The Major Head Protein of Bacteriophage T4 Binds Specifically to Elongation Factor Tu*

The Lit protease in *Escherichia coli* K-12 strains induces cell death in response to bacteriophage T4 infection by cleaving translation elongation factor (EF) Tu and shutting down translation. Suicide of the cell is timed to the appearance late in the maturation of the phage of a short peptide sequence in the major head protein, the Gol peptide, which activates proteolysis. In the present work we demonstrate that the Gol peptide binds specifically to domains II and III of EF-Tu, creating the unique substrate for the Lit protease, which then cleaves domain I, the guanine nucleotide binding domain. The conformation of EF-Tu is important for binding and Lit cleavage, because both are sensitive to the identity of the bound nucleotide, with GDP being preferred over GTP. We propose that association of the T4 coat protein with EF-Tu plays a role in phage head assembly but that this association marks infected cells for suicide when Lit is present. Based on these data and recent observations on human immunodeficiency virus type 1 maturation, we speculate that associations between host translation factors and coat proteins may be integral to viral assembly in both prokaryotes and eukaryotes.

Elongation factor (EF) Tu is the major host translation factor in *Escherichia coli* responsible for delivering charged tRNAs to the ribosome for protein synthesis. During its role in translation, EF-Tu interacts with a number of molecules, including the nucleotides GTP and GDP, aminoacylated tRNA molecules, mRNA-programmed ribosomes, and the nucleotide exchange factor EF-Ts (1). In addition to forming complexes with these molecules, EF-Tu is also known to serve as one of the substrates in the bacteriophage Q₈ replicate (2) and has recently been shown to have chaperone-like activity *in vitro*, promoting the renaturation of some denatured proteins (3). Hence, EF-Tu is capable of interacting with a variety of macromolecules and serving more than one biological function. In addition to these associations, EF-Tu also undergoes post-translation modifications, including methylation at a specific lysine residue, which appears to attenuate its GTPase activity and is linked to the growth phase of the cells (4), and phosphorylation, although the physiological role of this modification is uncertain (5).

EF-Tu is also the target of a bacteriophage exclusion system (6). Bacteriophage exclusion is a defense mechanism in which bacteria commit altruistic suicide in response to infection, thereby preventing propagation of the phage (7, 8). Although distinct to apoptosis in eukaryotes, similarities have been drawn between this form of suicide and programmed cell death events in multicellular organisms (9). Generally, these exclusion systems are mediated by the action of one or more nonsential proteins encoded by prophages, plasmids, or transposons. One of the best studied is that of *E. coli* K-12 strains, which exclude T₄ bacteriophage as well as other T-even phages through the action of a metalloprotease called Lit (Late Inhibitor of T₄), encoded by the defective prophage e14 (10). Following T₄ phage infection of a Lit-containing cell, EF-Tu is specifically cleaved by Lit between Gly⁵⁹ and Ile⁶⁰ in the RGITI motif of the effector I region resulting in the inhibition of translation (6). This region, which is involved in co-ordinating the γ-phosphate of GTP as well as the bound magnesium ion (11 and references cited therein), is conserved in other translation elongation factors such as EF-G and common to the homologous eukaryotic translation factors EF-1α and EF-2. The fact that neither EF-G nor heat-inactivated EF-Tu are cleaved by Lit suggests that the three-dimensional structure of EF-Tu, and not just the primary sequence of the proteolysis site, is required for cleavage and that the cleavage reaction is highly specific, implying the involvement of other regions of the translation factor (12).

Proteolysis of EF-Tu by Lit is activated by the appearance in the cell of a short peptide determinant approximately 29 amino acids long, beginning about 100 amino acids in from the N terminus of the unprocessed major head protein of the infecting T-even phage, gp23 (56 kDa). This short peptide sequence was named the Gol peptide sequence, because it was first identified as the terminal of the unprocessed major head protein of the infecting T-even phage, gp23 (56 kDa). This short peptide sequence was named the Gol peptide sequence, because it was first identified as the minimal gol region sequence. In this system, Lit-mediated cleavage of the Gly⁵⁹-Ile⁶⁰ bond of EF-Tu is dependent on the addition of the Gol peptide, with no additional viral or bacterial proteins being required (12).

