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Glucose Starvation Stimulon of *Escherichia coli*: Role of Integration Host Factor in Starvation Survival and Growth Phase-Dependent Protein Synthesis

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The DNA-binding protein IHF was found to be required for starvation survival and for the induction of 14 proteins of the glucose starvation stimulon. Many of these proteins have been shown previously to be general responders to diverse stress conditions. Overexpression of IHF during balanced growth was not sufficient to induce these proteins, but it resulted in an increased synthesis of *rpoH*-dependent heat shock proteins.

The integration host factor, IHF, of *Escherichia coli* participates in several cellular processes, including gene expression, site-specific recombination, transposition, phage packaging, and plasmid replication (9, 11). IHF is a heterodimer expressed from the *himA* and *hip* (also called *himD*) genes (18, 19). The heterodimer binds to specific DNA sequences and has been suggested to bend the DNA into a functional conformation (5, 11, 25). The *himA* and *hip* genes are at least partly growth phase regulated in that the transition to stationary phase induces their expression (2) and IHF is accumulated in growth-arrested cells (3, 6). The objective of this study was to determine whether IHF by itself functions as a global regulator of stationary-phase-induced gene expression. Towards answering this question, protein expression levels in cells lacking, overproducing, or exhibiting wild-type levels of IHF were analyzed by the global systems methodology.

Starvation survival and pattern of protein synthesis in an *E. coli himA* mutant. *E. coli himA* (K5302) and wild-type (K37) strains (provided by D. Friedman, University of Michigan) were grown aerobically in glucose minimal morpholinepropanesulfonic acid medium (GMM) with 0.02% glucose at 37°C (20, 23). In GMM with 0.02% glucose, growth is abruptly arrested at an optical density (420 nm) of 0.5 to 0.53 when glucose is exhausted (20). The growth rate (g = 55 min) of the *himA* mutant and that of the isogenic parent were indistinguishable in GMM. However, the *himA* mutant was found to be severely impaired in its ability to survive glucose starvation (Fig. 1). The colony forming capacities of the wild-type and *himA* strains were indistinguishable during the first 8 h of starvation, after which the rate of die-off increased markedly for the *himA* mutant (Fig. 1).

Cells were pulse-labelled with [^{35}S]methionine during exponential growth and at 5, 15, 30, and 60 min after growth ceased because of glucose depletion. Analysis by two-dimensional (2-D) gel electrophoresis, as described previously (23), revealed that 14 glucose starvation proteins (Gsps; Fig. 2, proteins 1 to 14) were not induced in the *himA* mutant during glucose starvation. The absolute levels of expression rather than the timing of induction of these Gsps were affected by the *himA* mutation (as exemplified by proteins 7 and 10 in Fig. 3). Six proteins (Fig. 2, proteins 15 to 20), including fumarase, glycerol-6-phosphate kinase, outer membrane protein C (OmpC), and DnaN, exhibited higher expression levels in the *himA* background, indicating that IHF, directly or indirectly, acts as a negative modulator of the genes encoding these proteins. IHF has previously been shown to inhibit *ompC* transcription both in vivo and in vitro (14). Interestingly, expression of fumarase and glycerol-6-phosphate kinase is increased in cells exposed to both uncouplers of oxidative phosphorylation (10) and carbon starvation, indicating that similar adjustments in the central metabolic pathways take place during these stresses.

Ten of the proteins subjected to control by IHF were identified as belonging to different starvation and stress stimulons (Table 1), and five of the proteins have previously been reported to be regulated by RpoS (21), Lrp (8, 28), OxyR (4, 28), or OmpR (26). Unfortunately, a correlation between the present study and the study of glucose starvation proteins by Matin and coworkers (12, 13, 15) was impossible because of differences in the 2-D protocols.

