Phosphorylation of *Escherichia coli* Translation Initiation Factors by the Bacteriophage T7 Protein Kinase†

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ABSTRACT: The lytic coliphage T7 encodes a serine/threonine-specific protein kinase which supports viral reproduction under suboptimal growth conditions. Expression of the protein kinase in T7-infected *Escherichia coli* cells results in the phosphorylation of 30S ribosomal subunit protein S1, and initiation factors IF1, IF2, and IF3, as determined by high-resolution two-dimensional gel electrophoresis and specific immunoprecipitation analysis. Phosphorylation occurs either exclusively on threonine (IF1, IF3, S1) or on serine and threonine (IF2). There is no phosphorylation of these proteins in uninfected cells or in cells infected with T7 lacking the protein kinase function. Phosphorylation of the initiation factors coincides with the onset of T7 late protein synthesis, occurring 9–12-min postinfection. T7 late protein synthesis, otherwise inhibited in ColIb plasmid-containing cells, is specifically supported by expression of the protein kinase. These results provide the first evidence for the functional involvement of protein phosphorylation in the control of bacterial translation.

Translational control mediated by protein phosphorylation is an essential gene regulatory mechanism in eukaryotic cells (Hershey, 1991). Specific enzyme-catalyzed phosphoryl transfer can alter the functions of proteins associated with the eukaryotic translational apparatus. For example, phosphorylation of factor eIF2α represses translation initiation (Chen et al., 1991). Phosphorylation of EF-1 can stimulate elongation (Venema et al., 1991), and phosphorylation of 40S subunit protein S6 is correlated with an activation of protein synthesis (Palen & Traugh, 1987). There is in contrast no current description of prokaryotic translational regulation occurring through phosphorylation (or other covalent modification) of the ribosome or its associated factors. Translational control in bacteria mainly relies on a diversity of specific noncovalent RNA–RNA or protein–RNA interactions, which influence the ability of the mRNA translation initiation region to interact with its complementary surface on the 30S subunit (Gold, 1988; McCarthy & Gualerzi, 1990).

The bacteriophage T7 expresses several proteins shortly after infection, which specifically activate viral protein synthesis (Hausmann, 1988). The early gene 1-encoded RNA polymerase directs synthesis of class II (middle) and class III (late) viral mRNAs, encoding proteins involved in phage DNA metabolism and capsid assembly, respectively. The early protein encoded by the 0.7 gene (gp0.7) stimulates T7 reproduction through support of viral late protein synthesis, especially during growth under certain suboptimal conditions, such as elevated temperature or carbon starvation (Hirsch-Kaufmann et al., 1975). The T7 gp0.7 exhibits a serine/threonine-specific, cAMP-independent protein kinase (PK)[1] activity (Rahmsdorf et al., 1974; Pai et al., 1975). Expression of gp0.7 PK in the infected cell results in the phosphorylation of a number of proteins (Rahmsdorf et al., 1974; Robertson & Nicholson, 1990). Two identified substrates are the β' subunit of RNA polymerase (Zillig et al., 1975) and ribonuclease III (Mayer & Schweiger, 1983). Phosphorylation by gp0.7 PK can alter protein function. With the modified RNA polymerase, there is evidence for stimulated transcription termination in vitro (Hesselbach & Nakada, 1977), and phosphorylated ribonuclease III possesses elevated processing activity in vitro (Mayer & Schweiger, 1983). The gp0.7 also shuts off host RNA polymerase-catalyzed transcription, by an unknown mechanism which is independent of the protein kinase activity (Rothman-Denes et al., 1973).

It was previously reported that 0.7 gene expression is required for efficient T7 growth in cells containing the ColIb plasmid (Gomez & Naulart, 1977). Specifically, late protein synthesis is apparently inhibited in T7 mutants which either lack or carry a defective 0.7 gene (Gomez et al., 1980). Since T7 gp0.7 also shuts off host transcription, it is not clear whether the gp0.7 protein phosphotransferase activity is responsible for suppressing or bypassing the ColIb plasmid block to late protein synthesis. It was therefore of interest to determine whether expression of gp0.7 PK results in phosphorylation of components of the translational apparatus and, if so, how this modification may affect T7 protein synthesis. The results presented below show that the translational factors IF1, IF2, IF3, and S1 are phosphorylated as a result of gp0.7 PK expression and that the phosphorylation is correlated with the support of late protein synthesis in the T7-infected ColIb$^+$ cell. These results represent the first demonstration of protein phosphorylation involvement in bacterial protein synthesis.

