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Vaccinia Virus Infection Induces a Stress Response That Leads to Association of Hsp70 with Viral Proteins

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We studied the impact of vaccinia virus infection on stress protein gene expression in human cells and investigated the possibility that eukaryotic heat shock proteins interact with viral components during assembly. Infection of human monocyte-macrophages by vaccinia virus caused a dramatic decrease in levels of cellular mRNAs such as those encoding actin and tubulin. In contrast, infection did not cause a significant reduction in the levels of Hsp90 and Hsp60 mRNAs and led to substantially increased levels of Hsp70 mRNAs. The accumulation of these stress protein mRNAs was due both to increases in their transcription rate and to their stability relative to other cellular mRNAs. The relative levels of the heat shock proteins and the other cellular proteins reflected the relative levels of their mRNAs. These results indicate that stress protein gene expression is relatively refractory to the generally deleterious effects of vaccinia virus infection on host cell gene expression. The continued expression of some of these stress proteins may be beneficial to the virus; the observations that the levels of Hsp70 are greatest at the peak of viral gene expression and that a large fraction of cellular Hsp70 is associated with vaccinia virus proteins suggest that Hsp70 is involved in vaccinia virus assembly.

Prokaryotic and eukaryotic cells respond to potentially damaging stimuli by increasing the synthesis of a family of proteins collectively known as stress proteins. The best studied of these stresses is heat shock (reviewed in references 20, 21, and 34), in which a sudden increase in temperature induces increased synthesis of heat shock proteins. Because many of the heat shock proteins are also induced by other stresses, they are also called stress proteins. Some of these proteins assist other proteins in folding, translocation, and assembly (10). The cell may exploit these functions of chaperonins to help protect it from the deleterious effects of stress.

Stress proteins can be induced in cells following infection by a variety of viruses in vitro (5, 9, 12, 15, 18, 19, 24, 28–30; reviewed in reference 35). When these studies were carried out, however, the lack of DNA and antibody probes prevented a thorough investigation of the effect of virus infection on the expression of the genes encoding Hsp90, Hsp70, and Hsp60. In addition, the ease with which laboratory manipulation of cells causes a stress response was not always fully appreciated, complicating the interpretation of some studies.

In bacteria, phage infection induces increased Hsp70 (DnaK) and Hsp60 (GroEL) synthesis (7, 16, 33), and these stress proteins are essential for the replication and assembly of a variety of bacteriophages (reviewed in reference 27). It is possible that eukaryotic viruses also exploit host stress protein functions during their life cycle. Recent reports have described associations between Hsp70 and components of adenovirus (22) and poliovirus (23).

We have studied the impact of vaccinia virus infection on the expression of three major stress protein genes: Hsp90, Hsp70, and Hsp60. In addition, we have investigated whether heat shock proteins are associated with vaccinia virus proteins within the cell. We find that Hsp mRNAs and proteins continue to be synthesized and accumulate in parallel with viral gene products, despite the rapid loss of other cellular mRNAs and proteins. Viral proteins are associated with a substantial fraction of Hsp70 in the cell, suggesting that Hsp70 is involved in the assembly of vaccinia virus.

MATERIALS AND METHODS

Cells and viruses. Vaccinia virus NYCBH was a kind gift of Dennis Panicali, Applied Biotechnology, Inc., Cambridge, Mass. U937 cells were obtained from American Type Culture Collection, Rockville, Md., and maintained as recommended by the supplier. Primary macrophages were prepared from human peripheral blood mononuclear cells by density gradient centrifugation and maintained as described previously (4).

DNA and antibody probes. DNA containing the mammalian stress protein Hsp90 (plasmid pHS801) was obtained from Lee Weber, University of Nevada (11), and DNA containing Hsp70 (plasmid pH2.3) was obtained from Rick Morimoto, Northwestern University (13). Human Hsp60 DNA was described previously (14). Genes for human actin (pHRL83-1VS4) and tubulin (21β3'UT) proteins were obtained from the American Type Culture Collection. Rabbit antiserum against human Hsp90 was obtained from Stephen Ullrich, National Institutes of Health, Bethesda, Md. Monoclonal antibody N27F3-4, which recognizes human Hsp70 protein, was provided by William Welch (31). Monoclonal antibody ML-30, derived against mycobacterial Hsp60 and cross-reactive with human Hsp60, was provided by Juraj Ivanyi, Medical Research Council, London, United Kingdom (8). Rabbit antiserum against mammalian actin protein was obtained from Don Doering, Whitehead Institute. Rabbit antiserum generated against purified killed vaccinia virus, which specifically recognizes intracellular vaccinia virus proteins, was obtained from Richard Condit, University of Florida.

