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Characterization of the *Agrobacterium tumefaciens* Heat Shock Response: Evidence for a σ^{32}-Like Sigma Factor

NICHOLAS J. MANTIS AND STEPHEN C. WINANS*

Section of Microbiology, Wing Hall, Cornell University, Ithaca, New York 14853-8101

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We have characterized the heat shock response of *Agrobacterium tumefaciens* and compared it with the well-characterized *Escherichia coli* heat shock response. Four major heat shock proteins with apparent molecular masses of 98, 75, 65, and 20 kDa were identified by pulse-labelling cultures after temperature upshift. The three largest proteins comigrated with proteins that were antigenically related to the *E. coli* heat shock proteins σ^{70}, DnaK, and GroEL, respectively. The heat shock proteins were also strongly induced by ethanol and cadmium chloride and were mildly induced by mitomycin C. To determine whether the *A. tumefaciens* heat shock regulatory system was similar to that of *E. coli*, we introduced the *E. coli dnaK* gene into *A. tumefaciens*. The *E. coli* DnaK protein was expressed in *A. tumefaciens*, and its synthesis was induced after heat shock. Primer extension analysis of the *E. coli dnaK* gene in *A. tumefaciens* indicated that transcription initiated from one or possibly both of the *E. coli* heat shock promoters. We conclude that *A. tumefaciens* has a heat shock response similar to that of *E. coli*, in that (i) similar proteins are induced by heat shock, (ii) synthesis of these proteins is induced in response to similar stimuli, and (iii) *A. tumefaciens* can recognize an *E. coli* heat shock promoter, suggesting that *A. tumefaciens* has a σ factor similar to σ^{32}.

The infection of a plant wound by the gram-negative bacterium *Agrobacterium tumefaciens* is a complex process that involves multiple levels of two-way chemical signalling between the bacterium and the plant host. Pathogenic strains of *A. tumefaciens* harbor a large plasmid, called the Ti plasmid, that confers the ability to elicit plant tumors (for reviews, see references 5, 13, 23, 37, 54, and 57). Infection involves the conjugationlike transfer of oncogenic DNA (T-DNA) from the bacterium to the plant cell nucleus. The genes required for T-DNA transfer (*vir* genes) are transcriptionally induced during infection by the VirA, VirG, and ChvE proteins (7, 22, 42) in response to phenolic compounds, sugars, and acid pH (7, 40, 41).

There are at least two reasons that an investigation of the *A. tumefaciens* heat shock response is warranted. First, the *virG* gene, which encodes the transcriptional activator of the *vir* genes, is induced in acidic media from a promoter whose −10 region has a strong sequence similarity to that of the family of *Escherichia coli* heat shock-inducible promoters (10, 32, 53). This promoter is thought to play an important role in the early stages of infection (53). Second, we were interested in the mechanisms by which *A. tumefaciens* responds to environmental stress, since plant wounds are potentially hostile environments. Plants have a number of wound-inducible or pathogen-inducible defense responses, including the synthesis of antimicrobial compounds (28). An understanding of the mechanisms by which plant pathogens respond and adapt to stressful environments may further our understanding of the infection process.

The heat shock response, in prokaryotes and eukaryotes, is the synthesis of a distinct set of proteins after temperature upshift or other toxic stresses (for reviews, see references 11, 17, 30, and 34). Seventeen heat shock proteins in *E. coli* have been identified (34), two of which, DnaK and GroEL, are members of a gene family of heat shock proteins found in all organisms (30). The *E. coli* heat shock response is regulated at the level of transcription by the σ factor σ^{32}, the product of the *rpoH* (*htpR*) gene (18). σ^{32} functions to direct and regulate the transcription of specific heat shock promoters (10, 19). A heat shock response similar to that of *E. coli* has been described for other bacterial species, including *Caulobacter crescentus* (15), *Pseudomonas aeruginosa* (2), and *Bacillus subtilis* (3). Heat shock-inducible promoter sequences that resemble the *E. coli* heat shock promoter consensus sequence have been identified for *C. crescentus* (14) and the enteric *Vibrio cholerae* (35); however, there is no direct evidence for a σ factor similar to σ^{32} in these or any other bacterial species.

