Genome-wide comparison of phage M13-infected vs. uninfected *Escherichia coli*

Fredrik Karlsson, Ann-Christin Malmborg-Hager, Ann-Sofie Albrekt, and Carl A.K Borrebaeck

Abstract: To identify *Escherichia coli* genes potentially regulated by filamentous phage infection, we used oligonucleotide microarrays. Genome-wide comparison of phage M13-infected and uninfected *E. coli*, 2 and 20 min after infection, was performed. The analysis revealed altered transcription levels of 12 *E. coli* genes in response to phage infection, and the observed regulation of phage genes correlated with the known in vivo pattern of M13 mRNA species. Ten of the 12 host genes affected could be grouped into 3 different categories based on cellular function, suggesting a coordinated response. The significantly upregulated genes encode proteins involved in reactions of the energy-generating phosphotransferase system and transcription processing, which could be related to phage transcription. No genes belonging to any known *E. coli* stress response pathways were scored as upregulated. Furthermore, phage infection led to significant downregulation of transcripts of the bacterial genes *gadA*, *gadB*, *hdeA*, *gadE*, *slp*, and *crl*. These downregulated genes are normally part of the host stress response mechanisms that protect the bacterium during conditions of acid stress and stationary phase transition. The phage-infected cells demonstrated impaired function of the oxidative and the glutamate-dependent acid resistance systems. Thus, global transcriptional analysis and functional analysis revealed previously unknown host responses to filamentous phage infection.

Key words: filamentous phage infection, global transcriptional analysis, AR, *Escherichia coli*.

Résumé : Afin d'identifier les gènes de *Escherichia coli* (*E. coli*) potentiellement régulés par l'infection d'un phage filamenteux, nous avons employé des microréseaux à oligonucléotides. Nous avons donc effectué une comparaison basée sur tout le génome de *E. coli* infecté ou non avec le phage M13, 2 et 20 minutes post-infection. Les résultats des analyses ont révélé des changements dans les niveaux de transcription de 12 gènes de *E. coli* en réponse à l'infection par le phage; la régulation observée des gènes de phage a corrélé avec les profils in vivo connus des ARNm de M13. Dix des douze gènes de l'hôte influencés ont pu être regroupés en 3 catégories fonctionnelles, indiquant une réponse coordonnée. Les gènes significativement modulés à la hausse codent des protéines impliquées dans les réactions du système de phosphotransférases (SPT) générateur d'énergie ainsi que dans la transcription reliée au phage. Nous n'avons noté aucun gène modulé à la hausse appartenant à des voies de stress connues chez *E. coli*. De plus, l'infection par le phage a mené à une régulation à la baisse significative des transcrits des gènes bactériens *gadA*, *gadB*, *hdeA*, *gadE*, *slp* et *crl*. Ces gènes régulés à la baisse fort normalement partie des mécanismes de réponse aux stress de l'hôte qui protège la bactérie dans des conditions de stress acide et de transition en phase stationnaire. Les cellules infectées par des phage démontraient des déficiences dans le fonctionnement de leurs systèmes de résistance au stress oxydatif et au stress acide dépendant du glutamate. Ainsi, l'analyse transcriptionnelle globale et l'analyse fonctionnelle ont mis en évidence des réponses à l'infection par un phage filamenteux auparavant inconnues.

Mots clés : infection par un phage filamenteux, analyse transcriptionnelle globale, résistance à l'acide (RA), *Escherichia coli*.