Infection and mock infection of cells. U937 cells were infected with vaccinia virus as follows. The cells were

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pelleted at 500 × *g* for 5 min at 37°C, most of the growth medium was removed and saved at 37°C, and the cells were suspended with vaccinia virus at a multiplicity of infection of 20. The cell suspension was incubated at 37°C for 1 h to permit virus adsorption, diluted with the saved growth medium, and further incubated at 37°C for various times. For infection of primary macrophages (which grow as adherent cells), the growth medium was removed and saved at 37°C and the virus was diluted in an aliquot of the saved medium and added directly to the flask at a multiplicity of infection of 20. After 1 h the saved growth medium was added back to the flask, and the cells were incubated at 37°C for 12 h. For every infection, equivalent numbers of cells were mock infected with the growth medium alone and treated in exactly the same fashion as infected cells. The U937 cells, primary human macrophages, and human H9 T lymphocytes supported vaccinia virus replication to a comparable degree.

Northern analysis. Total poly(A)$^+$ RNA from equal numbers of mock- and virus-infected cells was prepared by a guanidinium-CsCl method (1). Equivalent amounts of RNA (as determined by the optical density at 260 nm) were loaded on denaturing 1.2% agarose–formaldehyde gels for electrophoresis, and the gel was blotted onto a nitrocellulose filter as described previously (1). Purified DNA fragments, radiolabeled with ^{32}P by random-primer extension, were used to probe the nitrocellulose filter overnight at 42°C in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS)–5× Denhardt's solution (1× Denhardt's solution is 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, and 0.02% Ficoll). The filter was washed for 15 min in 1× SSC–0.1% SDS at room temperature and for 1 h in 0.2× SSC–0.1% SDS at 68°C and exposed to X-ray film for autoradiography.

Nuclear runoff transcription. The rate of transcription of specific genes was determined by nuclear runoff analysis (1). Fifty million U937 cells were either mock infected or infected with vaccinia virus at a multiplicity of infection of 20. The cells were harvested 12 h postinfection, washed three times in phosphate-buffered saline, lysed in a buffer containing 10 mM Tris-Cl (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, and 0.5% Nonidet P-40 (NP-40), and centrifuged to pellet the nuclei. The nuclear pellet was resuspended in the lysis buffer, pelleted again, and resuspended in buffer containing 50 mM Tris-Cl (pH 8.3), 40% glycerol, 5 mM $MgCl_2$, and 0.1 mM EDTA for storage at −70°C. [^{32}P]RNA was prepared from nuclei of the mock- and virus-infected cells as described previously (1). Purified DNA fragments containing genes for Hsp90, Hsp70, Hsp60, actin, or tubulin were blotted onto duplicate nitrocellulose filters by using a Minifold II slot blotter (Schleicher & Schuell, Keene, N.H.). Each filter was probed with equivalent counts of [^{32}P]RNA from mock-infected or virus-infected cells at 65°C for 36 h in hybridization solution containing 10 mM TES (pH 7.4) (1), 10 mM EDTA, 0.2% SDS, and 0.6 M NaCl. After hybridization, each membrane was washed twice in 2× SSC at 65°C for 1 h, treated with RNase A, washed again in 2× SSC at 37°C for 1 h, and subjected to autoradiography. The signals on the autoradiograph were quantitated by using ImageQuant software V3.0 (Molecular Dynamics, Sunnyvale, Calif.).