Overproduction of HimA and Hip is not sufficient to induce the IHF-dependent Gsps. Because IHF is accumulated in stationary-phase *E. coli* cells and several general starvation-inducible proteins require IHF for their induction during entry into stationary phase, the ability of IHF overproduction to induce these proteins during exponential growth was tested. Towards this end, plasmid pHNαβ was transformed into strain K37. Plasmid pHNβα (constructed by A. Granston, National Institutes of Health, from plasmid pHX3-8 [17]) carries the *lacI*^{q} gene and single copies of the *himA* and *hip* genes under control of the *tac* promotor. IHF protein was overexpressed in the exponential growth phase of strain K37/pHNβα growing in GMM containing 50 to 500 μM isopropyl-β-D-thiogalactopyranoside (IPTG), and the cells were pulse-labelled and analyzed by 2-D gel electrophoresis. The lowest IPTG concentration used (50 μM) was estimated by densitometric analysis of nonequilibrium pH electrophoresis gradient (NEPHGE) gels to cause a four- to eightfold increase in HimA (protein I010.5) levels (data not shown). As depicted in Fig. 4, none of the Gsps requiring IHF for induction during entry into stationary phase was induced by overproduction of IHF during the exponential growth phase. Thus, it appears that IHF is required, but not sufficient, for the induction of these Gsps. One protein requiring the presence of *himA* for full induction during entry into stationary phase was identified as universal stress protein A

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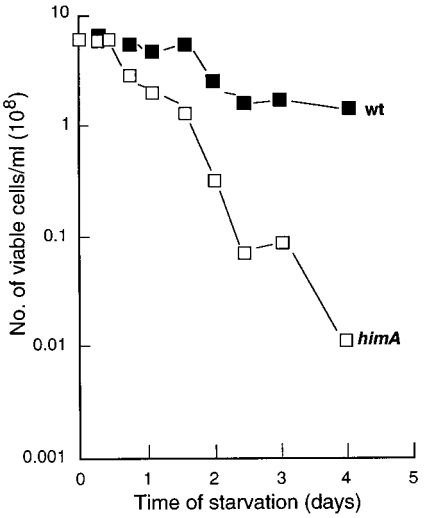

FIG. 1. Starvation survival of the wild-type (K37; wt) and *himA* mutant (K5302) strains during glucose depletion. Strains were grown in GMM with 0.02% glucose. After growth arrest commenced, incubation was continued for 4 days under the same conditions. Viable cells were counted as colonies plated on Luria-Bertani agar after appropriate dilutions.

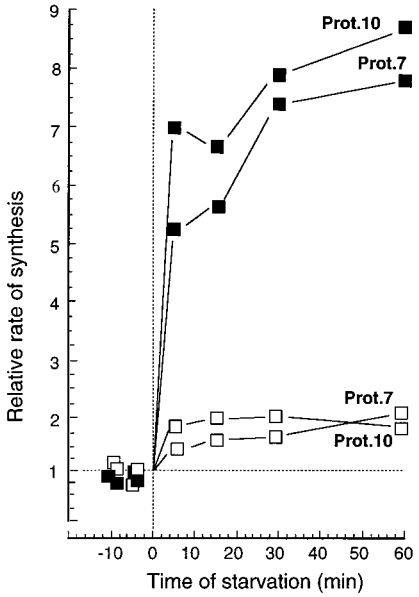

FIG. 3. Relative rates of synthesis of Gsps 7 and 10 in the wild-type strain (open squares) and the *himA* mutant (closed squares) throughout a starvation period of 1 h. The differential rates of synthesis were compared with the rate obtained during exponential growth, which was assigned a value of 1.0.

(UspA; Table 1) (23, 24). A role for IHF in tuning expression levels of *uspA* has been confirmed by using a *uspA-lacZ* fusion (22). However, *uspA* transcription is subjected to growth phase regulation in both *himA* mutants and IHF overproducers (22), indicating that IHF acts only as an auxiliary factor in the control of *uspA* transcription. Similarly, it has been demonstrated that IHF is involved in stationary-phase regulation of the *osmY* (16) and *dps* (1) genes and that additional factors, including Lrp, Cap, and RpoS (*osmY* [16] and *dps* [1]) participate in their regulation. Also, Lrp, RpoS, and OxyR have been reported previously (8, 21, 28) to be involved in the regulation of some Gsps reported here to be IHF dependent (Table 1).