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Introduction

The bacterium *Escherichia coli* can become infected by a number of different bacteriophages, i.e., viruses that infect only bacteria. Most bacteriophages that have been studied are temperate and have the ability to remain essentially quiescent with their viral DNA integrated in the host chromosome, whereas others are virulent, causing the host cell to burst (lyse) at the terminal release of new phage particles. However, some phages are released without lysis of their host cell. The filamentous phages (e.g., M13, fd, or f1) do not kill their *E. coli* hosts but establish a symbiotic relationship in which new virions are continually released by a secretory mechanism. The biological features of the

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filamentous bacteriophage have essentially been covered, including its infection mechanism, genome organization, and DNA replication and assembly process, leading to the secretion of progeny phage (Model and Russel 1988; Rasched and Oberer 1986; Webster 1996). It is also known that in the phage life cycle different host proteins are engaged, as exemplified by the bacterial TolQRA proteins involved in the infection process (Click and Webster 1997, 1998), the bacterial stress protein PspA induced by the phage gene IV protein during assembly and release of new phage particles (Brissette et al. 1990; Weiner and Model 1994), and the host endonuclease RNase E involved in processing phage mRNA (Goodrich and Steege 1999; Kokoska and Steege 1998; Kokoska et al. 1990). However, given the available new technologies, such as microarrays, it is now possible to explore the entire transcription profile of infected bacteria to look for transcriptional changes that might reveal other host proteins affected by the presence of filamentous phage.

Therefore, we studied host cell responses to phage infection by comparing the transcription profiles of bacteria infected with M13 bacteriophage for 2 or 20 min with those of uninfected bacteria, using oligonucleotide microarrays. Thus, for each of the 2 time points there was an uninfected control. We also compared cells infected for 20 min to cells infected for 2 min. The time points were carefully chosen to detect transcriptional changes that arise early after infection. The phage infection process and the following reproduction phase are rapid events. Phage transcripts are detected after 2 min (Blumer and Steege 1984; Steege 2000), and the major phage mRNAs are generated sequentially over a 10-min period after infection (Blumer and Steege 1984). The first release of phage progeny occurs 15–20 min after infection (Blumer and Steege 1984; Hofschneider and Preuss 1963). Thus, at this early phase after infection one can expect the level of adaptation within the infected cells to be the greatest.

Methods

We used Top10F′ *E. coli* (F′{*lac*Iq Tn*10* (TetR)} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *deo*R *rec*A1 *ara*D139 Δ(*ara-leu*) 7697 *gal*U *gal*K *rps*L *end*A1 *nup*G) (Invitrogen Corp. Carlsbad, Calif.) as host strain for the phage infection. Cells were grown in 2× yeast–tryptone (YT) medium (Sambrook et al. 1989) containing 10 μg tetracycline·mL^{-1} to log-phase (OD_{600} = 0.6). The growing culture was split in halves, of which 1 was subjected to phage infection and the other, not receiving any phage, served as a control. VCSM13 phage (Stratagene, La Jolla, Calif.) was used to infect the pili-expressing bacteria (multiplicity of infection ratio 100:1). Infection was allowed to proceed for 2 and 20 min before isolation of RNA. Phage infection was monitored both by titration of the cells resistant to kanamycin, the marker carried by the VCSM13 phage, on YT-agar plates, and by the protocol of Rapoza et al. (Rapoza and Webster 1995) with slight modifications. Briefly, after 2 (or 20) min the infection was stopped by rapid chilling to 0 °C. The infected culture was then filtered through an ice-cold Millipore type HA (0.45 μm) filter to remove free phage. The cells were washed with 2 volumes of cold media and then removed from the filter in 1 volume of cold media. Cells were diluted and plated onto YT-agar. The resulting colonies were picked onto lawns of Top10F′ to determine the rates of infection.

The Affymetrix (Affymetrix Inc., Santa Clara, Calif.) sense array GeneChip® contains 7312 probe sets complementary to 4285 protein coding genes plus 116 RNAs of the *E. coli* genome. The M13 bacteriophage genes gI, gII 5′, gII 3′, gIII, gIV, gV, gVI, gVII, gVIII, and gIX are also represented on the chips. A probe set is made up of 20 different probe pairs of a perfect match (PM) sequence and a mismatch (MM) sequence. The PM is complementary to the reference sequence, while the MM contains a single, homomeric base change at position 13 of the 25-mer oligonucleotide (Selinger et al. 2000).