Western immunoblot analysis. Equivalent numbers of U937 cells were mock infected or virus infected as described above. At 12 h postinfection, the cells were harvested and lysed in equivalent volumes of lysis buffer containing 50 mM Tris-Cl (pH 8.0), 150 mM NaCl, 0.2% sodium azide, 1% NP-40, and 2 mM phenylmethylsulfonyl fluoride. The cell debris were removed by centrifugation at 10,000 × *g* for 5 min at 4°C, and similar volumes of the supernatants from mock-infected and virus-infected cells were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 7.5% acrylamide gel. The separated proteins were transferred to nitrocellulose membranes and probed with antibodies against Hsp90, Hsp70, Hsp60, and actin.

Immunoprecipitation analysis. Equivalent volumes of the supernatants prepared from mock-infected and virus-infected cells were subjected to immunoprecipitation. The proteins were precipitated from the supernatants by using either rabbit polyclonal antiserum prepared against vaccinia virus proteins or control normal rabbit serum in a buffer containing 50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 0.1% NP-40, 1 mM EDTA, 1% gelatin, and 0.02% sodium azide. After incubation for 1 h at 4°C, protein A-Sepharose beads were added, and the mixture was further incubated for 1 h. The protein A-antigen-antibody complexes were collected by centrifugation at 12,000 × *g* for 1 min at 4°C and washed twice in 50 mM Tris-Cl (pH 7.5)–150 mM NaCl–0.1% NP-40–1 mM EDTA and then twice in 10 mM Tris-Cl (pH 7.5)–0.1% NP-40. The pellets were suspended in SDS-gel loading buffer and heated to 100°C for 5 min. The supernatants were subjected to SDS-PAGE and Western blot analysis with monoclonal antibody ML-30 against Hsp60 and monoclonal antibody N27F3-4 against Hsp70.

Sucrose gradient centrifugation. Lysates from equal numbers of mock-infected and virus-infected cells were layered onto a 5 to 20% sucrose gradient made in 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) containing 0.1 M NaCl, 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and 2 mM phenylmethylsulfonyl fluoride and centrifuged for 16 h in a Beckman SW 50.1 rotor at 49,000 rpm. Fifteen fractions of equivalent volume were collected from the bottoms of the tubes, and a portion of each fraction was subjected to Western blot analysis with monoclonal antibody N27F3-4 and a chemiluminscent detection system (ECL Western blot detection system; Amersham, Amersham, United Kingdom). The bands were quantitated by using ImageQuant software version 3.0.

RESULTS

Stress protein mRNAs accumulate in vaccinia virus-infected cells. We investigated the effect of vaccinia virus infection on stress protein gene expression in the human monocyte cell line U937. We chose these cells for our study of stress responses to viral infection because of the role of macrophages in antigen presentation during infection and because of our interest in the possibility that immune responses to stress protein determinants may play a role in immune surveillance of virus-infected cells (35, 36). Cells were infected with vaccinia virus or were mock infected. RNA was prepared from cells at various time intervals, and the levels of Hsp90, Hsp70, and Hsp60 mRNAs were investigated by Northern (RNA) blot analysis. The levels of actin and β-tubulin mRNAs were also studied, because these mRNAs encode major structural proteins that are relatively unaffected by stress. The peak of virus mRNA production occurred 12 h after infection.

Figure 1A shows that the levels of actin and β-tubulin mRNAs were dramatically reduced in the vaccinia virus-infected cells over the course of infection but were unchanged in the mock-infected cells. In contrast, the levels of Hsp90, Hsp70, and Hsp60 mRNAs were similar in virus-