The absolute dependence for proteolysis on the Gol peptide in the purified system raises the question of how the activation occurs. Few peptide-activated proteases have been described in the literature, although a well known example occurs in the

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¶ The abbreviations used are: EF, elongation factor; MOPS, 4-morpholinepropanesulfonic acid; HPLC, high pressure liquid chromatography; PAGE, polyacrylamide gel electrophoresis; GST, glutathione S-transferase; HIV, human immunodeficiency virus; NEM, N-ethylmaleimide; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide hydrochloride; GDP-PNP, guanosine 5′-O-(β,γ-imidotriphosphate).
matured of adenovirus in which an 11-residue C-terminal peptide of the viral coat protein activates the thiol protease Ad2 through thiol-disulfide exchange to cleave the same coat protein (15). In contrast, the Gol peptide acts in trans, and, although the peptide contains a single cysteine, this residue is not required for activation of Lit-mediated cleavage of EF-Tu, indicating a mechanism distinct to that of Ad2 (12).

Here we report the results of a number of different experimental approaches to show that the phage-derived Gol peptide binds to EF-Tu. We also show that this complex forms an activated substrate for Lit and that this association is sensitive to the identity of the nucleotide bound to the translation factor. Because the association of viral capsid proteins with host translation factors has also been demonstrated in eukaryotic systems, we discuss how this type of association may be a hitherto unrecognized requirement in the viral infection of eukaryotic cells.

**Experimental Procedures**

Glutathione-Sepharose 4B Redipack columns were purchased from Amersham Pharmacia Biotech. Vydac C8 and C18 reverse-phase columns purchased from Pharmacia Biotech. Vydac C8 and C18 reverse-phase columns were HPLC-purified on a Vydac C8 reverse-phase column that had been equilibrated in 90% water/10% acetonitrile containing 0.1% trifluoroacetic acid. The column was developed using a gradient up to 65% of acetonitrile with a flow rate of 1 ml/min over 35 min. Single radioactive peaks from all three pools were collected and concentrated using an Amicon Centricon 10 microconcentrator. For HPLC steps, the recovery of applied radioactivity was greater than 95%, and both the C8 and C18 columns were run at 18 °C while all other chromatographic conditions were held constant.

**Construction of the Gol Peptide S-Tag Fusion—** The plasmid, pET30PZ1, which was used to synthesize Gol peptide in *in vivo*, contains a 160-base pair PZ1 fragment extending from the natural I site in the T4 gp23 gene to an HindIII linker inserted at the end point of the Δ1 deletion downstream of the gol region (17) and then ligated into the PstI and HindIII sites of pET30b (Novagen). Once induced, the construct directs the synthesis of a 12-kDa polypeptide containing the gol region from gp23 fused to the C-terminal specific activity of the peptide (15.8 ± 8 Ci/mmol) was determined (5.9 nCi/nmol complex). This material was then proteolytically digested by the addition of 2% trypsin followed by incubation at 37 °C for 2 h. The proteolysis of bacterial EF-Tu by trypsin has been reported from a number of laboratories (20, 21). The conditions used in our study were similar to these and ensured the complete digestion of EF-Tu and the generation of small peptide fragments. The digestions were stopped by the addition of 10 mM octyl mercaptoethanol and 0.05 M EDTA to a final concentration of 2.5 mM. The reactions were then incubated at 18 °C for an additional amount of time, as described in the figure legend, prior to their quenching by the addition of Tris-HCl, pH 8.0, to 100 mM final concentration. The samples were analyzed by 10% SDS-PAGE.

To remove Gol peptide from the cross-linking reactions for analytical purposes, the samples were gel-filtered on an Amersham Pharmacia Biotech S75 column (26 × 300 mm) equilibrated in 50 mM Tris-HCl, pH 8.0, 10 mM β-mercaptoethanol, 2 mM MgCl2, 10 μM GDP at 1 ml/min.

**Tritiation of the Gol Peptide—** The Gol peptide (1AVMGMVRRAIPNLADICGVQPMNSPTG), corresponding to residues 93–122 of the Ad2 major capsid protein, was overexpressed in *E. coli* and purified by nickel chelation chromatography. Fractions containing active Gol peptide were confirmed by its ability to activate EF-Tu and cross-link to EF-Tu. At a concentration of 1 mM, in 25 mM Na2B4O7-HCl, pH 8.6, the Gol peptide was incubated with 20 μM [3H]GTP (0.125 Ci/mmol NEM) for 5 h at 37 °C. The labeled peptide was separated from excess [3H]GTP by gel-filtration on an Amersham Pharmacia Biotech S75 column (26 × 300 mm) equilibrated in water at a flow rate of 1 ml/min. Fractions containing [3H]Gol were pooled, lyophilized, and reconstituted into 1 ml of 50 mM MOPS, pH 7.9. The concentration and, hence, the specific activity of the peptide (15.8 ± 8 Ci/mmol peptide) were determined.