In this paper, we characterize the *A. tumefaciens* heat shock response and compare it with the *E. coli* response. *A. tumefaciens* heat shock proteins were identified by pulse-labelling after temperature upshift and by immunoblotting with antibodies against *E. coli* heat shock proteins. Stresses that induce the synthesis of the *E. coli* heat shock proteins were tested for the ability to induce the synthesis of the *A. tumefaciens* heat shock proteins. Finally, the *E. coli dnaK* gene was introduced into *A. tumefaciens* to test whether the heat shock regulatory systems were similar. Our results suggest that the *A. tumefaciens* heat shock response is similar to the *E. coli* response and is regulated by a σ^{32}-like σ factor.

MATERIALS AND METHODS

Reagents and enzymes. Antibiotics, *o*-nitrophenyl-D-galactopyranoside (ONPG), 2-(*N*-morpholino)ethanesulfonic acid (MES), 2-(hydroxyethyl)piperazine-3-propanesulfonic acid (EPPS), cadmium chloride ($CdCl_2$), mitomycin C, and diethyl pyrocarbonate (DEPC) were purchased from Sigma Co. (St. Louis, Mo.). $CdCl_2$ and mitomycin C were each dissolved in water and stored at 4 and −70°C, respectively. Coomassie brilliant blue G-250, bromophenol blue, prestained sodium dodecyl sulfate (SDS) molecular weight standards, Tween 20, gelatin, goat anti-rabbit immunoglobulin alkaline phosphatase, goat anti-mouse immunoglobulin

* Corresponding author.

alkaline phosphatase, 5-bromo-4-chloro-3-indoyl phosphate (BCIP), and *p*-nitroblue tetrazolium chloride (NBT) were purchased from Bio-Rad (Richmond, Calif.). Restriction endonucleases, T4 DNA ligase, T4 polynucleotide kinase, and Moloney murine leukemia virus reverse transcriptase were from Bethesda Research Laboratories (Gaithersburg, Md.). RNase Block II ribonuclease inhibitor was obtained from Stratagene (La Jolla, Calif.). Sequenase is a product of U.S. Biochemical (Cleveland, Ohio). L-[^{35}S]methionine and [γ-^{32}P]ATP were from Amersham (Arlington Heights, Ill.).

Strains and plasmids. *A. tumefaciens* A348 is a derivative of A136 containing pTiA6NC (38). *E. coli* MC4100 [F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flb-5301 deoC1 ptsF25 rbsR*] was obtained from C. Manoil (University of Washington). The plasmid pBB1, which contains the complete *E. coli dnaK-dnaJ* operon, was a gift from G. Walker (Massachusetts Institute of Technology). pNM109 was constructed by ligating the 8-kb *Bam*HI fragment containing *dnaK-dnaJ* and a spectinomycin cartridge from pBB1 into the broad-host-range plasmid pSW213 (8). This *Bam*HI fragment was also cloned into pTZ18R (U.S. Biochemical) to create pNM110. Plasmid DNA was introduced into *A. tumefaciens* strains by electroporation (33).

Pulse-labelling experiments. Strains were grown in AB medium (pH 7.0) (9) to an optical density (OD) of 0.4 at 600 nm, centrifuged, resuspended in 0.05 volumes of water containing 15% glycerol, and frozen at −70°C. For induction assays, cells were thawed, diluted 1:2,000 in AB medium (pH 7.0) in culture tubes (25 by 125 mm), and cultured at 30°C on a rotary aerator to an OD of 0.4 at 600 nm. For the heat shock experiments, 1-ml samples of cells were transferred to prewarmed test tubes (13 by 100 mm) in a 39°C water bath and shaken at 250 rpm for 10 min. The cultures required 1 min to reach the higher temperature. Cells were labelled with 5 μCi of L-[^{35}S]methionine for 3 min, chased with nonradioactive L-methionine (to a final concentration of 2 mM) for 3 min, and then transferred to 1.5-ml Eppendorf tubes containing 0.5 ml of 5% trichloroacetic acid. For ethanol, cadmium chloride, and mitomycin C treatments, 1-ml samples of cells were dispensed into culture tubes (13 by 100 mm) containing the appropriate amount of inducers, returned to the rotary aerator for 10 min, and then pulse-labelled. For pH shift experiments, cells were centrifuged at 8,000 × *g* for 10 min, resuspended in the precentrifugation volume of prewarmed AB minimal medium at the appropriate pH, and returned to the rotary aerator. Samples were removed for pulse-labelling as described above.