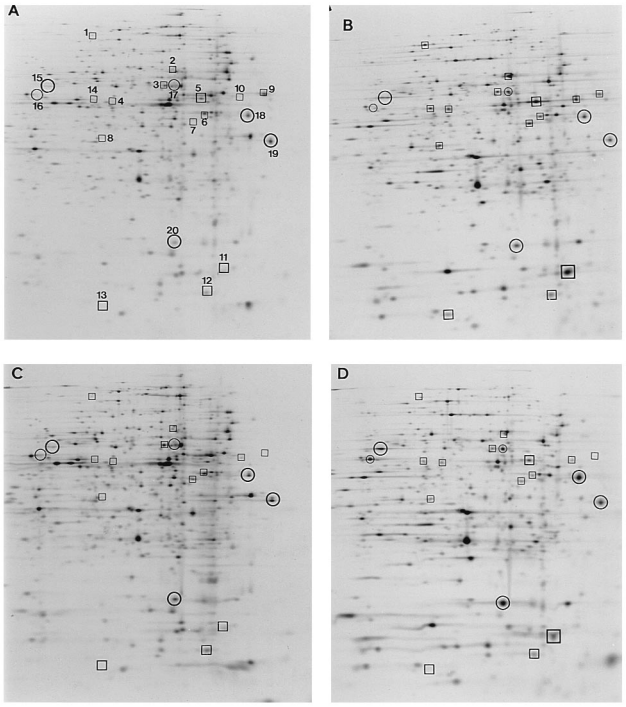

FIG. 2. Autoradiograms of two-dimensional gels obtained with wild-type (A and B) and *himA* (C and D) *E. coli* strains growing exponentially in GMM (A and C) and glucose starved for 15 min (B and D). Circled and boxed spots denote proteins that were found to exhibit higher and lower levels, respectively, of expression in the *himA* background.

Overproduction of HimA and Hip induces the *rpoH*-dependent heat shock response. While IHF overproduction was not sufficient to induce IHF-dependent Gsps in the exponential growth phase, it did significantly induce a group of proteins whose expression is dependent on the heat shock regulator σ^{32} (Fig. 4) (28). All the heat shock proteins (Hsps) resolved by 2-D analysis, except proteins C014.7 (HtpE), G013.5 (HtpN), D033.4 (HtpH), and D060.5 (LysU), were found to be markedly induced by IHF overproduction. Interestingly, all but one (D033.4) of the Hsps that were not induced by overexpression of IHF have been shown to require additional factors besides σ^{32} and are not induced by overexpression of σ^{32} in the normal temperature range (27). It may be argued that overproduction of IHF, or any other protein, could titrate out the negative modulators (DnaJ, DnaK, and GrpE) of the heat shock response if a fraction of the overproduced protein failed to fold properly. To test whether such overproduction artifacts could explain the observed induction of Hsps, *hip* alone (Fig. 5), *lacZ* (plasmid pAF604 constructed by A. Farewell, University of Göteborg), or *uspA* was overproduced using the same P_{tac} expression system. In no case did the overproduction of these proteins cause an induction of Hsps. In fact, overproduction of LacZ and UspA to 10% of the total protein content in the cells did not result in Hsp induction. However, overproduction of LacZ to 30% of the total protein content has been reported to induce DnaK and GroEL 2- and 1.3-fold, respectively (7). Also, induction of Hsps does not appear to be a simple result of a reduction in growth rate caused by protein overproduction, since expression of Hip, UspA, or LacZ alone reduced the growth rate to the same extent as HimA-Hip overproduction did and yet did not affect Hsp production. Thus, it is

TABLE 1. List of proteins with altered levels of production in a *himA* mutant compared with the isogenic parent during glucose starvation