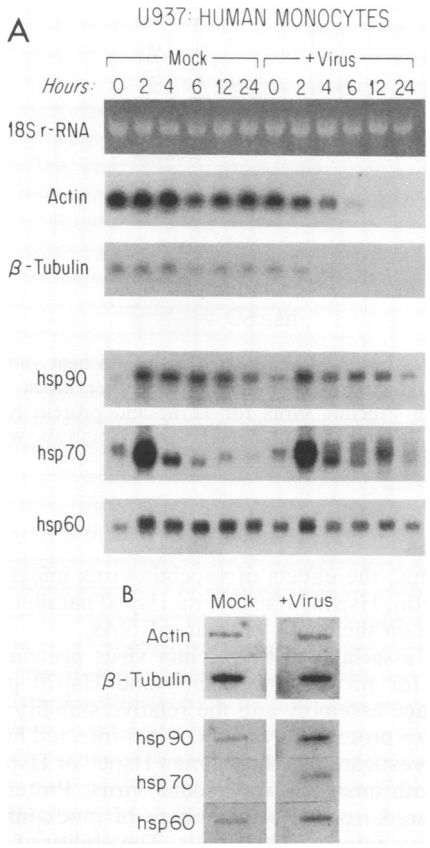

FIG. 1. (A) Northern analysis of actin, β-tubulin, and heat shock protein mRNAs in mock-infected or virus-infected U937 cells. RNA was isolated from cells at the indicated times after mock or genuine infection. The amount of RNA obtained per cell at each time point varied by no more than a factor of 2, whether or not the cells were genuinely infected. Equivalent amounts of RNA from each time point were subjected to gel electrophoresis and then transferred to nitrocellulose. The same blot was used to obtain all of the hybridization signals shown here. DNA probes are described in Materials and Methods. Ethidium bromide-stained 18S rRNA was used as a control. (B) Nuclear transcription runoff analysis of actin, β-tubulin, and heat shock protein genes. Nuclei were isolated from U937 cells which had been either mock infected or infected with vaccinia virus for 12 h. The isolated nuclei were incubated with ^{32}P-labeled nucleoside triphosphates, and labeled RNA was isolated and used to probe immobilized DNA fragments. The specific activity of the labeled RNA was comparable in the mock-infected (1.3×10^4 cpm/μg) and the virus-infected cells (1.0×10^4 cpm/μg). A signal for Hsp70 mRNA in mock-infected cells could be detected and used for quantitation after a more lengthy exposure of the autoradiograph.

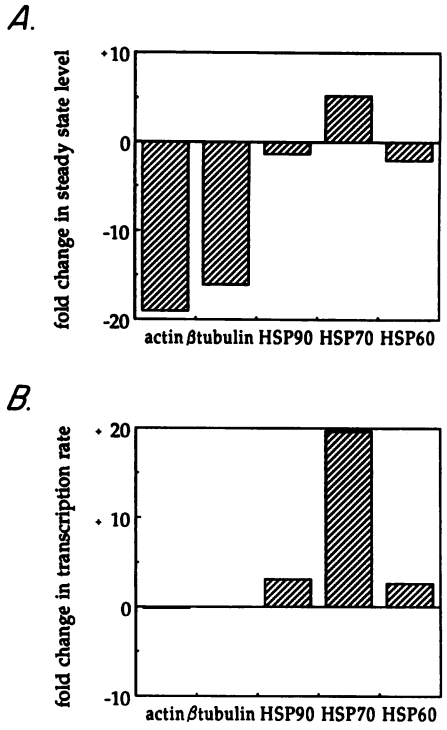

FIG. 2. (A) Change in the steady-state level of actin, β-tubulin, and heat shock protein mRNAs in vaccinia virus-infected cells relative to mock-infected cells. The increase or decrease in the levels of various mRNAs was calculated from densitometric analysis of the signals obtained at the 12-h time point in Fig. 1A. (B) Change in the transcription rate of various genes, calculated from densitometric analysis of the signals obtained at the 12-h time point in Fig. 1B.

infected and mock-infected cells. The manipulation of cells during infection (or mock infection) caused increased but transient accumulation of the Hsp mRNAs at the 2-h time point; this effect was particularly striking for the Hsp70 family. The most significant difference in Hsp mRNA levels between mock-infected and virus-infected cells was observed at the peak of virus production (12 h), when there was a relative increase in the level of Hsp70 mRNA in the virus-infected cells. Quantitation of the levels of various mRNAs at the peak of virus production revealed 19- and 16-fold decreases in actin and β-tubulin mRNA levels, respectively (Fig. 2A); the levels of other nonstress mRNAs (glyceraldehyde-3-phosphate dehydrogenase and glucose transporter) decreased to similar extents (data not shown). In contrast, there was little change in Hsp90 and Hsp60 mRNA levels and a more than fivefold increase in Hsp70 mRNA levels. Essentially identical results were obtained when infection was performed by introducing vaccinia virus directly into a flask of U937 cells to minimize stress due to manipulation of cells.