After pulse-labelling, cells were harvested by centrifugation at 10,000 × *g* for 3 min, washed once with acetone, vacuum dried, suspended in SDS sample buffer, heated to 100°C for 5 min, and size fractionated with SDS-polyacrylamide gels (27). Gels were stained with Coomassie brilliant blue, vacuum dried, and exposed on Kodak X-OMAT XAR-5 diagnostic film.

Immunoblotting. Cells were pulse-labelled, and the proteins were then size fractionated on SDS-10% polyacrylamide gels, as described above, and transferred to nitrocellulose by using a Hoeffer Transphor electrophoresis unit (6). Western blotting (immunoblotting) was performed either with polyclonal rabbit antiserum directed against *E. coli* DnaK, GroEL, or σ^{32} or with a murine monoclonal antiserum directed against *E. coli* σ^{70}. Gifts of antibodies were as follows: anti-DnaK from R. McMacken (1), anti-GroEL from R. Hendrix, anti-σ^{32} from N. Brot (39), and monoclonal anti-σ^{70} (2G10) and anti-σ^{32} from R. Burgess (43). Primary antibodies were detected with alkaline phosphatase-conjugated goat anti-rabbit antiserum or goat anti-mouse antiserum (49). To determine whether these immunostained proteins comigrated with the major heat shock proteins, the nitrocellulose filter was analyzed by autoradiography as described above.

RNA isolation and primer extension. Cells were grown in AB minimal medium (pH 7.0) at 30°C to an OD of 0.6 at 600 nm. For heat shock experiments, 15 ml of cells were removed and placed in prewarmed culture tubes (25 by 25 mm) in a 39°C water bath as described above. Total RNA was extracted from 10 ml of cells by lysozyme treatment in the presence of DEPC and quantitated by spectrophotometry at 260 nm (44). A 30-mer, complementary to the first 10 codons of *dnaK* (4), was synthesized at the Cornell Biotechnology Facility. This primer (10 pmol) was labelled at the 5′ end with [γ-^{32}P]ATP and T4 polynucleotide kinase, and the efficiency of the kinase reaction was determined by trichloroacetic acid precipitation (31).

For primer extension analysis, RNA (10 μg) and labelled primer (0.5 pmol, or approximately 10^7 cpm) were coprecipitated in ethanol and resuspended in 17 μl of hybridization buffer (50 mM Tris-HCl [pH 8.3], 70 mM KCl, 3 mM $MgCl_2$). The samples were heated to 80°C for 10 min and then cooled slowly to 30°C (1.5 h). Thirty-three microliters of reverse transcriptase mix (50 mM Tris-HCl [pH 8.3]; 70 mM KCl; 3 mM $MgCl_2$; 10 mM dithiothreitol; 1 mM [each] dGTP, dATP, dCTP, and dTTP; 0.5 U of RNase Block II; 200 U of Moloney murine leukemia virus reverse transcriptase) was added, and the samples were placed at 37°C for 45 min. The reaction was stopped by the addition of 25 mM EDTA (pH 8.0), and the sample was then extracted with 2 volumes of phenol-chloroform (1:1), ethanol precipitated, and resuspended in 4 μl of Tris-EDTA (pH 7.4) and 6 μl of Stop buffer from the Sequenase DNA sequencing kit. A *dnaK* sequencing ladder was generated with Sequenase, according to procedures recommended by the manufacturer, with the *dnaK* primer and single-stranded pNM110. The primer extension samples and the sequencing ladder were heated to 85°C for 5 min and size fractionated by electrophoresis on a 6% polyacrylamide gel. The gel was transferred to filter paper, vacuum dried, and exposed on Kodak X-OMAT XAR-5 diagnostic film at −70°C with an intensifying screen.

RESULTS

Heat shock response in *A. tumefaciens*. To identify proteins made in response to heat shock, log-phase cultures of *A. tumefaciens* were grown in minimal medium at 30°C and were pulse-labelled at 30°C or 10 min after a shift to 39°C (Fig. 1). Heat shock caused a 1.5-fold increase in the amount of L-[^{35}S]methionine incorporated into total protein, as determined by liquid scintillation counting of trichloroacetic acid-precipitable material (data not shown). Four major proteins with apparent molecular masses of 98, 75, 65, and 20 kDa were preferentially synthesized after heat shock (Fig. 1, lane 2). These proteins were maximally expressed at 10 min and were the only proteins detected after shifting bacterial cultures from 30 to 45°C (data not shown).