Enrichment, fragmentation, and labelling of sample mRNA and subsequent microarray analysis were performed according to the Affymetrix Expression Analysis Technical Manual (available at: www.affymetrix.com). Of note is that mRNA was prepared from samples and controls simultaneously using the same protocol throughout, allowing us to perform the subsequent comparative analysis without introducing asymmetric experimental errors. Fragmented and labelled mRNA (3.6 μg) was hybridized for 16 h at 45 °C to the *E. coli* GeneChip® arrays according to the Affymetrix Technical manual. The hybridized chips were washed and stained in the GeneChip® fluidics station (Affymetrix Inc.). The probe arrays were scanned with the Gene Array Scanner and analyzed.

For statistical analysis of the genechip results, we prepared mRNA samples from infected and uninfected cells on 2 separate occasions, thus obtaining 2 biological replicates for each time point (2 and 20 min) and experimental condition (±phage infection). We used MAS 5.0 New Statistical Algorithm from Affymetrix to generate lists of genes with significantly altered transcription. Each chip file was subjected to comparison analysis, i.e., 2 samples are compared with each other in order to detect and quantify changes in gene expression. One array is designated as the baseline and the other as the experiment. The analysis compares the difference values (between PM and MM) of each probe pair in the baseline array to its matching probe pair on the experimental array. A change algorithm generates a Change p value and an associated Change call, using statistically relevant tests (www.affymetrix.com). The p values range in scale from 0.0 to 1.0 and provide a measure of the likelihood of change and direction. Values close to 0.0 indicate an increase compared with the baseline, whereas values close to 1.0 indicate a decrease in transcript expression level. In this study, we set the average Change p value threshold for increase to $p \leq 0.005$ and the threshold for decrease to $p \geq 0.995$.

We first compared transcription 2 and 20 min after phage infection with corresponding uninfected controls. Then we monitored transcriptional changes within infected cells over time by comparing transcription in infected cells 2 and 20 min after infection. The control comparison between uninfected samples from the 2 time points showed that there was no significant change in gene transcription in the uninfected bacteria over the 20-min period. The duplicate sam-

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Table 1. Genes induced by phage M13 infection of *E. coli*, as determined by their Change *p*-values.

Gene*	b no.	Description*	Signal $\log_2$ ratio†	Change *p*-value‡
Infected (2 min) vs. noninfected (2 min)				
M13 gV		ssDNA binding protein Phage DNA replication	1.5	0.0009±0.0016
M13 gVIII		Structural protein of virion	3.0	0±0
M13 gIX		Structural protein of virion	1.2	0±0.0001
Infected (20 min) vs. noninfected (20 min)				
M13 gI		Protein required for phage assembly	2.7	0.0012±0.0020
M13 gII 5′		Protein required for viral replication	3.5	0±0
M13 gII 3′		Protein required for viral replication	2.2	0±0
M13 gIII		Structural protein of virion	4.1	0±0
M13 gIV		Protein required for phage assembly	2.8	0±0.0001
M13 gV		ssDNA binding protein Phage DNA replication	6.4	0±0
M13 gVII		Protein required for phage assembly	8.7	0±0
M13 gVIII		Structural protein of virion	6.8	0±0
M13 gIX		Structural protein of virion	6.0	0±0
dhaK	b1200	Putative dihydroxyacetone kinase (EC 2.7.1.2)	2.5	0.0014±0.0016
nagE	b0679	PTS system, N-acetylglucosamine specific enzyme, nagE operon	0.8	0.0020±0.0039§
Infected (20 min) vs. infected (2 min)				
M13 gI		Protein required for phage assembly	1.5	0.0006±0.0004
M13 gII 5′		Protein required for viral replication	2.1	0±0
M13 gII 3′		Protein required for viral replication	1.5	0.0001±0.0002
M13 gV		ssDNA binding protein Phage DNA replication	2.7	0±0
M13 gVII		Protein required for phage assembly	3.6	0±0
M13 gVIII		Structural protein of virion	2.6	0±0
M13 gIX		Structural protein of virion	2.7	0±0
b2431	b2431	Orf, hypothetical protein	0.6	0.0028±0.0047§
kefA	b0465	Mechanosensitive channel protein MscK	0.6	0.0017±0.0017
rraA	b3929	Protein inhibitor of RNase E activity	0.6	0.0017±0.0025§
rof	b0189	Anti-Rho termination factor	1.0	0.0010±0.0012
Control, uninfected (20 min) vs. noninfected (2 min)				
No genes passed the Change *p*-value threshold criteria				

*Names and descriptions were taken from http://bmb.med.miami.edu/EcoGene/EcoWeb/ and Affymetrix Expression Analysis Sequence Information Database.

