a lesser extent HSP90 mRNA levels were elevated. A listeriolysin-negative mutant of *L. monocytogenes* strain EGD and a noninvasive species of *Listeria*, *Listeria innocua*, did not induce PTP or HSP90 mRNA in infected macrophages. Mutations in other virulence genes did not affect transcription of PTP or HSP90. Expression of HSP60 mRNA remained constant over the time course studied in wild-type or mutant strains. These results suggest that phagocytosis of *L. monocytogenes* triggers transcription of HSP70 mRNA in macrophages; however, escape from the phagosome appears to be necessary for induction of PTP and HSP90 mRNA. Since both PTP and HSP90 may have links with signal transduction pathways in eukaryotic cells, the induction of these mRNAs suggests a role for *L. monocytogenes* in influencing the signal transduction routes of the host cell.**

Eukaryotic cells synthesize a number of distinct proteins called stress proteins in response to potentially detrimental environmental stimuli. One of the most studied stress stimuli is heat, which gives rise to a group of polypeptides called heat shock proteins (HSPs) in eukaryotic cells (1). In general, HSPs act as molecular chaperones, carrier proteins that bind to unfolded nascent polypeptides, protecting these newly formed proteins from damaging protein–protein interactions and allowing the proper folding of the new polypeptides (2). These HSPs have been characterized on the basis of the size of the polypeptide that is expressed and include HSPs of 60, 70, and 90 kDa (1). Other stimuli can also activate HSP genes (3), including oxidative stresses associated with superoxide production and hydrogen peroxide formation in macrophages (4). Phagocytosis of erythrocytes (4), live bacteria (5), or heat-killed bacteria (6) can also induce elevated levels of HSP70 protein in host cells. This induction of HSP70 probably protects the phagocytes from their own effector mechanisms used to deal with the phagocytized material.

Another stress protein that has been recently identified is a protein-tyrosine phosphatase (PTP) (7). This protein is induced in response to oxidative stress and heat shock in human fibroblast cells. It has been characterized as an immediate early gene in the growth cycle of the cell and is stimulated by several mitogens (8). Structurally, the PTP protein has significant homology with the VH1 vaccinia serine/tyrosine phosphatase (9) and shares some features with the nonreceptor class of protein tyrosine phosphatases (7) that are identified with several regulatory events within eukaryotic cells (10). In addition to the structural similarities with other phosphatases, the enzymatic phosphatase activity associated with PTP suggests a possible role in the phosphorylation/dephosphorylation of signal transduction pathways.

With the exception of the analysis of HSP70 noted above, little is known about what occurs at the gene level during host cell responses to bacterial pathogens capable of invasion. Such perturbations by the bacteria must cause considerable stress upon the host cell. As a model system for studying host stress gene responses, we used *Listeria monocytogenes* invasion of J774 mouse macrophage cells (11). *L. monocytogenes* is a facultative intracellular pathogen which is taken up by phagocytic and normally nonphagocytic mammalian cells, replicates within their cytoplasm, causes actin polymerization, and spreads from one cell to another (12, 13). Several virulence genes (including those encoding a metalloprotease, *mpl*; a phosphatidylinositol-specific phospholipase, *plcA*; and a listeriolysin, *hly*; and a gene involved in actin polymerization, *actA*) have been identified, and their proteins have been associated with intracellular survival of the bacteria (14). In this study, we demonstrate that stress genes of the host cells are differentially transcribed after specific events associated with *L. monocytogenes* invasion. The onset of induction of mRNA for HSP90 and PTP, two stress proteins with possible linkage to signal transduction pathways (1, 7, 8), at later time points when intracellular bacterial replication and actin polymerization occur, suggests that bacterial subversion of the signal transduction routes in the host cell may be critical for intracellular survival of the bacteria.

MATERIALS AND METHODS

Bacterial Strains. Wild-type *L. monocytogenes* strain EGD Sv 1/2a was provided by S. H. E. Kaufmann (Ulm University, Ulm, F.R.G.). *Listeria innocua* 6a (NCTC 11288) was obtained from the special *Listeria* culture collection of the Institute of Hygiene and Microbiology, Würzburg, F.R.G. The *hly*$^-$, *actA*$^-$, *plcA*$^-$, and *mpl*$^-$ mutants of *L. monocytogenes* strain EGD have been previously described (15). All *Listeria* used for cell culture infection were grown in brain heart infusion (BHI) broth (Difco) at 37°C with shaking and prepared as noted (15), adding erythromycin to the medium at 5 μg/ml for growth of the mutants.