***E. coli* and *A. tumefaciens* heat shock proteins.** To characterize the *A. tumefaciens* heat shock proteins, we first compared these proteins with the *E. coli* heat shock proteins (Fig. 2). *E. coli* MC4100 and *A. tumefaciens* A348 were pulse-labelled before or after heat shock, and total protein was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). In agreement with earlier studies, *E. coli* synthesized two major heat shock proteins with apparent

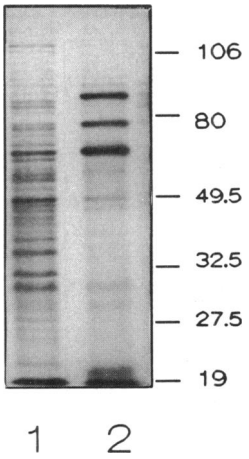

FIG. 1. *A. tumefaciens* protein synthesis after heat shock. A log-phase culture of *A. tumefaciens* was pulse-labelled at 30°C (lane 1) or 10 min after a shift to 39°C (lane 2) as described in Materials and Methods. The mobilities of molecular mass markers (in kilodaltons) are shown on the right.

molecular masses of 78 and 65 kDa (Fig. 2A, lane 2) (11). To determine whether the three *A. tumefaciens* heat shock proteins were antigenically related to any known *E. coli* heat shock protein, we immunoblotted total size-fractionated protein from heat shocked cells by using antibodies directed against *E. coli* DnaK, GroEL, and σ^{70} (RpoD). The anti-DnaK antiserum reacted strongly with an *E. coli* 78-kDa protein and with an *A. tumefaciens* 75-kDa protein (Fig. 2B). These proteins comigrated with major heat shock proteins (Fig. 2A). The anti-GroEL antibody reacted with a 65-kDa protein from *E. coli* and *A. tumefaciens* (Fig. 2C). These proteins also comigrated with major heat shock proteins (Fig. 2A). Finally, anti-σ^{70} antiserum reacted strongly with a 90-kDa *E. coli* protein and more weakly with a 98-kDa *A. tumefaciens* protein (Fig. 2D). σ^{70} migrates as a 90-kDa protein on Tris-buffered SDS-polyacrylamide gels (16). The 98-kDa *A. tumefaciens* cross-reacting protein comigrated with the largest major heat shock protein (Fig. 2A, lane 3), leading us to suspect that they were the same protein. We

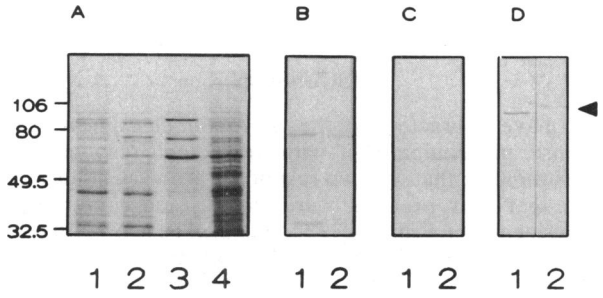

FIG. 2. Identification of the *A. tumefaciens* heat shock proteins. *E. coli* MC4100 or *A. tumefaciens* A348 was pulse-labelled before or after heat shock as described in Materials and Methods. (A) *E. coli* at 30°C (lane 1) or 3 min after shift to 42°C (lane 2) and *A. tumefaciens* at 30°C (lane 4) or 10 min after shift to 39°C (lane 3); (B to D) immunoblots of heat-shocked *E. coli* (lane 1) and *A. tumefaciens* (lane 2) with anti-DnaK (B), anti-GroEL (C), and anti-σ^{70} (D). The mobilities of molecular mass markers (in kilodaltons) are shown on the left. Arrow at right indicates the *A. tumefaciens* cross-reacting protein.