†Log_2 ratios (mean) of transcript levels from each of the condition comparisons (bold subheadings), in the order sample vs. baseline.

‡Data are means ± SD. The average Change *p*-value threshold for increase was set to $p \leq 0.005$. e.g., for "infected (2 min) vs. non-infected (2 min)", replicates #1 and #2 were each compared with replicates #1 and #2 of noninfected bacteria, thus giving 4 Change call *p*-values per gene, from which the mean could be calculated. Only genes having a mean Change *p*-value ≤ 0.005, and an increase call in at least 3 of the comparisons, are shown.

§Only 3 out of 4 comparisons resulted in an increase call, but all 4 Change *p*-values were included in the calculation of the mean and SD.

ples (biological replicates) for each time point and condition gave 4 comparison files for each of the 4 situations under study (Tables 1 and 2).

Results

Two minutes after infection an average of 58% of the cells from which the mRNA had been isolated were infected. Nevertheless, increase in transcription of 3 M13 genes, gV, gVIII, and gIX, was detected at this stage (Table 1). All 3 upregulated phage genes are part of the primary phage transcripts known to be present in vivo (Blumer and Steege 1984; Goodrich and Steege 1999; Model and Russel 1988). Transcripts from gene VII and II, which are also part of the primary phage transcripts A and B (Cashman et al. 1980; Model and Russel 1988), had Change *p* values (p = 0.007 and 0.017, respectively) somewhat above the cutoff set in this study (p = 0.005). Thus, the genechip technology is able to accurately detect transcriptional changes occurring in a fraction of the sampled cells, although no bacterial transcripts were significantly upregulated 2 min after infection.

At 20 min after infection an average of 97% of the cells were infected. All phage transcripts (except gVI) were significantly upregulated compared with the uninfected control (Table 1). Gene VI protein is responsible for the termination of phage assembly (Rakonjac et al. 1999), which might indicate that the reproduction phase had not reached completion in enough cells at this time point. Furthermore, many of the phage transcripts were abundant, as can be seen from the level of expression (Table 1, Signal Intensity). Interestingly, only 2 *E. coli* genes (*nagE* and *dhaK*) demonstrated a significantly increased level of transcription, as compared with the uninfected control at 20 min (Table 1). The *nagE* gene encodes the *N*-acetylglucosamine-specific phosphotransferase system transporter (Plumbridge 2001). This major active transport system depends on phosphoenolpyruvate (PEP) (Plumbridge 2002), the principle high-energy phosphate of many bacteria. The other upregulated gene (*dhaK*) encodes a dihydroxyacetone (Dha) kinase involved in phospho-

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Table 2. Genes suppressed by phage M13 infection of *E. coli*, as determined by their Change *p*-values.

Gene*	b no.	Description*	Signal $\log_2$ ratio[†]	Change *p*-value[‡]
Infected (2 min) vs. non-infected (2 min)				
No genes passed the Change *p*-value threshold criteria				
Infected (20 min) vs. non-infected (20 min)				
gadA	b3517	Glutamate decarboxylase A	–2.8	0.9990±0.0021
gadB	b1493	Glutamate decarboxylase B	–3.0	1.0±0
hdeA	b3510	Periplasmic chaperone of acid-denatured proteins	–1.5	1.0±0
gadE	b3512	Required for stationary phase-, pH- and EvgA-induced acid resistance	–1.5	0.9991±0.0013
Infected (20 min) vs. infected (2 min)				
crl	b0240	Transcriptional regulator of cryptic csgA gene for curli surface fibers	–1.0	0.9968±0.0064[§]
slp	b3506	Outer membrane protein, starvation and stationary phase inducible	–1.2	0.9997±0.0004
Control, uninfected (20 min) vs. uninfected (2 min)				
No genes passed the Change *p*-value threshold criteria				

*Names and descriptions were taken from http://bmb.med.miami.edu/EcoGene/EcoWeb/ and Affymetrix Expression Analysis Sequence Information Database.