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Abbreviations: PTP, protein-tyrosine phosphatase; HSP, heat shock protein; β-Act, β-actin.

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Mammalian Cell Culture, Infection, and Cytochalasin D Treatment. The macrophage-like J774 cell line (11) was cultured and infected according to Kuhn and Goebel (15). A multiplicity of infection of 50 bacteria per eukaryotic cell was used. Some macrophage monolayers were pretreated with cytochalasin D (0.5 μg/ml final concentration), and total RNA was extracted as noted below after a 45-min exposure to *Listeria*.

Total RNA Isolation and cDNA Synthesis. At various times after infection with *Listeria* total RNA was extracted from the macrophage cells by the guanidinium thiocyanate procedure (16), and cDNA was synthesized from 6 μg of total RNA as previously noted (15).

Polymerase Chain Reaction. The polymerase chain reaction (PCR) amplification (17) was performed in a Thermocycler 60/2 (bio-med, Theres, F.R.G.) for 30 cycles: denaturation of 1 min, 94°C; annealing of 40 sec, 57°C; and extension of 45 sec, 72°C. Primers used for PCR were prepared with an automated synthesizer (model 380B, Applied Biosystems) and used as follows: PTP3 5′-TACCCCTCTCTACGATCAGG-3′ and PTP4 5′-GGAGATGCCGGCCTGGCAAT-3′ (8), HSP60A 5′-GTCAAAGCTCCAGGTTTTGG-3′ and HSP60B 5′-CTTCAACAGCTGCTCTTGTAG-3′ (18), HSP68A 5′-AAGCAGACGCAGACCTTCAC-3′ and HSP68B 5′-CACCTCCTCGATGGTGGG-3′ (19), HSP90A 5′-GACGAGAAATCCTGATGATGACATC-3′ and HSP90B 5′-TGAGAGTCCTCGTGAATTCC-3′ (20); and β-actin (β-Act)A 5′-TGGAATCCTGTGGCATCCATGAAAC-3′ and β-ActB 5′-TAAAACGCAGCTCAGTAACAGTCC-3′ (21). For limiting dilution PCRs, the same primer pairs and conditions were used with the following modifications: cDNA was diluted 2-fold in H_2O before use and 32 cycles were run.

Northern Blot Hybridization. Twenty micrograms of total RNA per lane was run as previously described (22). The probes were the PCR products listed above isolated and labeled as previously described (22). Each probe was hybridized to RNA on nitrocellulose filters at 46°C for 16–18 hr (22). Each filter was washed twice in 1× SSC (1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0)/0.5% SDS for 20 min at room temp, followed by two washes at 50°C in 0.5× SSC/0.5% SDS, and a final wash in 2× SSC. Filters were exposed to x-ray film at −80°C for 16 hr to 7 days.

Immunoblotting. SDS/PAGE was performed with a 12.5% separating gel, using 10 μl of macrophage cell total lysate in sample buffer (23). Proteins were electrotransferred to nitrocellulose membranes (24). Membranes were blocked with bovine serum albumin-containing Tris-buffered saline for 1 hr, washed, and then allowed to react with monoclonal anti-HSP70 (Affinity BioReagents, Neshanic Station, NJ) overnight at 4°C. Horseradish peroxidase-conjugated anti-mouse immunoglobulin antibody (Dako, Hamburg, F.R.G.) was added after washing and the filter was incubated for 2 hr at room temp. Visualization of bands was done with 4-chloro-1-naphthol (Sigma).