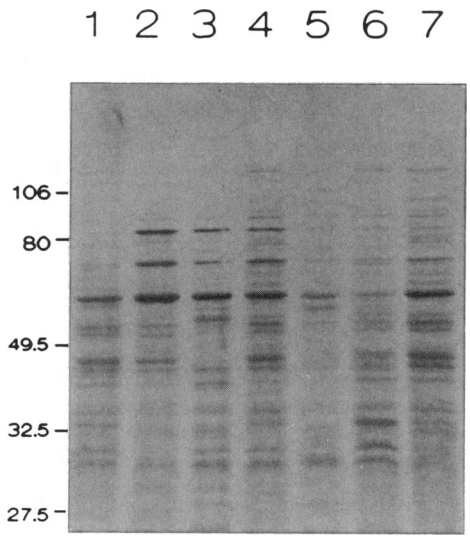

FIG. 3. Protein synthesis in response to various stresses. *A. tumefaciens* A348 grown at 30°C (pH 7.0) was pulse-labelled and prepared 10 min after no treatment (lane 1), heat shock at 39°C (lane 2), the addition of ethanol (4% [vol/vol]) (lane 3), the addition of $CdCl_2$ to 270 μM (lane 4), incubation at pH 5.0 (lane 5), or incubation at pH 8.7 (lane 6) or 60 min after the addition of mitomycin C (10 μg/ml) (lane 7), as described in Materials and Methods. The mobilities of molecular mass markers (in kilodaltons) are shown on the left.

also autoradiographed the Western blot shown in Fig. 2D and confirmed that these proteins did exactly comigrate (data not shown).

We tested whether polyclonal or monoclonal antibodies raised against the *E. coli* σ^{32} reacted with any protein present in *A. tumefaciens* lysates. We did not detect a protein in *A. tumefaciens* cell lysates that reacted with these antibodies, although a 32-kDa protein was observed in *E. coli* extracts (data not shown).

Stress induction of the heat shock proteins. The synthesis of the *E. coli* heat shock proteins is induced by a variety of stresses that include ethanol, heavy metals (50), DNA-damaging agents (26), and extreme pH (20, 21, 45). To determine whether the *A. tumefaciens* heat shock proteins are induced by these treatments, we tested strain A348 in a wide range of concentrations of each agent and pulse-labelled it with L-[^{35}S]methionine at time points over a 90-min period. The results of these assays are shown in Fig. 3. The synthesis of the major heat shock proteins was induced 10 min after treatment with ethanol (4% [vol/vol]) or $CdCl_2$ (270 μM) (Fig. 3, lanes 3 and 4). The 20-kDa protein was also induced by these treatments (data not shown). Treatment with mitomycin C for 60 min mildly induced the synthesis of the 75- and 65-kDa proteins but not the 98-kDa protein (Fig. 3, lane 7). This induction by mitomycin C was not observed after treatment for shorter time intervals (data not shown). We tested the effect of a shift in external pH from neutral pH to acidic (as low as pH 5.0) or to alkaline (as high as pH 8.75) conditions. We did not detect the synthesis of the major heat shock proteins after extreme pH shift (Fig. 3, lanes 5 and 6). However, a major protein of 58 kDa was induced by a shift to acidic pH (Fig. 3, lane 5).

Expression of the *E. coli* DnaK protein in *A. tumefaciens*. We noticed earlier that the *A. tumefaciens* and *E. coli* DnaK proteins have slightly different electrophoretic mobilities

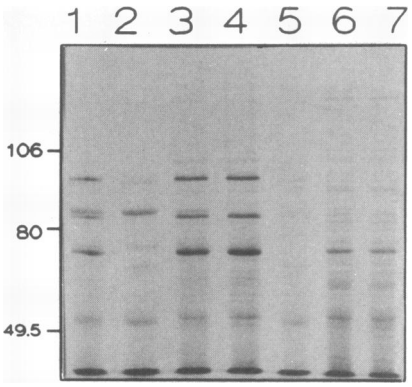

FIG. 4. Expression of the *E. coli dnaK* gene in *A. tumefaciens*. Cultures were pulse-labelled at 30°C (lanes 5 to 7) or 10 min after a shift to 39°C (lanes 2 to 4) as described in Materials and Methods. Equal amounts of *E. coli* and *A. tumefaciens* proteins isolated after heat shock and pulse-labelling were loaded into the same lane (lane 1). Lanes 2 and 5, *E. coli* MC4100(pBB1); lanes 3 and 6, *A. tumefaciens* A348(pNM109); lanes 4 and 7, A348(pSW213). The mobilities of molecular weight markers (in kilodaltons) are shown on the left.