[†]Log_2 ratios (mean) of transcript levels from each of the condition comparisons (bold subheadings), in the order sample vs. baseline. A negative value denotes a decrease in transcription, compared to the baseline file.

[‡]Data are means ± SD. The average Change *p*-value threshold for Decrease was set to $p \geq 0.995$. e.g., for “infected (2 min) vs. non-infected (2 min)”, the replicates #1 and #2 were each compared to the replicates #1 and #2 of non-infected bacteria, thus giving 4 Change call *p*-values per gene from which the mean could be calculated. Only genes having a mean Change *p*-value ≥ 0.995, and a Decrease call in at least 3 of the comparisons, are shown.

[§]Only 3 out of 4 comparisons resulted in a Decrease call, but all 4 Change *p*-values were included in the calculation of the mean and SD.

transferase system reactions, in which Dha is phosphorylated at the expense of PEP (Gutknecht et al. 2001). Thus, phage infection upregulated 2 genes that, during the initial steps of cellular energy generation, are involved in carbohydrate-dependent phosphotransfer of the high-energy carrier molecule PEP.

Over time, the infected bacteria were found to upregulate *rraA*, *rof, kefA*, and *b2431* transcripts (Table 1). The gene *kefA* encodes MscK, a protein forming a mechanosensitive channel (McLaggan et al. 2002), and *b2431* seems to be an *E. coli*-specific open reading frame of unknown function. However, it was recently proven that *rraA* encodes a polypeptide that binds to the essential *E. coli* endonuclease RNase E, inhibiting its RNA processing activity (Lee et al. 2003). Overexpression of *rraA* stabilizes numerous transcripts (Lee et al. 2003), and it has been shown that filamentous phage primary transcripts are subject to both RNase E-dependent and RNase E-independent cleavage (Goodrich and Steege 1999; Kokoska and Steege 1998; Kokoska et al. 1990). The products of *rof* bind to and inhibit the transcription termination factor Rho, giving Rof antiterminator activity (Pichoff et al. 1998). The phage genome contains 2 Rho-dependent terminators, but phage transcription of the most frequently transcribed region is followed by Rho-independent termination at a common site (Model and Russel 1988).

Nevertheless, both RraA and Rof are involved in transcription processing and affect the function of proteins related to phage transcription, and may thus play important roles in processing phage transcripts. Furthermore, as RraA inhibits the major *E. coli* endonuclease RNase E, known to process transcripts prominently encoding proteins involved in energy-generating pathways (Bernstein et al. 2002; Lee et al. 2002), it may provide a possible regulatory link to the upregulated *nagE* and *dhaK* genes. RNase E is intimately coupled to RNA processing of filamentous phage mRNA (Kokoska and Steege 1998; Kokoska et al. 1990; Stump and Steege 1996), and inactivation of its function leads to differential stabilization of primary phage transcripts and 10-fold lower phage titres (Kokoska and Steege 1998). Therefore, the RNase E-inhibiting capacity of RraA can represent a potential host defence mechanism. Another possibility is that the upregulation of *rraA* can account for the different steady-state levels of mRNAs and proteins observed for filamentous phages, by stabilizing the primary phage transcript RNA A.

The reason why the phage shock protein A (*pspA*) gene was not induced by the phage infection is at present not clear, but our results were obtained using cells in mid log-phase in contrast to the late log-phase cells used by Brissette et al. (1990). Moreover, since Model et al. (1997) have shown that it is the insertion of pIV into the membrane during phage assembly that triggers *pspA* induction, it appears this stage was not reached in enough cells to allow detection of the host response. Neither the control samples nor the infected samples analyzed after 2 min generated any bacterial genes with altered transcription levels. However, this does not imply that the transcription level of an individual gene is identical in the 2-min infected sample and the 20-min uninfected control.