Experimental Procedures

Materials. Escherichia coli strains MC4100 and BB1553 were provided by J. McCarty and G. Walker. T7 phage were provided by F. W. Studier, and were grown and purified as described (Robertson & Nicholson, 1990). Carrier-free inorganic [^{32}P]phosphate (8500–9120 Ci/mmol), ^{35}S-labeled Express protein labeling mix (1000 Ci/mmol), and [^{3}H]uridine (37.5 Ci/mmol) were obtained from Dupont–New England Nuclear (Boston, MA). Ampholines were purchased from Pharmacia–LKB (Piscataway, NJ). Purified IF1 and antisera

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[1] Abbreviations: PK, protein kinase; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing; NEPHGE, nonequilibrium pH gel electrophoresis; 2D, two-dimensional; 1D, one-dimensional; ColIb, colicin Ib; moi, multiplicity of infection; WT, wild-type.

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to IF1, IF2, and IF3 were provided by J. W. B. Hershey; antisera to S1 were provided by B. Wittmann-Liebold. Protein A–Sepharose, phosphoamino acid standards, and plastic-backed thin-layer cellulose plates were purchased from Sigma (St. Louis, MO).

T7 Infection and Radiolabeling of E. coli Cells. Cells were grown at 30 °C in glucose minimal media and infected with phage at an moi of 10 as described (Robertson & Nicholson, 1990). Depending on the assay, [^{32}P]phosphate (50 μCi/mL) and/or ^{35}S-labeled Express labeling mix (50 μCi/mL) was then added, with continued incubation at 30 °C until the reaction was stopped with cyanide–phosphate buffer. For 1D SDS–PAGE analysis, RNase-treated cell lysates were prepared essentially as described (Robertson & Nicholson, 1990); for 2D PAGE, lysates were prepared according to a protocol provided by R. van Bogelen.

One-Dimensional Gel Electrophoresis of Protein. 1D SDS–polyacrylamide slab gel electrophoresis was carried out as described (Robertson & Nicholson, 1990). Following electrophoresis, gels were dried directly and exposed to Fuji RX film.

Two-Dimensional Gel Electrophoresis of Protein. Two-dimensional gel electrophoresis was carried out using either IEF or NEPHGE in the first dimension, and discontinuous SDS–PAGE in the second dimension, essentially as described (Phillips et al., 1987; van Bogelen et al., 1990), with several modifications as suggested by R. van Bogelen. The IEF gels used 1.6% pH 5–7/0.4% pH 3.5–10 ampholines. The NEPHGE analysis used 2% pH 3.5–10 ampholines. Following second-dimension electrophoresis, the gels were dried directly, and differential autoradiography was performed according to Cooper and Burgess (1982). This technique provided a ^{32}P autoradiogram essentially free of ^{35}S radioactivity and an ^{35}S autoradiogram essentially free of ^{32}P radioactivity.

Immunoprecipitation Analysis. IP-PAGE analysis was performed according to Anderson & Blobel (1983) and Bardwell et al. (1989), using polyclonal antisera specific for the proteins analyzed. Immunoprecipitated proteins, labeled with ^{32}P and ^{35}S, were analyzed by 1D SDS–PAGE, and differential autoradiography was performed as described above.

Phosphoamino Acid Analysis. Regions of the dried 2D gels which contained individual ^{32}P-labeled proteins were excised and then processed for phosphoamino acid analysis as described (Cooper et al., 1983), with several modifications as suggested by A. Laudano. *E. coli* MC4100 or BB1553 cells were the source of ^{32}P-labeled IF2 and S1, respectively. HCl hydrolysates of the ^{32}P-labeled protein were combined with unlabeled phosphoserine, phosphothreonine, and phosphotyrosine, spotted on thin-layer, plastic-backed cellulose plates, and electrophoresed at pH 1.9 in the first dimension and at pH 3.5 in the second dimension as described (Cooper et al., 1983). ^{32}P-Labeled phosphoamino acids were identified by autoradiography at −70 °C using intensifying screens.

Determination of in Vivo RNA Synthesis Rates. Infected cell RNA synthesis rates were determined by measuring the incorporation of [^{3}H]uridine into acid-precipitable RNA, according to McAllister and Barrett (1977). Cells were infected with phage and then labeled for 2 min with [^{3}H]uridine (20 μCi/mL) at successive times following infection. Aliquots were treated with trichloroacetic acid, the precipitates were collected on glass fiber filters, and the levels of ^{3}H radioactivity were measured by scintillation counting.