RESULTS

Kinetics of HSP and PTP mRNA Induction in J774 Cells. To determine differences in HSP (HSP60, HSP70, HSP90) and PTP mRNA levels and when these changes occurred after infection, J774 cells were infected with *L. monocytogenes* strain EGD, and at various time points transcriptional analyses were performed, using reverse transcription of total RNA and PCR analysis of the resulting cDNA. HSP70 mRNA was induced within 15–30 min after exposure to *L. monocytogenes* (Fig. 1*A*). Two forms of the HSP70 mRNA were clearly induced at the 45-min time point, with a weaker induction of both forms at later times. Mouse L cells have at least three different populations of HSP70 mRNA, based on differences in their nucleotide sequence (19). The HSP70

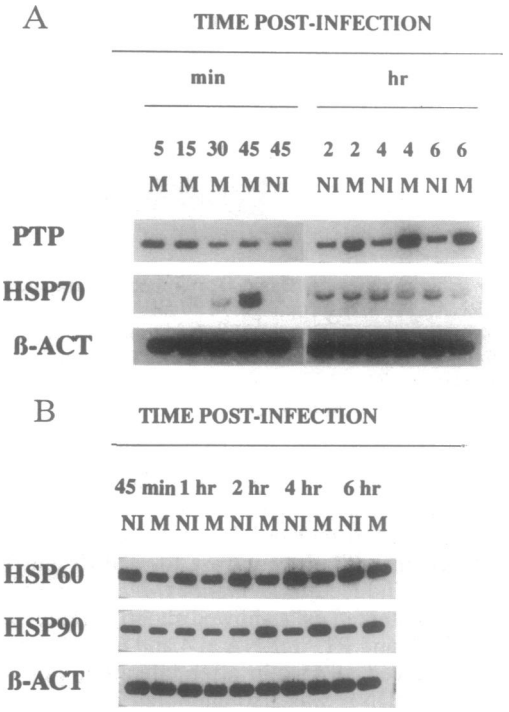

FIG. 1. Kinetics of PTP and HSP mRNA induction in J774 macrophages infected with wild-type *L. monocytogenes*. The mRNAs were detected by PCR of cDNA from *L. monocytogenes*-infected (M) or noninfected (NI) cells loaded onto 1.5% agarose gels. (*A*) Detection of PTP, HSP70, and β-Act mRNAs over a time course 5 min to 6 hr. The PCR product sizes were as follows: PTP, 275 bp; HSP70, 651 bp; and β-Act, 348 bp. (*B*) Detection of HSP60, HSP90, and β-Act mRNAs over a time course from 45 min to 6 hr. The PCR product sizes were as follows: HSP60, 402 bp; HSP90, 471 bp; and β-Act, 348 bp.

mRNA level peaked at 45 min (the time after phagocytosis at which gentamicin is added is designated as 0 hr) after incubation with the bacteria, and then it declined to the level of noninfected cells by 2 hr and remained at this level through 6 hr. Noninfected cells showed a slight induction of HSP70 mRNA beginning at 2 hr through 6 hr after infection that was higher than the 45-min time point. Western blotting (Fig. 2) confirmed that HSP70 protein was induced in J774 cells over constitutive levels within 30 min of exposure to *L. monocytogenes*. This protein level remained elevated for at least 6 hr after infection. Cytochalasin D treatment before infection with *L. monocytogenes* strain EGD prevented the induction of HSP70 at 45 min after infection, whereas untreated infected cells displayed a highly inducible band (Fig. 3). Noninfected cells showed no induction of HSP70 mRNA with or without cytochalasin D exposure at the same time.

At 2 hr after infection with *L. monocytogenes*, HSP90 (Fig. 1*B*) and PTP mRNAs (Fig. 1*A*) were elevated, and the amplified mRNAs were maintained at the higher level

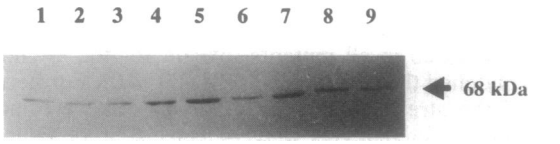

FIG. 2. Detection of HSP70 protein induction after infection of J774 cells with *L. monocytogenes*. Western blots of whole cell extracts were probed with an anti-HSP70 monoclonal antibody. Lanes 1 and 6 represent noninfected cells and lanes 2–5 and 7–9 represent whole cell extract from *L. monocytogenes*-infected J774 cells at 5 min (lane 2), 15 min (lane 3), 30 min (lane 4), 45 min (lane 5), 2 hr (lane 7), 4 hr (lane 8), or 6 hr (lane 9) after infection.