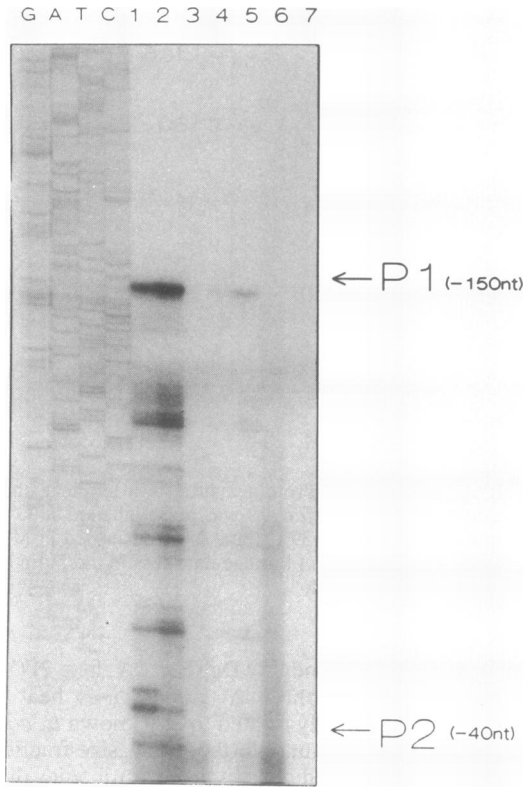

FIG. 5. Transcription of the *E. coli dnaK* gene in *E. coli* and *A. tumefaciens*. Total RNA was isolated from *E. coli* MC4100(pBB1) (lanes 1 and 4), *A. tumefaciens* A348(pNM109) (lanes 2 and 5), or A348(pSW213) (lanes 3 and 6) at 30°C (lanes 4 through 6) or 10 min after a shift to 39°C (lanes 1 through 3) and was used for primer extension analysis as described in Materials and Methods. Lane 7 was a negative control with yeast RNA (Sigma Co.) as template. A sequence ladder of the *dnaK* promoter region was loaded alongside the primer extension products. P1 and P2 indicate the two *dnaK* σ^{32}-dependent promoters. The distances, in nucleotides (nt), of the promoters from the translational start site are indicated in parentheses.

(Fig. 2B), suggesting that they could be resolved when present in the same cell extract. We confirmed this by mixing *A. tumefaciens* and *E. coli* extracts from cells that had been heat shocked and pulse-labelled (Fig. 4, lane 1). The ability to distinguish these proteins allowed us to test whether the *E. coli dnaK* gene could be regulated in *A. tumefaciens*. We therefore cloned the *E. coli dnaK* gene onto a broad-host-range plasmid, creating pNM109, and introduced this plasmid into *A. tumefaciens* A348.

When cultured at 30°C, strain A348(pNM109) produced an 85-kDa protein (Fig. 4, lane 6) which was not synthesized by a strain containing a negative-control plasmid (Fig. 4, lane 7). This protein comigrated with the *E. coli* DnaK protein (Fig. 4, lane 2). After heat shock, this protein was expressed much more strongly than most other *A. tumefaciens* proteins, although not as strongly as the other major heat shock proteins (Fig. 4, lane 3). An *A. tumefaciens* strain containing a negative-control plasmid did not produce this protein after heat shock (Fig. 4, lane 4). This result indicates that *A. tumefaciens* preferentially expressed the *E. coli* DnaK protein after heat shock, although it does not prove whether increased expression was mediated at the level of transcription.

The *dnaK* transcription start site. The *dnaK* gene is transcribed in *E. coli* at two σ^{32}-dependent heat shock promoters, P1 and P2 (10). We used primer extension analysis to determine whether these promoters are preferentially utilized in *A. tumefaciens* after heat shock. A reverse transcript whose 3′ end mapped precisely to the P1 promoter was detected after primer extension analysis of RNA isolated from bacteria grown at 30°C (Fig. 5, lane 5). A number of minor bands, probably due either to degradation of mRNA initiating from P1 or to incomplete reverse transcription of a P1 transcript, were also seen. A band was also seen which corresponded to the P2 promoter (Fig. 5, lane 5). Whether this band is due to transcription initiating at P2 or is due to an artifact is not known. The intensities of most or perhaps all of these bands were dramatically enhanced by using total RNA from bacteria that had been heat shocked (Fig. 5, lane 2), indicating that transcription from promoter P1 and possibly also P2 is dramatically enhanced by this treatment. These data strongly suggest that *A. tumefaciens* has a σ factor which can respond to heat shock by preferentially transcribing an *E. coli* heat shock promoter.