Results

The strategy for identifying T7-infected cell phosphoproteins involved a combination of high-resolution two-dimensional gel electrophoresis and specific immunoprecipitation analysis. Essential to the success of this approach was the availability of an *E. coli* gene-protein index, which currently lists over 600 identified cellular proteins that have been characterized by their unique positions in the 2D IEF and NEPHGE systems (Phillips et al., 1987; van Bogelen et al., 1990). Cells were infected with T7(WT) phage and concurrently labeled with ^{32}P and ^{35}S, and total cell protein was resolved in two dimensions, using either IEF or NEPHGE in the first dimension and SDS–polyacrylamide gel electrophoresis in the second dimension. Differential autoradiography (Cooper & Burgess, 1982) was carried out such that the ^{32}P-labeled proteins could be directly located on the ^{35}S-labeled protein pattern. Direct comparison to the standard gene-protein index pattern (Phillips et al., 1987; van Bogelen et al., 1990) could provide a preliminary identification of specific phosphoproteins, which would be confirmed by immunoprecipitation analysis.

Figure 1 displays specific portions of the 2D IEF and NEPHGE patterns of ^{35}S,^{32}P-labeled protein from uninfected or T7(WT)-infected *E. coli*. The IEF patterns (Figure 1A–C) display acidic proteins (p*I* range approximately 4.7–6.5) having molecular masses between 50 and 160 kDa. Figure 1A,B displays the ^{35}S-labeled and ^{32}P-labeled uninfected cell protein patterns, respectively. Figure 1C displays the ^{32}P-labeled, T7(WT)-infected cell proteins. Comparison of the uninfected and T7(WT)-infected cell phosphoprotein patterns reveals approximately 10 additional phosphoproteins occurring in this gel region as a result of infection. The T7(WT) infection-dependent phosphoproteins are a consequence of gp0.7 PK expression, since the IEF pattern of ^{32}P-labeled proteins from cells infected with T7(A23) phage (carrying a point mutation in the 0.7 gene which abolishes PK activity)[2] is essentially identical to the uninfected cell phosphoprotein pattern (E. S. Robertson and A. W. Nicholson, unpublished experiments). One of the T7 infection-specific phosphoproteins exhibits an electrophoretic mobility close to that of translation initiation factor IF2, while another phosphoprotein overlaps the position of ribosomal protein S1 (indicated by the arrows in Figure 1A,C).[3] Neither of these species is phosphorylated in the uninfected cell (compare Figure 1B and Figure 1C), or in the T7(A23)-infected cell. The phosphorylation of IF2 and S1 was confirmed by immunoprecipitation analysis (see below).

The displayed portion of the 2D NEPHGE patterns (Figure 1D–F) contains basic proteins (p*I* range approximately 8–11) varying in molecular mass from 7 to 25 kDa. The uninfected cell phosphoprotein pattern (Figure 1E) shows approximately 13 species exhibiting relatively minor amounts of ^{32}P radioactivity. T7(WT) infection results in the appearance of approximately 20 additional phosphoproteins in this region (compare Figure 1E with Figure 1F). Similar to the results of IEF analysis, the T7(WT) infection-specific phosphoproteins are dependent on gp0.7 PK expression, since the T7(A23)-

[2] The T7(A23) mutant, isolated by Studier (1973), does not express gp0.7 PK activity (Robertson & Nicholson, 1990). The point mutation has been identified and is predicted to change Asp_{100} to Asn in the gp0.7 sequence (Michalewicz & Nicholson, 1992). This site is directly downstream from a putative ATP-binding domain, and is probably involved in substrate recognition (Michalewicz & Nicholson, 1992).

[3] Protein S1 and heat shock protein DnaK comigrate in the 2D IEF gels, using Pharmacia–LKB ampholines which provide the standard gene-protein index pattern (Phillips et al., 1987; van Bogelen et al., 1990). Our initial 2D PAGE analyses used Bio-Rad ampholines, which permitted separation of the two proteins. However, the 2D pattern was qualitatively different from the standardized pattern. Therefore, we used the *dnaK* deletion strain BB1553 as a source of radiolabeled protein in the 2D IEF–PAGE analyses which utilized Pharmacia–LKB ampholines. The isogenic strain MC4100 was used in the 2D NEPHGE analyses.