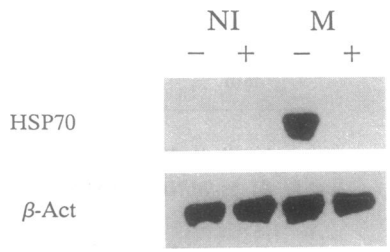

FIG. 3. Effects of cytochalasin D treatment on the induction of HSP70 mRNA in J774 macrophages. The mRNAs were detected by PCR of 45 min postinfection cDNA from noninfected (NI) or *L. monocytogenes*-infected (M) cells with (+) or without (−) cytochalasin D treatment. The PCR products of HSP70 and β-Act cDNA were loaded onto 1.5% agarose gels.

through 6 hr after infection. The amplified PTP DNA from *L. monocytogenes*-infected J774 macrophages showed 100% nucleotide sequence homology (data not shown) with a previously published mouse PTP sequence derived from a fibroblast cell line (8). In contrast to HSP70 and HSP90, HSP60 was not induced in *L. monocytogenes*-infected cells (Fig. 1*B*). Noninfected cells showed slightly greater levels of HSP60, HSP90, and PTP mRNA at 4 and 6 hr after infection than was observed at 45 min after infection. For all PCR amplifications, cDNA was also amplified concurrently with the β-Act set of primers to ensure equal loadings of cDNA between samples.

Differences in mRNA After Infection with Mutants of *L. monocytogenes*. Infections with wild-type *L. monocytogenes* demonstrated differences in stress protein mRNA levels compared with noninfected cells. Various mutants of *L. monocytogenes* strain EGD were tested to assess if a particular event in *L. monocytogenes* invasion may trigger differential transcription of these mRNAs. At the 45 min time point no differences were observed for PTP (Fig. 4*A*); however, a marked difference was shown in HSP70 comparing the listeriolysin-negative (*hly*) mutant (lane 2) with wild-type *L. monocytogenes* (lane 1). The *plcA* and *mpl* mutants (lanes 4 and 5, respectively) also showed only slightly, if at all, reduced levels of HSP70 mRNA compared with wild-type bacteria, and HSP70 was clearly induced above noninfected cell levels in both mutants. The *actA* mutant (lane 3) exhibited an induction of HSP70 mRNA similar to the wild-type bacteria. No differences among the mutants were revealed for either HSP60 or HSP90 mRNA at 45 min after infection (Fig. 4*B*).

By 4 hr after infection, no significant differences were demonstrated for HSP60 (Fig. 4*B*). Examination of HSP70 mRNA levels showed no difference between the wild type, the *hly* mutant, and the *actA* mutant after infection compared with the noninfected cells (Fig. 4*A*). Only a slight elevation in mRNA intensity was observed for the *plcA* and *mpl* mutants. Wild-type *L. monocytogenes* as well as the *plcA*, *actA*, and *mpl* mutants all exhibited an induction of PTP mRNA (Fig. 4*A*). In contrast, the listeriolysin-negative mutant showed essentially a background level of PTP similar to noninfected cells. Also at 4 hr after infection, HSP90 mRNA levels were slightly elevated in all mutants, except for the *hly* mutant, which matched the noninfected cells (Fig. 4*B*). Furthermore, none of the stress gene mRNA populations were induced above noninfected levels in J774 cells 2 hr or 6 hr after infection with the noninvasive species *L. innocua* (data not shown).

Quantitative Determinations of mRNA Induction After Infection. The PCR analyses described above examined qualitative differences among samples. A more accurate determination of induction was tested by 2-fold limiting dilutions of the cDNA used for PCR. As shown in Fig. 5*A*, the level of