There was a small but detectable change in the average size of Hsp70 mRNAs over the course of the experiment shown in Fig. 1A. These changes, and the broad signal in the 2-h samples, most probably reflect the appearance of multiple transcripts from members of the closely related Hsp70 gene family (34).

Transcription of stress genes increases during infection. We investigated the effect of vaccinia virus infection on the rate of transcription of genes encoding stress and nonstress proteins by using nuclear runoff transcription analysis. Mock-infected and virus-infected cells were harvested 12 h after infection, and cell nuclei were isolated. The isolated nuclei were incubated with ^{32}P-labeled nucleoside triphosphates to permit elongation and labeling of previously initiated transcripts. The labeled RNA was isolated and used to probe nitrocellulose slot blots containing DNA fragments encoding actin, β-tubulin, Hsp90, Hsp70, and Hsp60 (Fig. 1B). The rates of transcription of actin and tubulin genes were essentially identical in uninfected and vaccinia virus-infected cells at the peak of virus production. In contrast, the rates of Hsp90 and Hsp60 mRNA synthesis increased approximately threefold and that of Hsp70 increased 20-fold.

The impact of vaccinia virus infection on the rate of

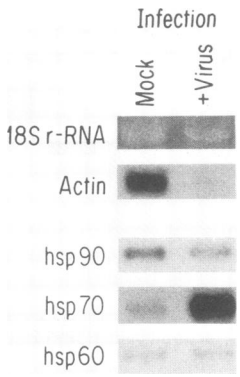

FIG. 3. Northern analysis of mRNAs after virus infection of primary macrophages. Primary macrophages prepared from peripheral blood mononuclear cells were either mock infected or infected with vaccinia virus for 12 h. Total RNA was isolated and subjected to Northern analysis with DNA probes as described for Fig. 1A.

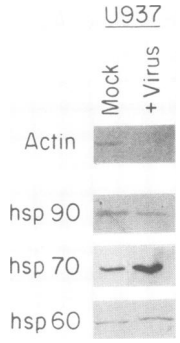

FIG. 4. Western blot analysis of actin and heat shock proteins after virus infection. U937 cells were either mock infected or infected with vaccinia virus for 12 h, and protein lysates were prepared and subjected to Western blot analysis with various antibody probes (see Materials and Methods).

transcription of genes encoding actin, β-tubulin, Hsp90, Hsp70, and Hsp60 and on the levels to which the mRNAs accumulate is summarized in Fig. 2. The rates of transcription of actin and β-tubulin are not significantly affected by vaccinia virus infection, but the levels to which these transcripts accumulate drop approximately 20-fold, indicating that their rates of degradation must increase substantially. These results are consistent with previously reported experiments that indicate that actin and α-tubulin mRNAs become significantly less stable during vaccinia virus infection (32). The effects of vaccinia virus infection on Hsp gene expression are clearly different. There is about a 3-fold increase in the rate of transcription of Hsp90 and Hsp60 genes and a 20-fold increase in the rate of transcription of Hsp70 genes. Although the levels to which the Hsp90, Hsp70, and Hsp60 transcripts accumulate do not match the increase in transcription rates, the Hsp transcripts appear to be significantly more stable than the actin and β-tubulin transcripts.

Virus-induced stress responses in primary macrophages. Since transformed cells may produce aberrant levels of stress proteins (2, 24), we investigated the impact of vaccinia virus infection on primary macrophages. Primary macrophages were prepared from peripheral blood mononuclear cells by density gradient centrifugation and permitted to adhere to plastic culture flasks. Equivalent numbers of cells were mock infected or infected with vaccinia virus. The cells were harvested 12 h postinfection, RNA was isolated, and equivalent amounts of total RNA were subjected to Northern blot analysis. The results were similar to those obtained with the U937 cell line (Fig. 3). There was a dramatic loss of actin mRNA, little or no change in the levels of Hsp90 and Hsp60 mRNAs, and a very substantial increase in Hsp70 mRNA levels.