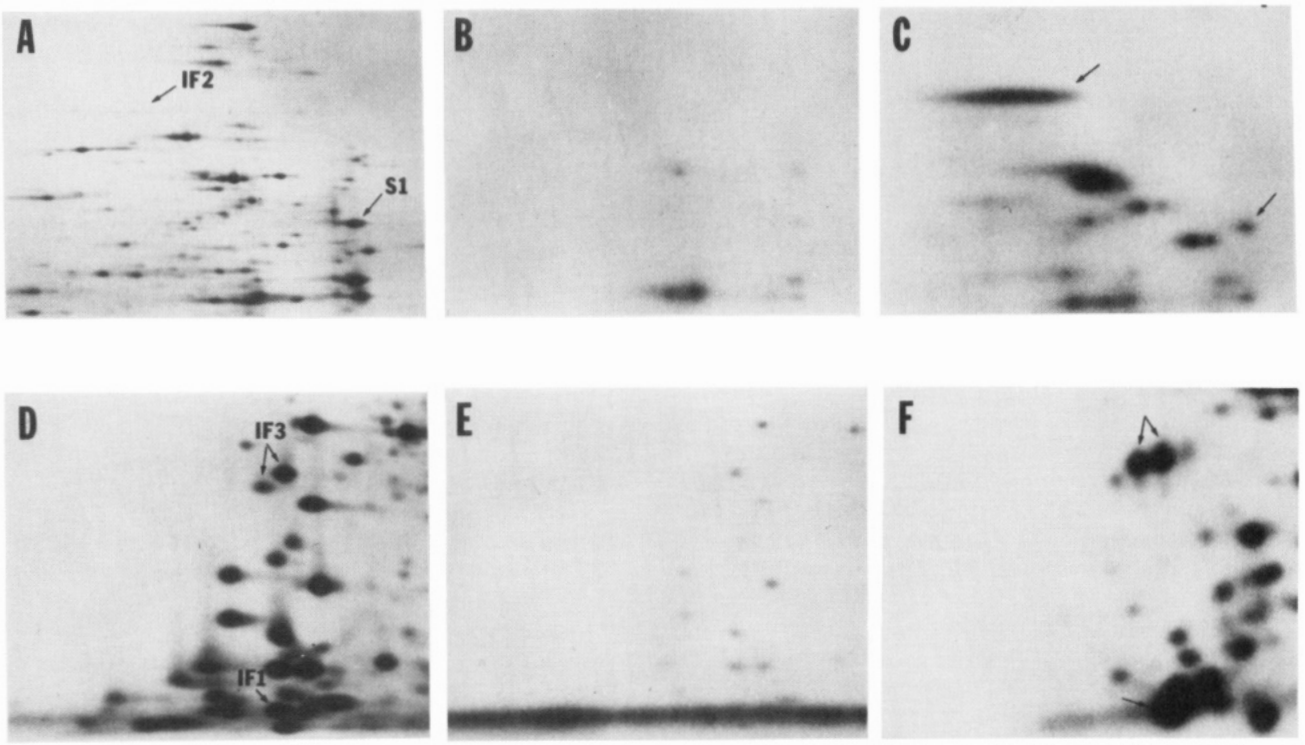

FIGURE 1: Two-dimensional gel electrophoretic analysis of ^{32}P-labeled proteins from T7-infected cells. *E. coli* MC4100 or BB1553 cells were infected with T7(WT) and labeled with ^{32}P and ^{35}S and IEF and NEPHGE analysis of protein was performed as described under Experimental Procedures. Panels A–C, IEF analysis (BB1553 cells); panels D–F, NEPHGE analysis (MC4100 cells). Panel A displays ^{35}S-labeled proteins and panel B the corresponding ^{32}P-labeled proteins from uninfected cells. Panel C displays the ^{32}P-labeled proteins from T7(WT)-infected cells. Indicated in panel A are the positions of IF2 and S1 (Phillips et al., 1987; van Bogelen et al., 1990); indicated in panel C are the ^{32}P-labeled proteins that overlap the positions of IF2 and S1. Panel D displays ^{35}S-labeled proteins and panel E the corresponding ^{32}P-labeled proteins from uninfected cells. Panel F shows the ^{32}P-labeled proteins from T7(WT)-infected cells. Indicated in panel D are the positions of IF1 and IF3 (Phillips et al., 1987; van Bogelen et al., 1990). The ^{32}P-labeled species that comigrate with IF1 and IF3 are indicated by the arrows in panel F.

infected cell pattern is essentially identical to the uninfected cell protein pattern (E. S. Robertson and A. W. Nicholson, unpublished experiments). One of the T7(WT)-infected cell phosphoproteins has a gel electrophoretic mobility similar to initiation factor IF1 (indicated in Figure 1D,F). Since the IF1 spot occurs in a relatively crowded region of the gel, the position of this protein was confirmed in a separate NEPHGE experiment, which included purified IF1 that was located by Coomassie staining (E. S. Robertson and A. W. Nicholson, unpublished experiments). The two other phosphoproteins indicated (see arrows in Figure 1D,F) correspond to the two forms of initiation factor IF3. The occurrence of two IF3 species has been observed elsewhere (Lee-Huang & Ochoa, 1973; van Bogelen et al., 1990). The phosphorylation of IF1 and IF3 was verified by immunoprecipitation analysis (see below).