**Tryptic Digests—** Cross-linking reactions between the tritiated Gol peptide and EF-Tu and all the relevant controls (i.e., [3H]Gol and EF-Tu alone) were performed as described previously except that the radiolabeled peptide was in a 20-fold molar excess to that of EF-Tu. Following gel-filtration of the cross-linked complex to remove excess peptide, appropriate fractions containing both radioactivity and EF-Tu were pooled and concentrated using an Amicon Centrificon 10 microconcentrator. Upon addition of 100-fold excess of Gol peptide, EF-Tu and Gol peptide were mixed and incubated at 18 °C for 30 min before the addition of EDC to a final concentration of 2.5 mM. The reactions were then incubated at 18 °C for an additional amount of time, as described in the figure legend, prior to their quenching by the addition of Tris-HCl, pH 8.0, to 100 mM final concentration. The samples were analyzed by 10% SDS-PAGE.

**Conversion of EF-Tu-GDP into EF-Tu-GTP—** To convert the EF-Tu-GDP form into EF-Tu-GTP, 20 μM EF-Tu-GDP was incubated with 20 μM guanosine 5′-o-(3-thiotriphosphate), 200 μM phosphoenolpyruvate, and 0.05 μg/ml pyruvate kinase for 30 min at 37 °C, essentially as described by Canosa et al. (22). The reaction was quenched on ice.

**EF-Tu GTPase Assays—** Determination of the intrinsic GTPase ac-
tivity of EF-Tu was based upon the measurement of $\gamma^{32}$P liberated from $[\gamma^{32}\text{P}]\text{GTP}$ upon its hydrolysis as described by Mesters et al. (23).

All assays were carried out in 64 mM Tris-HCl, pH 7.6, 80 mM NH$_4$Cl, 10 mM MgCl$_2$, 10 mM $\beta$-mercaptoethanol, 83 mM phosphoenolpyruvate, 17 $\mu$g/ml pyruvate kinase, 1 $\mu$g EF-Tu, and Gol peptide as indicated in the figure legend. For the rate experiments, a 270-$\mu$l solution containing EF-Tu-GDP, phosphoenolpyruvate, pyruvate kinase, and 500 $\mu$mol Gol peptide was preincubated at 37 °C for 30 min. Then, $[\gamma^{32}\text{P}]\text{GTP}$ (1200 dpm/pmol), also preincubated with phosphoenolpyruvate and pyruvate kinase, was added to give a final volume of 300 $\mu$l and a final concentration of $[\gamma^{32}\text{P}]\text{GTP}$ of 5 $\mu$M. Aliquots of 30 $\mu$l were removed from the reaction at the times indicated in the figure legend and quenched by the addition of silicic tungstate in 1 mM H$_2$SO$_4$ to a final concentration of 3.7 mM. Following the addition of ammonium molybdate in 2 mM H$_2$SO$_4$ to a final concentration of 0.74% (w/v), the liberated $\gamma^{32}\text{P}$, as a dodecamolybdate complex, was extracted into a 1:1 toluene/butan-2-ol mixture (24) and counted on a scintillation counter. For the titration experiments, the concentration of Gol peptide in separate 30-$\mu$l reactions was increased as indicated in the figure legend. The reactions were then preincubated at 37 °C for 30 min before the addition of $[\gamma^{32}\text{P}]\text{GTP}$, then they were incubated for a further 60 min before being quenched and analyzed as described above.

RESULTS

Detection of Gol Peptide-EF-Tu Complexes by Affinity Chromatography—To monitor the association of EF-Tu with Gol peptide, separate extracts were prepared of bacterial cells containing a glutathione S-transferase-EF-Tu (GST-EF-Tu) fusion protein and a Gol peptide-S-Tag fusion (see “Experimental Procedures”). In this construct, a 60-amino acid peptide of the gp23 protein that spanned the Gol region was fused to an S-Tag. The extracts were mixed and loaded onto a glutathione-Sepharose column. Fig. 1A shows that the GST-EF-Tu fusion protein bound to the column along with some of the Gol peptide-S-Tag fusion, which could then be eluted when the GST-EF-Tu fusion protein was stripped from the column by the addition of excess glutathione to the wash buffer (lanes d, e, and f). Much less S-Tagged peptide was retained in identical control experiments in which the S-Tag was not fused to the Gol sequence (Fig. 1B) or if the GST protein was not fused to EF-Tu (Fig. 1C). These data demonstrate that the peptide was retained on the column due to a direct interaction between the Gol peptide and EF-Tu rather than through nonspecific interactions between, for example, GST and the S-Tag. Overall, these results indicate that the Gol peptide is retained on the column by virtue of its interaction with EF-Tu.