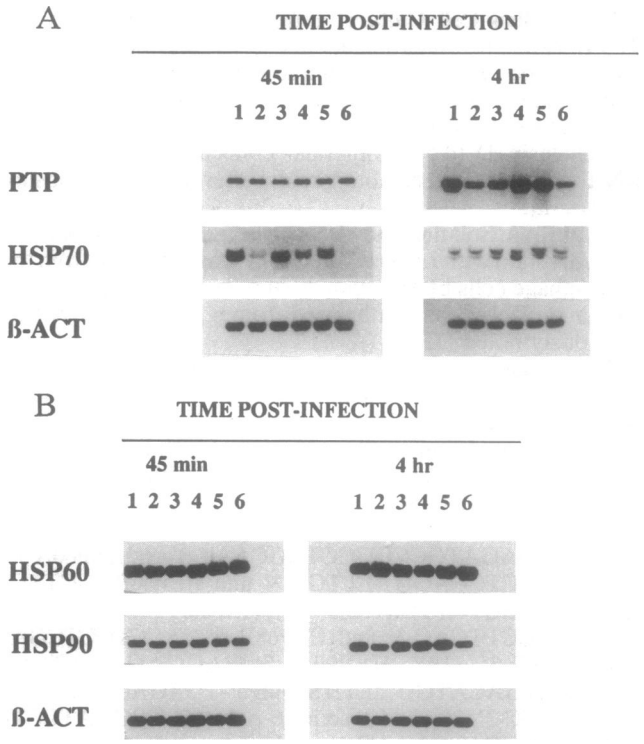

FIG. 4. Comparison of mRNA induction of wild-type *L. monocytogenes* strain EGD and various mutants of this strain. Lanes were loaded as follows: 1, *L. monocytogenes* wild-type; 2, *L. monocytogenes hly* mutant; 3, *L. monocytogenes actA* mutant; 4, *L. monocytogenes plcA* mutant; 5, *L. monocytogenes mpl* mutant; and 6, noninfected cells. (*A*) Detection of PTP, HSP70, and β-Act mRNA by PCR at 45 min and 4 hr after infection. (*B*) Detection of HSP60, HSP90, and β-Act mRNAs at 45 min and 4 hr after infection. The PCR product sizes on 1.5% agarose gels are as noted for Fig. 1.

PTP cDNA amplified at 2 hr after infection was between 1/4th (*L. monocytogenes hly*) and 1/8th (*L. innocua* and noninfected) of wild-type *L. monocytogenes*. The level of HSP90 mRNA was also between 1/2 and 1/4 of that in noninfected, *L. monocytogenes hly*, and *L. innocua* lanes compared with wild-type *L. monocytogenes*. Additionally, Northern blot hybridizations of total RNA at 45 min and 2 hr after infection were also analyzed. A PTP mRNA band was detected only in the wild-type *L. monocytogenes* lane (Fig. 5*B*, lane 2) 2 hr after infection, whereas no transcript was detected in the other lanes or any lanes after 45 min. No significant differences were noted between lanes probed for HSP60, HSP90, or β-Act mRNAs. However, RNA probed for HSP70 transcripts at 45 min after infection with wild-type *L. monocytogenes* showed a significant induction of HSP70 mRNA (lane 2), whereas *L. innocua* (lane 3) and *L. monocytogenes hly* (lane 4) both demonstrated weaker inductions, and no transcript was detected in the control lane (lane 1). By 2 hr after infection, all lanes showed approximately equivalent levels of HSP70 mRNAs that were lower than that observed for wild-type *L. monocytogenes* at 45 min after infection.

DISCUSSION

Differential transcription of stress genes in eukaryotic cells infected with virulent *L. monocytogenes* is established in this study. It is well known that environmental stresses can induce HSPs in these cells, but HSPs induced in host cells by infecting microorganisms have not been extensively studied. Previously, it has been reported that HSP70 protein levels are elevated by bacterial infections with *Mycobacterium* (5) or