To confirm the phosphorylation of IF1, IF2, IF3, and S1, immunoprecipitation analysis was carried out using polyclonal antisera specific for each of the proteins. The 1D SDS–PAGE analysis of immunoprecipitated protein from extracts of ^{32}P,^{35}S-labeled cells is shown in Figure 2, where it is seen that antisera can selectively precipitate ^{32}P-labeled protein from T7(WT)-infected cell extracts. Parallel analysis of extracts from uninfected cells or from T7(A23)-infected cells did not reveal these ^{32}P-labeled species (Figure 2), demonstrating that the incorporation of ^{32}P radioactivity is dependent on T7 infection and gp0.7 PK expression. The corresponding ^{35}S autoradiograms for IF2, IF3, and S1 show immunoprecipitated, ^{35}S-labeled protein species which comigrate with the ^{32}P-labeled species in the corresponding T7(WT) infection experiments (the amount of ^{35}S radioactivity immunoprecipitated in the IF1 experiment was very low and was not observable in this experiment). The IF2 analysis reveals two phosphoproteins (indicated by the arrows in Figure 2, panel D) which presumably correspond to IF2α (97.3 kDa; the upper band) and IF2β (79.7 kDa; the lower band) (Sacerdot et al., 1982). IF2α is the more heavily radiolabeled of the two and corresponds to the species observed in the 2D gel analysis (Figure 1). The apparent molecular mass of IF2α in the 1D and 2D gel systems [approximately 115 kDa; see above and Robertson and Nicholson (1990)] is significantly greater than its actual molecular mass (97.3 kDa). The position of IF2β in the 2D gel system is currently not known (van Bogelen et al., 1990). The immunoprecipitation analysis of IF3 (Figure 2, panels E and F) reveals a closely-spaced doublet in the 20-kDa molecular mass range (Figure 2, panel E); the two forms of IF3 are consistent with the results of 2D NEPHGE analysis (Figure 1D).

To identify the phosphorylated amino acid(s) in IF1, IF2, IF3, and S1, the 2D gel regions containing the ^{32}P-labeled protein species were excised and subjected to standard phos-

Table I: Summary of Phosphoamino Acid Analysis of ^{32}P-Labeled Initiation Factors[a]

protein	serine-P	threonine-P	tyrosine-P
IF1	–	+	–
IF2	+	+	–
IF3[b]	–	+	–
S1	–	+	–

[a] Analyses were performed as described under Experimental Procedures. The plus (+) and minus (–) signs indicate the presence or absence, respectively, of ^{32}P radioactivity appearing in the region of the 2D chromatogram staining for the indicated phosphoamino acid. [b] The two forms of ^{32}P-labeled IF3 (see Figure 1F) were combined for this analysis.