Attempts were made to recapitulate these chromatographic experiments using the chemically synthesized 29-residue Gol peptide. However, we were unable to detect retention of EF-Tu on a Gol peptide affinity column nor indeed retention of the peptide to an EF-Tu affinity column (data not shown). It is clear from estimates of the binding constant for the Gol peptide-EF-Tu complex (see below) that the synthetic peptide binds weakly to EF-Tu, which explains why complexes could not be detected chromatographically using the peptide. The fact that binding could be detected for the 60-mer version of the Gol peptide in the GST and S-Tag experiments implies that the extra flanking amino acids from gp23 likely increase the binding affinity for EF-Tu. Lastly, we could not detect Lit retention on the EF-Tu or Gol-peptide affinity columns. This implied that binary complexes between Lit and the other components did not occur or were too weak to be detected.2

The Gol Peptide-EF-Tu Complex Is the Substrate for Lit Cleavage—The chromatographic experiments suggested that the Gol peptide formed a complex with EF-Tu. However, these experiments did not address the question of whether the resulting complex is relevant to the mechanism by which the Gol peptide activates proteolysis of EF-Tu by Lit. To investigate this, we employed chemical cross-linking, which is capable of capturing even weakly bound complexes, and, to simplify interpretation, we focused on the chemically synthesized Gol peptide and purified EF-Tu. Using the bifunctional, zero-length cross-linking reagent EDC, it was possible to specifically cross-link the synthetic 29-residue Gol peptide to EF-Tu bound with GDP (Fig. 2, lane 1) demonstrating that a complex is detectable. A cross-linked adduct was observed of ~47 kDa, corresponding to EF-Tu (44 kDa) covalently bound with a single

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2 R. Bingham and C. Kleanthous, unpublished observations.
copy of the 3-kDa Gol peptide (cross-linking efficiency ~20% by laser densitometry). Increasing concentrations of either the cross-linking reagent or Gol peptide did not yield species of higher molecular mass (data not shown), suggesting that a 1:1 complex was being cross-linked. Cross-linking was also used to probe for Gol peptide binding to Lit, but no cross-linked adducts could be detected (data not shown).

Having demonstrated cross-linking between EF-Tu and the Gol peptide, the functional relevance of this complex could then be tested by incubating the mixture of cross-linked and un-cross-linked EF-Tu with the Lit protease after excess peptide had been removed by gel-filtration chromatography (see “Experimental Procedures”). Only the EF-Tu to which the Gol peptide was covalently attached was a substrate for Lit cleavage as deduced by the loss of the upper band at 47 kDa but not the lower band at 44 kDa (Fig. 2, lane 2). The specific cleavage of cross-linked EF-Tu is also apparent from the size of the cleavage fragments. Lit-mediated proteolysis of native EF-Tu ordinarily yields two cleavage fragments of approximately 37 kDa (amino acids 60–393) and 7 kDa (amino acids 1–59), respectively, with only the larger fragment being visible on 10% SDS-polyacrylamide gels. Proteolysis of the cross-linked complex, however, gave rise to a larger cleavage fragment of ~40 kDa, corresponding to the 37-kDa fragment covalently bound to the Gol peptide. These results not only demonstrate the high specificity of cross-linking between the Gol peptide and EF-Tu, because the complex still acts as a substrate for Lit proteolysis, but also shows that the cross-linking between Gol peptide and EF-Tu is to amino acids C-terminal to the cleavage site (Gly286–Ile286). To demonstrate that EDC does not inactivate the remaining native EF-Tu, excess Gol peptide was added along with Lit. Then the uncleaved EF-Tu was also digested, generating the normal 37-kDa fragment in addition to the 40-kDa cross-linked fragment (Fig. 2, lane 3).