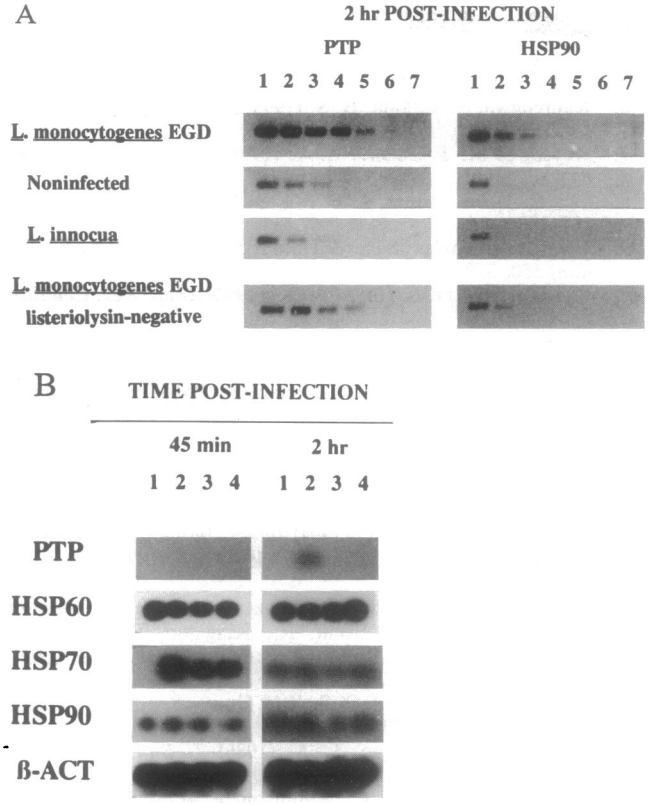

FIG. 5. Quantitative determinations of mRNA induction in infected J774 cells. (*A*) Limiting dilution analysis of cDNA at 2 hr after infection with wild-type *L. monocytogenes*, listeriolysin-negative *L. monocytogenes*, or *L. innocua*. The PTP and HSP90 PCR primer pairs were used to amplify 2-fold serially diluted cDNA. All PCR products were run on 1.5% agarose gels to visualize. The lanes represent the following diluted cDNA: lane 1, undiluted; lanes 2–7, 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64, respectively. (*B*) Northern blot hybridization analysis of total RNA from noninfected or *Listeria*-infected J774 cells. Total RNA was extracted at 45 min or 2 hr after infection. The probes used to detect PTP; HSPs 60, 70, and 90; and β-Act are as described above. The lanes are as follows: 1, noninfected; 2, *L. monocytogenes* wild-type; 3, *L. innocua*; and 4, *L. monocytogenes hly*.

phagocytosis of heat-killed *Staphylococcus aureus* (6), but the kinetics of the induction were not determined. Here, we demonstrate that infection by *L. monocytogenes* causes a rapid induction (within 15–30 min) of HSP70 mRNA and HSP70 protein synthesis. Prior work has demonstrated that HSP70 mRNA can be induced in as little as 5–7 min after exposure to heat (25). This quick response in *L. monocytogenes*-infected macrophages suggests that the initial phagocytosis of the bacteria can already activate HSP70 synthesis. Treatment with cytochalasin D, a potent inhibitor of microfilaments (26), confirmed that phagocytosis and not merely adherence was necessary. Macrophage cells pretreated with cytochalasin D and then infected with *L. monocytogenes* failed to induce HSP70 mRNA, whereas untreated infected cells exhibited a strong induction (Fig. 3). A listeriolysin-negative mutant of *L. monocytogenes* and the nonhemolytic species *L. innocua* also induced HSP70, albeit at lower levels than wild-type *L. monocytogenes*, suggesting listeriolysin may accentuate the HSP70 mRNA response initiated by phagocytosis.

Although HSP70 mRNA levels were quickly elevated by infection, HSP60 mRNA levels remained constant during the time course studied. Previously, it has been shown that the HSP60 protein was not induced 3 hr after phagocytosis of *S. aureus* cells (6). Apparently, phagocytosis of bacteria does not affect HSP60 mRNA levels inside the host cell.

These data suggest that bacterial uptake by phagocytosis triggers HSP70 mRNA transcription in J774 cells. However, HSP90 is not induced until 2 hr after bacterial invasion by these macrophage cells. After 2 hr after infection, *L. monocytogenes* cells have escaped from the phagosome, replicated, and initiated actin polymerization (27). HSP90 is affiliated with several tyrosine kinases and steroid hormone receptors that have roles in signal transduction (1, 2). Two possible roles of HSP90 protein in signal transduction are (*i*) preserving a particular unfolded formation and preventing premature activation until the proper cellular compartment is reached or (*ii*) perhaps functionally activating these signal transduction proteins by helping to mold them into an active conformation (2). In addition, HSP90 protein can be associated with actin polypeptides (28) and in this capacity may be involved in actin polymerization. Similar to those of HSP90, the kinetics of PTP transcriptional induction are delayed in *L. monocytogenes*-infected macrophages, although the mRNA activation is higher than HSP90 (Fig. 5*A*). The gene for PTP encodes a phosphatase with dual specificities for tyrosine and threonine (29). This PTP protein apparently modulates mitogen-activated protein kinase by dephosphorylation (29) and is also suggested to be linked with signal transduction events associated with oxidative stress in the eukaryotic cell (7). Additionally, mediators of both protein kinase C-dependent and -independent pathways can induce transcription of PTP (8).