DISCUSSION

We have characterized the *A. tumefaciens* heat shock response and compared it with the *E. coli* response. Our results indicate that the two responses are very similar. We found that four proteins were rapidly and preferentially synthesized after a shift to 39°C (Fig. 1). These proteins were transiently expressed and were the only proteins induced at temperatures above 45°C (data not shown). These kinetics of protein synthesis after temperature shift are typical of the heat shock proteins (11). At least 17 proteins are induced in *E. coli* (34), and we suspect that significantly more than 4 *A. tumefaciens* heat shock proteins could be detected with two-dimensional electrophoresis. It is not surprising that the *A. tumefaciens* heat shock proteins are induced at a slightly lower temperature than those of *E. coli*, since *Agrobacterium* species grow optimally between 25 and 30°C (24).

We tentatively identified the *A. tumefaciens* 75- and

65-kDa heat shock proteins as DnaK and GroEL, respectively. These proteins had molecular weights similar to those of the *E. coli* DnaK and GroEL proteins and were antigenically related to the *E. coli* proteins (Fig. 2). DnaK and GroEL are highly conserved among most or all organisms (4, 30). It was recently hypothesized that DnaK functions as the direct cellular sensor of temperature (11). In addition, DnaK negatively regulates the heat shock response (48). GroEL is required for cell viability at normal temperatures and is thought to function in protein folding and export (29). We did not identify an *E. coli* major heat shock protein which corresponded to the *A. tumefaciens* 20-kDa heat shock protein (Fig. 1). However, *E. coli* has several minor heat shock proteins of unknown function whose molecular masses are approximately 20 kDa (34).

We provided evidence suggesting that the *A. tumefaciens* 98-kDa heat shock protein could be the vegetative σ factor. This protein comigrated with a protein that immunoreacted with monoclonal antiserum directed against conserved region 3 of the *E. coli* σ^{70} (Fig. 2) (43). A 98-kDa protein that copurifies with the *A. tumefaciens* RNA polymerase core enzyme has previously been identified (25). The vegetative σ factor of *P. aeruginosa* is also slightly larger than *E. coli* σ^{70} (2). Both σ^{70} and the *P. aeruginosa* vegetative σ factor are induced by heat shock (2, 47), as is the *A. tumefaciens* 98-kDa protein (Fig. 1 and 2). However, the *E. coli* Lon protein, which is heat shock inducible, has a similar molecular weight (36), and we have not excluded the possibility that the 98-kDa heat shock-inducible protein could be an *A. tumefaciens* Lon homolog.

The synthesis of the *A. tumefaciens* heat shock proteins was induced rapidly by ethanol and cadmium chloride and more slowly by mitomycin C (Fig. 3). The kinetics of heat shock protein synthesis in response to these treatments are similar to those observed with *E. coli* (50). Ethanol most closely mimics the effect of heat in the immediacy and magnitude of its ability to induce the heat shock proteins (34). Cadmium chloride induces over 35 proteins in *E. coli*, including members of the SOS, oxidative stress, and heat shock regulons (50). Treatment of *E. coli* by DNA-damaging agents such as UV and nalidixic acid induces GroEL and DnaK proteins within 20 min (26, 50). The *A. tumefaciens* GroEL and DnaK proteins were stimulated by mitomycin C in a similar way (Fig. 3). Induction of the heat shock proteins in *E. coli* by heat, ethanol, and UV is *rpoH* dependent (26, 34). It was proposed that the heat shock response is induced by the presence of denatured proteins in the cell and that stresses such as high temperature, ethanol, and other toxic compounds function to increase the concentration of denatured proteins (11).

We did not detect the synthesis of the major *A. tumefaciens* heat shock proteins after an extreme pH shift (Fig. 3). In *E. coli*, by contrast, a shift from pH 6.0 to 8.0 is sufficient to induce DnaK and GroEL proteins in an *rpoH*-dependent manner (47). Acidic pH induces a subset of the *E. coli* heat shock proteins which can be detected by two-dimensional electrophoresis (20, 21). Our experiments using one-dimensional electrophoresis do not rule out the possibility that a subset of the *A. tumefaciens* heat shock proteins were induced by acidic pH. In separate studies, we observed that low pH strongly induced a set of proteins, including the 58-kDa protein of Fig. 3, but did not induce the major heat shock proteins (32).