Protein no.[a]	Alphanumeric designation[b]	Gene name	Expression in wt[c]		Regulator(s)[d]	Other stimulus or stimuli producing induction[e]
			Log phase	Stationary phase		
1	G080.1		±	+++		Ile, DNP, Cd, QN, HP, 42, 50
2			±	+++		
3	F049.0		+	++		PS, Ile
4			−	+++		
5			±	+++		PS, NS
6	D035.8		+	++		NS
7	D038.3		−	+++	RpoS	PS
8			−	+++		
9			+	++		
10			−	+++		
11	B015.5		±	+++	OxyR	Cd, QN, HP,
12	C013.5	*uspA*	+	++		PS, NS, Ile, Cd, QN, HP, DNP, 42
13			−	++		
14			−	+++		
15	H049.2		±	++		NS
16	H080.4	*fumC*	±	+		DNP
17	E048.7	*glpK*	±	++	RpoS	DNP, 42
18	A036.1	*dnaN*	±	±		
19	A035.5	*ompC*	±	±	OmpR, Lrp	
20	E017.6		±	++	Lrp, RpoS	

[a] Protein numbers are those of Fig. 2.
[b] Designations are those of VanBogelen et al. (28).
[c] wt, wild type; −, not detectable; ±, low-level but detectable expression; +, weak induction; ++, medium induction; +++, strong induction during glucose starvation of wild-type *E. coli* K37.
[d] Regulators that have been shown previously to be involved in the regulation of the corresponding proteins (21, 28). Lrp, leucine-responsive protein; OxyR, oxidation stress regulator; RpoS, RNA polymerase sigma factor for stationary phase; OmpR, outer membrane protein regulator.
[e] NS, nitrogen starvation; PS, phosphate starvation; Ile, isoleucine starvation; DNP, dinitrophenol exposure; Cd, cadmium chloride exposure; HP, hydrogen peroxide exposure; QN, quinone 6-amino-7-chloro-5,8-dioxoquinoline exposure; 42, temperature shift from 28 to 42°C; 50, temperature shift from 28 to 50°C.

possible that the induction of Hsps caused by overexpression of *himA* and *hip* is a direct effect of normal IHF functions rather than the result of overproduction artifacts or growth rate regulation.

Several Hsps have been demonstrated to be general responders to stresses causing growth arrest (15, 28), but the regulatory mechanisms involved have not been elucidated. It is possible that IHF plays a role in Hsp induction under one or more such conditions. However, it should be noted that IHF is not required for Hsp induction during heat stress, since the *himA* mutant K5302 exhibited a normal heat shock response during a temperature upshift from 28 to 42°C (data not shown). Because there are no known IHF-binding sequences proximal to the *hsp* promoters, the effect of IHF overproduction on Hsp induction most likely results from increased levels of σ^{32}. Increased levels of functional IHF may affect the half-life of σ^{32}, the efficiency of translation of the *rpoH* transcript, or the activity of one or several of the *rpoH* promoters. It is noteworthy that the results for overproduction of σ^{32} (27) and those for overproduction of IHF (this study) are strikingly similar.

In summary, the patterns of protein production in a wild-

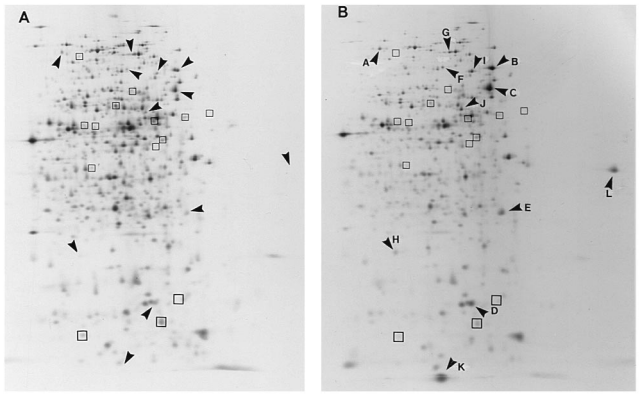

FIG. 4. Autoradiograms of two-dimensional gels obtained with *E. coli* K37/pNHαβ growing without (A) and with (B) 100 μM IPTG. Boxed spots denote Gsps 1 to 14 (Fig. 2), which were found to require the presence of the *himA* gene for full induction during glucose starvation. Arrows with letters indicate proteins that were significantly induced by IHF overproduction. Increasing the IPTG concentration to 500 μM did not alter the pattern of protein synthesis.