Differential transcription of stress genes appears to be a consequence of *L. monocytogenes* uptake. Although some stress is placed on the host cells during the steps leading to extraction of total RNA as indicated by the slight increase of all stress gene mRNAs during the experiments, the substantial activation of HSP70 mRNA at an early time point and the inductions of PTP and HSP90 mRNAs at later time points are clearly caused by the host cell response to the bacteria. These events are depicted in Fig. 6, and they point to the probable involvement of host cell signal transduction pathways. Signal transduction pathways appear to be stimulated by *Salmonella* invasion, based on an initial interaction with the epidermal growth factor receptor (30). Signals transmitted through contact with this receptor mobilize a signaling cas-

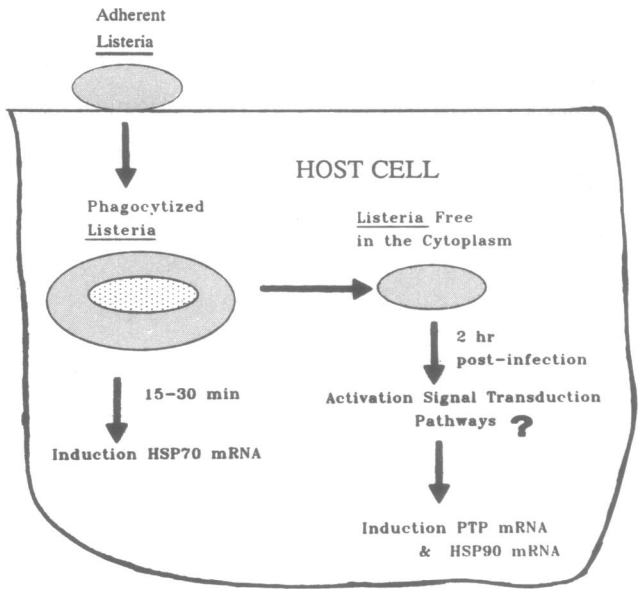

FIG. 6. A model for stress gene mRNA induction and possible linkage to signal transduction events in the host cell after infection with *L. monocytogenes*.

cade that causes phosphorylation and activation of mitogen-activated protein kinase and phospholipase A_2 (31). Other bacterial pathogens, such as *Yersinia* and enteropathogenic *Escherichia coli*, also induce tyrosine phosphorylation of host proteins; however, unlike *L. monocytogenes*, they remain in the phagosome of the host cell (32).

Mutants of *L. monocytogenes* were used to try to understand what mechanisms may be involved in stress gene mRNA activation. Escape from the phagosome and entry into the cytoplasm appear to be crucial steps for activation of the two signal transduction-associated stress gene mRNA populations (HSP90 and PTP) because the listeriolysin-negative mutant and the nonhemolytic, noninvasive species *L. innocua*, which remain in membrane-bound vacuoles (ref. 12; W.R.S., A. Demuth, G. Krohne, M. Kuhn, and W.G., unpublished data) both failed to induce these two groups of mRNAs. Several signal transduction pathways are actively involved in actin polymerization within the host cell (33). Both the Mpl and ActA proteins from *L. monocytogenes* are linked to host cell actin polymerization (14), but the respective mutants were unchanged with respect to stress gene mRNA levels. This suggests that signal transduction pathways other than those involved in actin polymerization may be affected.

In this study, we have used *L. monocytogenes* infection of macrophage-like cells to determine the effect on some stress genes in the host. Apparently, uptake of *L. monocytogenes* by host macrophages can induce differential transcription of several stress genes: activation of HSP70 mRNA primarily as a result of phagocytosis, induction of HSP90 and PTP mRNAs upon entry of the bacteria into the cytoplasm, and no response for HSP60 mRNA. It is conceivable that some factor or factors expressed by intracellular *Listeria* which are released into the cytoplasm activate specific genes in signal transduction routes of the host cell, leading to elevated transcription of HSP90 as well as PTP mRNA. Activation of these mRNAs and presumably elevated levels of the corresponding proteins may be part of a cross-talk between host and pathogen, helping the bacteria to survive intracellularly and at the same time keeping the eukaryotic cell alive.