The comparative analysis also detected downregulation in the transcription of bacterial genes in response to phage infection, albeit not at 2 min after infection (Table 2). After 20 min of infection, the transcripts of 4 bacterial genes (*gadA, gadB, hdeA*, and *gadE*) were detected as significantly downregulated compared with uninfected cells (Table 2). The function of all these downregulated genes is related to acid resistance (AR) (De Biase et al. 1996; Gajiwala and Burley 2000; Hartl and Martin 1995; Hersh et al. 1996; Hommais et al. 2004), demonstrating that phage infection downregulated genes with closely related functions.

Furthermore, in infected cells, we identified transcripts downregulated over time. This procedure revealed 2 bacterial genes (*slp* and *crl*) having downregulated transcription (Table 2). Both *slp* and *crl* have been implicated in the genes involved in the stress response induced by transition into sta-

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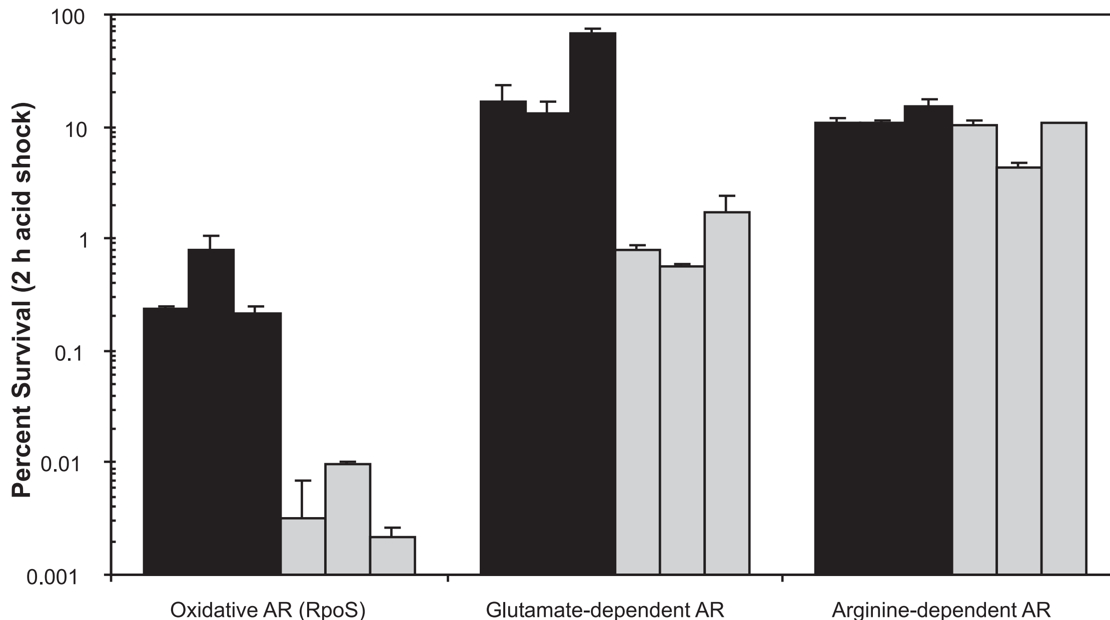
Fig. 1. Three independent experiments showing the cell survival of uninfected (black) and phage-infected (grey) *E. coli* Top10F′ cells, following 2 h of challenge at acidic pH. The test for the oxidative, RpoS-dependent, acid resistance (AR) system involved overnight growth in Luria–Bertani (LB)–morpholineethanesulfonic acid (pH 5.5), followed by dilution to 5×10^5 to 1×10^6 CFU·mL^{-1} in E medium with glucose (EG) (pH 3.0). The glutamate and arginine systems required overnight growth in LB–glucose (glutamate system) or brain heart infusion broth (arginine system) cultures, which were diluted to 5×10^5 to 1×10^6 CFU·mL^{-1} in EG (pH 2.5) containing 1.5 mmol·L^{-1} glutamate or EG (pH 3.0) containing 0.6 mmol·L^{-1} arginine, respectively. Percent survival is calculated as the number of CFU·mL^{-1} remaining after the acid treatment, divided by the initial CFU·mL^{-1} at time zero. Error bars represent SD of the mean.