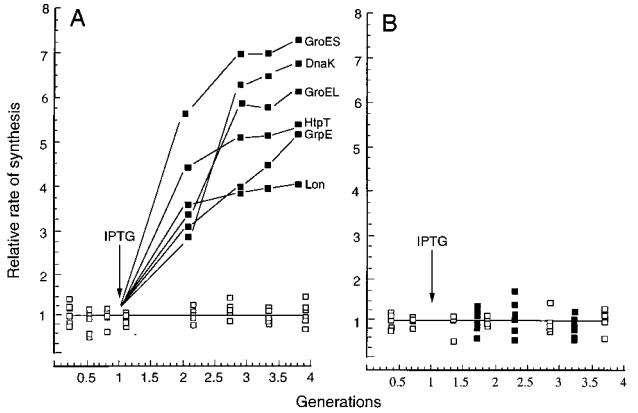

FIG. 5. Relative rates of Hsp synthesis during exponential growth after IPTG induction (100 μM) of *himA* and *hip* (A) and *hip* alone (B). The *hip*-overproducing plasmid was made by digesting pHNβα with *Hin*dIII to completion (17). The differential rates of synthesis were compared with the rate obtained for cells growing without IPTG, which was assigned a value of 1.0.

TABLE 2. List of proteins with increased levels of production during IPTG induction of *himA* and *hip*

Protein designation[a]	Alphanumeric designation[b]	Gene name	Protein name	Other stimulus or stimuli producing induction[c]
A	H094.0	*lon*	Lon or La	42, 50, DNP, PS, NA
B	B066.0	*dnaK*	DnaK	42, 50, DNP, CS, PS, NS, NA, Ile, Cd, QN, HP
C	B056.5	*mopA*	GroEL	42, 50, DNP, CS, PS, NA
D	C015.4	*mopB*	GroES	42, 50, DNP, CS, PS, NA, Cd
E	B025.3	*grpE*	GrpE	42, 50, DNP, CS, PS, NA, Cd, Ile
F	G072.0	*clpB*	ClpB	42, 50, CS, PS, NA, Cd
G	F084.1	*clpB*	ClpB	42, 50, CS, PS, NA, Cd
H	G021.0	*htpO*	HtpO	42, CS, DNP, PS, NA
I	C062.5	*htpG*	HtpG	42, 50, NA, Cd
J	D048.5	*htpI*	HTpI	42, 50, DNP
K	F010.1	*htpK*	HtpK	42, 50, PS, NS, Cd
L	A029.5	*htpT*	HtpT	42

[a] Protein designations are those of Fig. 4.
[b] Alphanumeric designations are those of VanBogelen et al. (28).
[c] CS, carbon starvation; NA, nalidixic acid exposure. Consult Table 1 for definitions of other abbreviations.

type strain and an isogenic *himA* mutant were very similar when cells were growing in exponential phase. In contrast, significant abnormalities in protein expression were observed in the *himA* mutant after it entered stationary phase. Most of the proteins whose expression levels were altered because of the *himA* mutation were not induced in the normal wild-type fashion during stasis, indicating that IHF may act as a positive modulator of expression for these proteins. However, in no case was IHF overproduction sufficient to induce these proteins in the exponential growth phase. In contrast, overexpression of the two subunits of IHF concomitantly resulted in a surprising induction of nearly all *rpoH*-dependent heat shock proteins. Finally, while the *himA* mutation had little or no effect on the growth rate of cells, it dramatically reduced the ability of cells to withstand growth arrest caused by carbon starvation.

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