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1. Lindquist, S. & Craig, E. A. (1988) *Annu. Rev. Genet.* **22**, 631–677.
2. Craig, E. A., Gambill, B. D. & Nelson, R. J. (1993) *Microbiol. Rev.* **57**, 402–414.
3. Ananthan, J., Goldberg, A. L. & Voellmy, R. (1986) *Science* **232**, 522–524.
4. Clerget, M. & Polla, B. S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1081–1085.
5. Mistry, Y., Young, D. B. & Mukherjee, R. (1992) *Infect. Immun.* **60**, 3105–3110.
6. Kantengwa, S. & Polla, B. S. (1993) *Infect. Immun.* **61**, 1281–1287.
7. Keyse, S. M. & Emslie, E. A. (1992) *Nature (London)* **359**, 644–647.
8. Charles, C. H., Abler, A. S. & Lau, L. F. (1992) *Oncogene* **7**, 187–190.
9. Guan, K., Broyles, S. S. & Dixon, J. E. (1991) *Nature (London)* **350**, 359–362.
10. Charbonneau, H. & Tonks, N. K. (1992) *Annu. Rev. Cell Biol.* **8**, 463–493.
11. Ralph, P., Prichard, J. & Cohn, M. (1975) *J. Immunol.* **114**, 898–905.
12. Kuhn, M., Kathariou, S. & Goebel, W. (1988) *Infect. Immun.* **56**, 79–82.
13. Tilney, L. G. & Portnoy, D. A. (1989) *J. Cell Biol.* **109**, 1597–1608.
14. Portnoy, D. A., Chakraborty, T., Goebel, W. & Cossart, P. (1992) *Infect. Immun.* **60**, 1263–1267.
15. Kuhn, M. & Goebel, W. (1994) *Infect. Immun.* **62**, 348–356.
16. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
17. Coen, D. M. (1990) in *Current Protocols in Molecular Biology*, eds. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (Wiley, New York), pp. 15.01–15.1.7.
18. Picketts, D. J., Mayanil, C. S. K. & Gupta, R. S. (1989) *J. Biol. Chem.* **264**, 12001–12008.
19. Lowe, D. G. & Moran, L. A. (1986) *J. Biol. Chem.* **261**, 2102–2112.
20. Moore, S. K., Kozak, C., Robinson, E. A., Ullrich, S. J. & Appella, E. (1989) *J. Biol. Chem.* **264**, 5343–5351.
21. Platzer, C., Richter, G., Überla, K., Müller, W., Blöcker, H., Diamantenstein, T. & Blanckenstein, T. (1992) *Eur. J. Immunol.* **22**, 1179–1184.
22. Schwan, W. R., Seifert, H. S. & Duncan, J. L. (1992) *J. Bacteriol.* **174**, 2367–2375.
23. Laemmli, U. K. (1979) *Nature (London)* **227**, 680–685.
24. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
25. Parsell, D. A. & Sauer, R. T. (1989) *Genes Dev.* **3**, 1226–1232.
26. Cooper, J. A. (1987) *J. Cell Biol.* **105**, 1473–1478.
27. Tilney, L. G. & Tilney, M. S. (1993) *Trends Microbiol.* **1**, 25–31.
28. Koyasu, S., Nishida, E., Uadowaki, T., Matsuzaki, F., Iida, K., Harada, F., Kasuga, M., Sakai, H. & Yahara, I. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8054–8058.
29. Alessi, D. R., Smythe, C. & Keyse, S. M. (1993) *Oncogene* **8**, 2015–2020.
30. Galan, J. E., Pace, J. & Hayman, M. J. (1992) *Nature (London)* **357**, 588–589.
31. Pace, J., Hayman, M. J. & Galan, J. E. (1993) *Cell* **72**, 505–514.
32. Bliska, J. B., Galan, J. & Falkow, S. (1993) *Cell* **73**, 903–920.
33. Stossel, T. P. (1993) *Science* **260**, 1086–1094.