tionary phase (Alexander and St. John 1994; Pratt and Silhavy 1998; Tao et al. 1999). In addition, the gene *slp* is upregulated during acid stress (Tucker et al. 2002), and Crl has been shown to stimulate the activity of a general stress response regulator involved in AR (Pratt and Silhavy 1998).

Thus, all of the genes found to be downregulated in response to phage infection have been implicated in acid stress responses. The interpretation of this is not clear at present, but we still decided to confirm the downregulation of these AR genes at the protein level. We therefore tested whether phage-infected cells were less tolerant to conditions of low pH, by way of AR assays. Detailed studies of AR mechanisms in *E. coli* have exposed 3 systems that can protect cells against pH as low as 2.0 (Hersh et al. 1996; Lin et al. 1995, 1996). The first of these AR systems is termed the RpoS-dependent oxidative system and involves the alternative sigma factor RpoS, an RNA polymerase subunit expressed by the *rpoS* gene (Lin et al. 1995). Expression of the RpoS-dependent AR system is induced by Luria–Bertani (LB) medium and repressed by glucose (Castanie-Cornet et al. 1999). The glucose repression of the oxidative AR system is necessary for testing of the second, glutamate-dependent AR system, which requires growth in LB–glucose. The third, arginine-dependent, AR system requires growth in brain heart infusion broth before the pH-shock treatment (Castanie-Cornet et al. 1999). Furthermore, the last 2 AR systems require the addition of glutamate or arginine, respectively, during acid challenge to survive the low pH (Castanie-Cornet et al. 1999; Hersh et al. 1996; Lin et al. 1995).

Phage infection negatively affected 2 *E. coli* AR systems at pH ≤ 3 (Fig. 1). The RpoS-dependent AR system exposed a minimum 75-fold difference in acid survival between infected and uninfected cells, and the glutamate-dependent AR system exposed an average 25-fold difference in acid survival. In comparison, deletion of *gadE*, a gene imperative in acid survival, has been shown to result in a 25-fold decrease in survival relative to that of wild type cells (Tucker et al. 2002). Thus, acid shock survival was considerably reduced by phage infection. The level of survival for glutamate-dependent AR, reported in Fig. 1, is comparable to that of previous reports (Castanie-Cornet et al. 1999; Lin et al. 1995). None of the acid-sensitive control samples survived the AR assays (data not shown), demonstrating that the levels of response to acid challenge were dependent on the respective AR system. Furthermore, the difference in survival between infected and uninfected cells is pH-dependent, in that challenge at pH ≥ 3.5 did not produce any difference in AR between infected and uninfected cells (data not shown). The arginine-dependent AR, for which we did not detect any transcriptional changes, was included as a control, showing no difference in the response to acid challenge for infected and uninfected cells.

The increased acid sensitivity of phage-infected cells presented here is likely to depend on the downregulation of AR genes. The AR assays used in this study only affect a group of proteins involved in these specific pathways of *E. coli* AR. The protein products of all the genes we found downregulated by phage infection belong to this group. However, a recent study, in which *E. coli* cell division was

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inhibited, also reported the downregulation of the same AR-related genes (Arends and Weiss 2004). The authors made the interpretation that these genes are exquisitely sensitive to changes in cell physiology. Consequently, the link between phage infection and acid sensitivity may be indirect, illustrating that further investigations are needed.

In summary, this genome-wide analysis of the dynamic interaction of 2 genomes at the early phase of phage infection demonstrates that host genes associated with transcription processing and phosphotransferase reactions are upregulated and that those associated with AR are downregulated. Furthermore, the host response to phage infection was limited, lacking immediate upregulation of known stress genes.

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