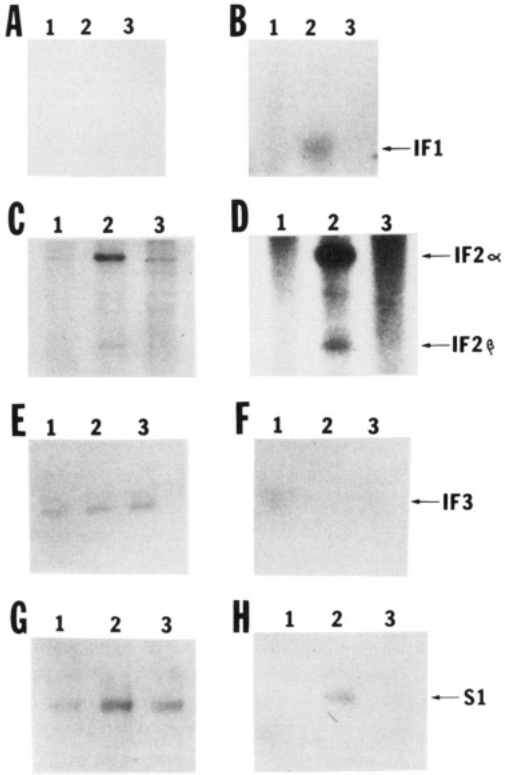

FIGURE 2: Immunoprecipitation PAGE analysis of ^{35}S,^{32}P-labeled proteins from T7-infected cells. Infection and radiolabeling of W3110 cells were carried as described under Experimental Procedures. Panels A, C, E, and G displays ^{35}S-labeled, immunoprecipitated protein, while panels B, D, F, and H displays the corresponding ^{32}P pattern. Panels A and B present the IF1 analysis, showing protein from uninfected cells (lane 1), from T7(WT)-infected cells (lane 2), and from T7-(A23)-infected cells (lane 3). The ^{32}P-labeled IF1 is indicated in panel B. Panels C and D display the IF2 analysis; the order of experiments is the same as with the IF1 analysis. The two IF2 species [IF2α (upper and IF2β (lower)] are indicated in panel D; the fainter band in the middle has not been identified. A shorter exposure time revealed no specific ^{32}P radioactivity in the T7(A23) experiment (lane 3). Panels E and F display the IF3 analysis: lane 1, T7(WT)-infected cell proteins; lane 2, uninfected cell protein; lane 3, T7(A23)-infected cell protein. Note the doublet appearing in the lanes in panel E. Panels G and H display the S1 analysis: lane 1, uninfected cell protein; lane 2, T7(WT)-infected cell protein; and lane 3, T7(A23)-infected cell protein. The relatively darker bands seen in panels C and G (lane 2) result from the slight exposure of the ^{35}S-specific film to ^{32}P radioactivity, which is an unavoidable consequence of the differential autoradiographic technique used (Cooper & Burgess, 1982).

phoamino acid analysis (Cooper et al., 1983). Table I summarizes the results of the analysis. IF1, IF3, and S1 are phosphorylated only on threonine, while IF2 is modified on threonine and serine. Thus, IF2 is phosphorylated on at least two sites. No data are yet available on the placement of the phosphorylation site(s) within the primary sequences of the proteins.

It was previously shown that T7 mutants which lack the 0.7 gene grow poorly in cells containing the ColIb plasmid (Gomez & Nualart, 1977). The inhibition of T7(0.7$^-$) growth in ColIb$^+$ cells has been correlated with an inhibition in phage late protein synthesis (Gomez et al., 1980). It was therefore of interest to examine the time course for phage protein production in ColIb$^+$ cells infected with T7(0.7$^-$) mutants that specifically fail to express the gp0.7 PK activity. We assessed the ability of T7(A23)-infected ColIb$^+$ cells to support phage protein synthesis throughout the infection cycle. Figure 3 displays the 1D SDS–PAGE analysis of ^{35}S-labeled protein from T7-infected ColIb$^+$ cells. In both the T7(WT) and T7(A23) infections, there occurs essentially the same patterns

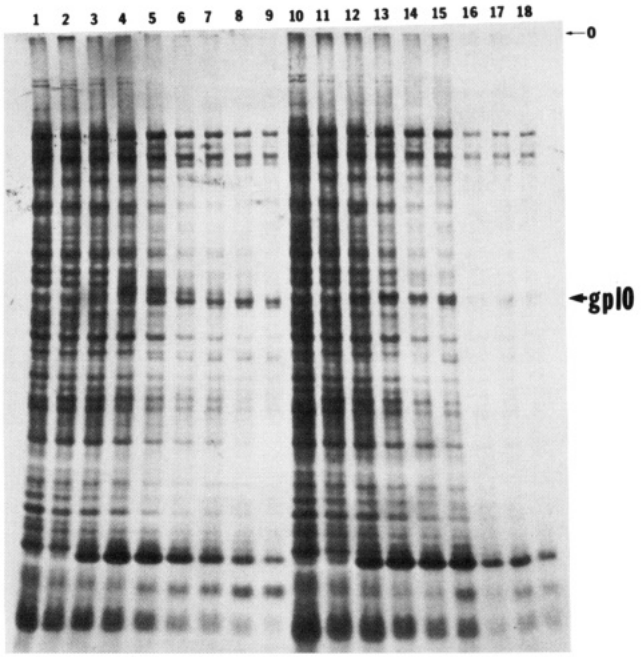

FIGURE 3: Effect of gp0.7 PK expression on protein synthesis in T7-infected ColIb$^+$ cells. *E. coli* W3110 cells containing the ColIb plasmid were infected with T7(WT) and T7(A23) and then pulse-labeled with ^{35}S for 3 min, at successive intervals postinfection. Proteins were electrophoresed in a 10–20% linear gradient polyacrylamide gel. Lanes 1 and 10, uninfected cell protein; lanes 2–9, T7(WT) infection, with the radiolabeling initiated at 0-, 3-, 6-, 9-, 12-, 15-, 18-, and 21-min postinfection. Lanes 11–18 T7(A23) infection, with the same times as for T7(WT) infection. "0" marks the origin of electrophoresis. The position of T7 gp10 is indicated.

and levels of phage protein production, up to 15-min postinfection. However, there subsequently occurs a strong reduction in protein synthesis in the T7(A23) infection, which is especially noticeable with gp10 (indicated by the arrow in Figure 3). The inhibition of T7(A23) late protein synthesis is dependent on the presence of the ColIb plasmid, since identical protein time course patterns are observed in plasmid-free cells infected with T7(WT) or T7(A23) phage. Moreover, infection of ColIb$^+$ cells with a T7(0.7$^-$) mutant which retains gp0.7 PK activity, but lacks gp0.7-dependent host transcription shut-off activity, produces normal levels of late proteins (E. S. Robertson and A. W. Nicholson, unpublished experiments).