Stress proteins are relatively stable in vaccinia virus-infected cells. The effect of vaccinia virus infection on the accumulation of actin and of Hsp90, Hsp70, and Hsp60 proteins was analyzed by Western blot analysis. Protein lysates were prepared from equivalent numbers of mock-infected and virus-infected cells 12 h after infection, subjected to SDS-PAGE, transferred to nitrocellulose, and probed with specific antibodies (Figure 4). Although quantitation of Western blots is not highly accurate, it is clear that vaccinia virus infection caused about a threefold reduction in the level of actin, little or no change in the levels of Hsp90 and Hsp60, and a significant increase in the level of Hsp70 protein. Thus, the effects of vaccinia virus infection on the levels of actin, Hsp90, Hsp70, and Hsp60 parallel the effects of infection on the levels of their mRNAs.

Hsp70 is associated with vaccinia virus proteins. The requirement for prokaryotic Hsp60 and Hsp70 proteins in bacteriophage assembly and the relative stability and abundance of Hsp proteins in vaccinia virus-infected human cells led us to investigate whether human Hsp60 or Hsp70 protein interacts with intracellular vaccinia virus. Protein extracts were prepared from equal numbers of mock-infected and vaccinia virus-infected U937 cells. The ability of the monoclonal antibodies to detect Hsp70 and Hsp60 proteins in extracts prepared from the virus-infected cells was confirmed by Western blot analysis before the extracts were subjected to immunoprecipitation (Fig. 5A). Proteins in the extracts were immunoprecipitated with a rabbit polyclonal antibody directed against a broad spectrum of vaccinia virus proteins. The immunoprecipitates were resuspended, subjected to SDS-PAGE, transferred to nitrocellulose, and probed with monoclonal antibodies against Hsp60 or Hsp70 (Fig. 5A). Hsp70 protein was clearly observed in the immunoprecipitated material obtained from vaccinia virus-infected cells but not in the material obtained from mock-infected cells. In contrast, no Hsp60 was found in the vaccinia virus immunoprecipitates. We estimate that the amount of Hsp70 associated with vaccinia virus proteins is between 5 and 30% of the total cellular Hsp70 recognized by the monoclonal antibody.

To further investigate the association of Hsp70 with vaccinia virus proteins, protein extracts prepared from mock-infected and vaccinia virus-infected U937 cells were subjected to sucrose gradient centifugation. Fractions were collected from the gradient, and each fraction was analyzed for the presence of vaccinia virus proteins and Hsp70 by Western blot analysis. In mock-infected cell lysates, all of the Hsp70 protein sedimented as a single peak approximately one-third of the way down the gradient. In the vaccinia virus-infected cell lysates, the Hsp70 peak was significantly diminished and Hsp70 was found cosedimenting with viral proteins throughout much of the gradient. Most of the Hsp70 in the virus-infected cell lysate sedimented more rapidly than Hsp70 in the mock-infected cell lysate. Although this result may reflect an increase in the association of Hsp70 with both viral and cellular proteins during infection, the data in Fig. 5 indicate that a substantial fraction of

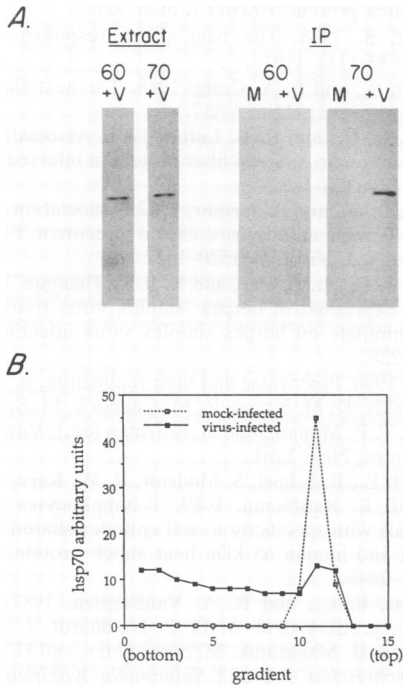

FIG. 5. (A) Coimmunoprecipitation of Hsp70 with vaccinia virus proteins. Whole-cell extracts were prepared from mock-infected (M) and vaccinia virus-infected (+V) U937 cells. The ability of the Hsp-specific antibodies to detect Hsp70 and Hsp60 in the vaccinia virus-infected whole-cell extracts is shown on a Western blot at the left. These extracts were subjected to immunoprecipitation with rabbit antiserum specific for intracellular vaccinia virus proteins. The immunoprecipitates (IP) were resuspended, subjected to SDS-PAGE, and transferred to nitrocellulose. The immobilized proteins were probed with antibodies specific for Hsp70 and Hsp60 proteins. (B) Sucrose gradient sedimentation analysis of Hsp70 in mock-infected and virus-infected cells. Whole-cell extracts prepared from mock-infected and vaccinia virus-infected U937 cells were subjected to sucrose density gradient centrifugation, and fractions were collected from the bottom of the gradient and analyzed by Western blot for Hsp70 or for vaccinia virus proteins by using specific antibodies and a chemiluminescence detection system.