Our results suggest that the *A. tumefaciens* heat shock response is regulated by a σ factor similar to *E. coli* σ^{32}. The *E. coli* DnaK protein was expressed in *A. tumefaciens* at 30°C and induced upon heat shock (Fig. 4). Furthermore, the *E. coli dnaK* gene was transcribed from at least one and possibly both of the *dnaK* heat shock promoters (Fig. 5). However, the *E. coli* DnaK protein was not induced as strongly in *A. tumefaciens* as the native protein (Fig. 4). It is unclear whether this is due to less efficient transcription or translation or to poor stability of mRNA or protein. Overexpression of the *dnaK* gene in *E. coli* causes a reduced heat shock response at high temperatures (48). It is thought to function to enhance the instability of σ^{32} (11, 17). However, overproduction of the *E. coli* DnaK protein in *A. tumefaciens* did not detectably affect induction of heat shock proteins (Fig. 4). It is possible, therefore, that the *E. coli* DnaK protein cannot modulate the *A. tumefaciens* response.

In other studies, we have shown that the *virG* gene is induced by acidic pH from a promoter (P2) whose −10 region resembles that of *E. coli* heat shock promoters, and it was hypothesized that P2 was transcribed by RNA polymerase containing a σ^{32}-like sigma factor (53). This hypothesis is feasible, since *A. tumefaciens* recognizes *E. coli* σ^{32}-dependent promoters (Fig. 5). However, P2 was not efficiently induced by treatments that were found to induce heat shock proteins, indicating that it is not a typical heat shock promoter (32). It is possible, nevertheless, that the σ^{32}-like sigma factor is required for P2 transcription but that additional regulatory proteins are also needed; alternatively, a different σ factor may be utilized.

Our results are significant, since they suggest a similarity between the heat shock regulatory systems in two disparate bacterial genera. *E. coli* is classified in the γ subdivision of the purple bacteria, which includes enterics, vibrios, and the fluorescent pseudomonads (55). *A. tumefaciens* is a member of the α subdivision, which includes, among others, the rhizobacteria and *Caulobacter* species (55). Evidence exists for similar heat shock regulatory systems in at least two other bacterial species within these subdivisions. The *htpG* gene of *V. cholerae* is expressed by a promoter which resembles *E. coli* heat shock promoters and which, when introduced into *E. coli*, is stimulated by overproduction of σ^{32} (35). Second, the heat-inducible promoter of the *C. crescentus dnaK* gene is similar to the *E. coli* heat shock promoters (14). The inability of antibodies directed against *E. coli* σ^{32} to react with any *A. tumefaciens* protein suggests that considerable divergence must have occurred between these proteins (data not shown). The heat shock response in the gram-positive bacterium *B. subtilis* is also similar to the *E. coli* response in that the major heat shock proteins are immunologically related and are induced by stimuli such as ethanol (3). However, the *E. coli* heat shock promoters are nonfunctional in *B. subtilis* (52), suggesting that the heat shock regulatory systems of gram-positive and gram-negative bacteria may be significantly different.

A primary function of the heat shock response may be to protect cells from the toxic effects of elevated temperatures and also from a variety of other stresses (30). Pathogenic microbes encounter a variety of stress stimuli in the course of infection, since plant and animal hosts have evolved many mechanisms to prevent infection (12, 28). Induction of heat shock genes during infection could explain the finding that these proteins are often the major immunodominant antigens in a variety of prokaryotic and eukaryotic pathogens of mammals (56). Moreover, it is reasonable that genes involved in pathogenicity may be regulated by global stress regulons. Two examples in which genes involved in pathogenicity are regulated by the heat shock and SOS responses include the regulation of ToxR, the positive regulatory

protein of the toxin genes in *V. cholerae*, which is modulated by the heat shock response, albeit inversely (35), and a gene encoding a pectin lyase in *Erwinia carotovora*, which is induced by DNA-damaging agents in a *recA*-dependent fashion (58). Similarly, toxin genes of *P. aeruginosa* and *Corynebacterium diphtheriae* are induced by iron starvation (46, 51). Finally, the *virG* gene of *A. tumefaciens* is induced by acid pH and by phosphate starvation (53). These observations suggest that microbial pathogens encounter a variety of stressful environments during infection and that genes involved in adapting to such environments and genes involved in pathogenicity are often coregulated.

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