A possible explanation for the reduction in T7(A23) late protein synthesis in ColIb$^+$ cells is a specific reduction in phage late mRNA synthesis. To address this possibility, ColIb$^+$ cells were infected with T7(WT) or T7(A23) phage, and total RNA synthesis rates were measured by [^{3}H]uridine incorporation. The results are shown in Figure 4, where it is seen that the absence of gp0.7 PK does not cause a decrease in total RNA synthesis rates but in fact produces a relative stimulation, compared to the wild-type infection. With either infection, there occurs an initial drop in the RNA synthesis rate, which is due to expression of gp0.7-dependent host shut off; the subsequent increase corresponds to the production of the phage RNA polymerase, which is insensitive to gp0.7-dependent shut off (Rothman-Denes et al., 1973; McAllister & Barrett, 1977). The reason for the relative increase in the RNA synthesis rate in the T7(A23) infection, compared to the T7(WT) infection, is not known. Gel electrophoretic analysis of RNA synthesized during the two infections revealed no selective decrease in the levels of any particular phage RNA (E. S. Robertson and A. W. Nicholson, unpublished experiments). These results indicate that the ColIb plasmid exerts its inhibitory effect on T7 late protein production at the translational level and that

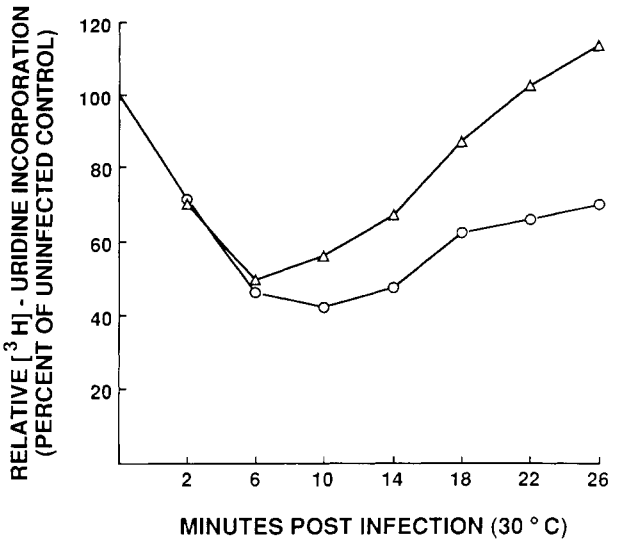

FIGURE 4: Effect of protein kinase expression on total RNA synthesis in T7-infected ColIb$^+$ cells. *E. coli* W3350 cells containing the ColIb plasmid were infected with T7(WT) or T7(A23) and then pulse-labeled with [^{3}H]uridine for 2 min, at successive intervals following infection, as described under Experimental Procedures. The acid-precipitable radioactivity was measured and expressed as a percent of RNA synthesis in uninfected cells. (○) T7(WT) infection; (△) T7(A23) infection.

the gp0.7 PK is responsible for suppressing or bypassing the plasmid-dependent inhibition.

Discussion

This study has shown that translation initiation factors IF1, IF2, and IF3 and the 30S ribosomal subunit protein S1 are phosphorylated following T7 infection. These proteins are not detectably phosphorylated in the uninfected cell, under the experimental conditions used, and the phosphorylation is specifically dependent on gp0.7 PK activity, as these factors are not modified in cells infected with a T7(0.7$^-$) mutant that fails to express gp0.7 PK. The simplest (and testable) interpretation is that IF1, IF2, IF3, and S1 are directly phosphorylated by gp0.7 PK; in this regard, the β' subunit of RNA polymerase and ribonuclease III are in vitro substrates for gp0.7 PK (Zillig et al., 1975; Mayer & Schweiger, 1983). Phosphoamino acid analysis reveals threonine as a target in all the initiation factors, and IF2 in addition is modified on serine. Whether there is an overall preference for threonine will require analysis of additional phosphoproteins. Identification of phosphorylation site sequences may reveal a conserved motif, which would be of value in predicting other gp0.7 PK targets, and in designing model peptide substrates. IF1, IF2, IF3, and S1 perform vital roles in translation, including the accurate selection of fMet-tRNA and mRNA translation initiation regions (IF3), the binding and functional commitment of fMet-tRNA (IF2), providing a helper function for IF3 and IF2 (IF1), and promoting 30S subunit binding of mRNAs that are otherwise inefficiently recognized (S1) (Gold, 1988; van Knippenberg, 1990; Gualerzi et al., 1990). Characterization of the changes in activity as a result of phosphorylation should provide important information on the mechanism of initiation, and on the possible regulated function of these factors.