Hsp70 in vaccinia virus-infected cells is associated with viral proteins at the peak of virus production.

DISCUSSION

Vaccinia virus infection causes a stress response in human monocyte-macrophages. This response is characterized by a 3-fold increase in Hsp90 and Hsp60 mRNA synthesis and a 20-fold increase in Hsp70 mRNA synthesis. Despite a dramatic loss of major cellular mRNAs and proteins in the infected cell, Hsp mRNAs and proteins, particularly those of Hsp70, continue to accumulate. A large fraction of the Hsp70 protein becomes associated with vaccinia virus proteins, suggesting that it may be involved in virus assembly.

Hsp mRNAs escape rapid degradation. Previous reports have demonstrated that vaccinia virus infection causes a dramatic decrease in the levels of host cell gene products (reviewed in references 6 and 25). Actin and α-tubulin mRNAs, for example, are rapidly degraded in vaccinia virus-infected cells (32). The molecular mechanisms involved in the loss of host cell mRNAs are not well understood, but it appears that loss of cellular mRNAs accounts for the shutoff of cell-specific protein synthesis. The results described here indicate that actin and β-tubulin genes continue to be transcribed at normal rates in vaccinia virus-infected cells but accumulate to only very low levels, reflecting their short half-lives.

Our results indicate that Hsp mRNAs are somewhat less stable in infected cells than they are in uninfected cells but are largely exempt from the very rapid rate of degradation apparent for actin and tubulin. Indeed, Hsp90, Hsp70, and Hsp60 mRNAs accumulate to substantial levels in the infected cells, as a result of both increases in their transcription rate and their stability relative to other cellular mRNAs. It will be interesting to learn why Hsp mRNAs are largely exempt from the rapid degradation observed for other cellular mRNAs during vaccinia virus infection.

Interaction of Hsp70 with vaccinia virus. Bacteriophage infection induces Hsp70 and Hsp60 synthesis in prokaryotes, and these stress proteins are essential for the replication and assembly of a variety of bacteriophages (7, 16, 27, 33). It seems likely that eukaryotic viruses also exploit host stress protein functions during their life cycle. Two lines of evidence indicate that Hsp70 interacts with vaccinia virus proteins during infection. First, Hsp70 coprecipitated with intracellular vaccinia virus proteins when they were immunoprecipitated from infected-cell extracts. Under the same conditions, Hsp60 did not coprecipitate with these viral components. Second, a substantial fraction of Hsp70 in vaccinia virus-infected cells was associated with viral proteins at the peak of virus gene expression; most of the cellular Hsp70 cosedimented with viral proteins in sucrose gradients. These data suggest that Hsp70 may be involved in the assembly of vaccinia virus. Hsp70 has also been reported to associate with components of adenovirus (22) and poliovirus (23).

Implications for immune response to infection. The results described here may have implications for immune recognition of stressed cells. Pathogen-derived stress proteins are frequent targets of T cells during the immune response to infection (35, 36). Healthy individuals have stress protein T lymphocytes which can recognize self stress protein determinants as well (3, 17, 26). These observations are consistent with the suggestion that T lymphocytes that recognize self stress proteins may recognize virus-infected cells via an increase in the level of stress protein determinants presented by the infected cells (36). This capability would provide a first line of defense against infection by permitting recognition and elimination of stressed autologous cells, and this defense need not await the development of immunity to novel antigens. Our results indicate that during vaccinia virus infection, some stress proteins accumulate to substantial levels, especially relative to other cellular proteins. These stress proteins, if appropriately presented, could thus target infected cells for destruction by stress protein-specific T lymphocytes.

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