Expression of gp0.7 PK is required for efficient T7 growth in ColIb$^+$ cells. The ColIb plasmid inhibition of T7(0.7$^-$) reproduction is exerted following the onset of class III gene expression. The inhibition occurs at the translational level, since there is no reduction in phage late mRNA synthesis. The gp0.7 PK activity is maximally expressed 9–12-min postinfection (Robertson & Nicholson, 1990) and enhances the production of phage late proteins. Since an array of proteins are phosphorylated in response to gp0.7 PK expression, it is not yet clear whether the modification of initiation factors is sufficient for the manifestation of the gp0.7 PK helper function in T7-infected ColIb$^+$ cells. The identification of additional phosphoproteins is currently underway, and there is preliminary evidence that other ribosomal components are phosphorylated in a gp0.7 PK-dependent manner (E. S. Robertson and A. W. Nicholson, unpublished experiments).

Evaluation of a previous study by Gomez et al. (1980) is complicated by the fact that the experiments used a T7(0.7$^-$) deletion mutant, which also lacked other early genes. Thus, besides failing to express gp0.7 PK, the T7 deletion mutant was unable to express gp0.7-dependent host transcription shut off, as well as other early functions. Moreover, prior to infection, the ColIb$^+$ cells were irradiated with UV light, which is known to stimulate colicin Ib production and cause cell death (Pugsley & Oudega, 1987). We have shown that the specific absence of gp0.7 PK activity is sufficient to allow ColIb plasmid inhibition of T7 late protein synthesis. However, the gp0.7-catalyzed host transcription shut-off activity is also important for T7 growth in ColIb$^+$ cells, apparently through a mechanism that does not involve or otherwise influence phage late protein synthesis (E. S. Robertson and A. W. Nicholson, unpublished experiments).

Results of other studies provide additional clues to the functional consequences to protein synthesis resulting from gp0.7 PK-catalyzed phosphorylation. Yamada and Nakada (1976) described the stimulated in vitro translational activity of ribosomes isolated from T7-infected cells, compared to ribosomes from uninfected cells. Strome and Young (1980b) noted an increase in polysome size following T7 infection, and a shift of the 70S monosome pool to 30S and 50S subunits. A free 30S subunit is a requirement for initiation complex formation. Perhaps phosphorylation activates the translational apparatus in a generalized manner. On the other hand, there is no evidence that gp0.7 PK-catalyzed phosphorylation directly controls specific cistron selection (which could be argued as a mechanism for the shift of translation from host and T7 early mRNA to T7 late mRNA). The relative abundance and intrinsically high translation initiation efficiencies of T7 late mRNAs are sufficient determinants for the observed translational control in the T7-infected cell (Strome & Young, 1980a,b; Olins & Rangwalla, 1989).

Phosphorylation of the bacterial translational apparatus has until now not been definitively described. An early study indicated only negligible amounts of phosphoproteins associated with the ribosome in uninfected *E. coli* cells (Gordon, 1971). It was subsequently reported that T7 infection causes the phosphorylation of ribosome-associated proteins (Rahmsdorf et al., 1973). However, the weakly-labeled proteins were not identified, nor was it conclusively shown whether the in vivo phosphorylation was a specific consequence of T7 gp0.7 PK expression. The potential involvement of phosphorylation in prokaryotic protein synthesis was suggested in two studies (Fakunding et al., 1972; Traugh & Traut, 1972) which showed that the catalytic subunit of mammalian cAMP-dependent protein kinase can phosphorylate *E coli* IF2, as well as several 30S and 50S proteins. However, there was no detectable change in IF2 activity following phosphorylation (Fakunding et al., 1972). Our study suggests that phosphorylation control of protein synthesis is a universal mechanism. Perhaps a bacterial cell-encoded protein kinase exists which

regulates translational activity under special conditions. Further studies on the T7 protein kinase should provide additional insight into the role of protein phosphorylation in translation, and the functional and evolutionary relationships of prokaryotic protein kinases.

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