Gol Peptide Contacts Domains II and III of EF-Tu—Because the Gol peptide is specifically cross-linked to EF-Tu at a site where it can activate cleavage by Lit, mapping the sites of cross-linking on EF-Tu should begin to reveal where the Gol peptide binds. We therefore identified the positions of some of the cross-links by peptic mapping (see “Experimental Procedures”). These experiments capitalized on two observations. First, it was possible to specifically radiolabel the Gol peptide at its single cysteine residue (Cys19) by chemical modification using tritiated N-ethylmaleimide ([3H]NEM). This modification did not inhibit the ability of Gol to activate Lit-mediated cleavage of EF-Tu nor its cross-linking with EF-Tu (data not shown), in agreement with our previous data on the ability of a Cys19 → Ala mutant of the Gol peptide to sustain Lit cleavage of EF-Tu (12). Second, radioactive Gol peptide cross-linked to EF-Tu was not degraded by trypsin, which allowed the isolation of EF-Tu peptide fragments that retained Gol-associated radioactivity. The Gol peptide contains two arginines (at positions 8 and 9 in the 29-residue peptide; see “Experimental Procedures”) both of which are readily digested by trypsin in the unbound peptide. However, no tryptic fragments corresponding to cleaved Gol peptide were observed for [3H]Gol cross-linked to EF-Tu, implying protection of the peptide proteolysis sites when bound to the translation factor (data not shown).

Following cross-linking of [3H]Gol to EF-Tu by EDC and the removal of excess peptide by gel-filtration, the pooled and concentrated [3H]Gol peptide-EF-Tu cross-linked complex was digested with trypsin and fractionated on a reverse-phase C18 column with an acetonitrile gradient (described under “Experimental Procedures”). Peptides eluted between 5 and 30 min, with three distinct radioactive pools eluting at 16.5, 18.5, and 20 min (designated pools A–C, respectively) with little starting material remaining. The three pools were subjected to further purification on a reverse-phase C18 column and these data are shown in Fig. 3 (A–C). Homogeneous radio-labeled peptide fractions were isolated from each of these pools (S1 from pool A, S2 and S3 from pool B, and S4 and S5 from pool C) and were subjected to N-terminal sequencing during which the eluted radioactivity was monitored after each cycle.

Of the purified peptide fractions from the C18 column, S2 and S5 did not yield clear data and so were not studied further; S2 contained trypsin sequence as well as an unassigned sequence, whereas S5 gave multiple amino acids during each sequencing cycle that could not be assigned to EF-Tu, trypsin, or the Gol peptide. In both cases the radioactivity associated with the peptide eluted throughout all the sequencing cycles. Interpretable sequencing data were obtained for the remaining three peptides S1, S3, and S4, and these data are shown in Table I. Peptide fraction S1, isolated in three independent experiments, corresponded to residues 326–333 of E. coli EF-Tu from domain III, but no discernible Gol sequence could be identified. The radioactivity associated with each sequencing cycle of this peptide was only just above background (normally ~25 cpm) and eluted across all the residues, making sequence-specific assignment of the cross-linking site impossible. In contrast, peptide S3 was derived from residues 254–261 of domain II and contained a single unassigned residue (Cys256), which also corresponded to the elution position of the radioactivity (Table I). The absence of Gol peptide sequence infers that, although Gol is bound (because radioactivity is retained), its N terminus is most likely blocked. Peptide S4 contained sequence corresponding to both EF-Tu (residues 271–278 of domain II) and Gol peptide (residues 1–3). After three cycles, however, the Gol sequence was lost and this corresponded to the appearance of a single unassigned residue in the EF-Tu sequence (Glu273) and the elution of all the associated radioactivity (Table I). The data indicate that Cys256 and Glu273 of domain II are sites of Gol-peptide cross-linking (both are consistent with the known chemistry of EDC) as well as residues 326–333 of domain III, although no single residue can be identified in this domain. It is important to note that the three identified sequences all lie C-terminal to the Lit cleavage site at Gly286–Ile286, consistent with the original cross-linking data in Fig. 2.

Gol Peptide Inhibits the Intrinsic GTPase of EF-Tu—EF-Tu is a member of the GTPase family of proteins, and this enzymatic activity is central to its role as a translation factor. Consequently, molecules that affect its properties as a trans-
Proteolysis of EF-Tu Induced by Phage Peptide Binding

**Fig. 3. Peptide mapping of [3H]Gol-EF-Tu cross-links.** Purified [3H]Gol-EF-Tu cross-linked complex (4.5 nmol) was digested with 2% trypsin and fractionated on a C8 reverse-phase HPLC column (see "Experimental Procedures"). Three radioactive pools (A, B, and C), eluting at 18, 18.5, and 20 min, respectively, were collected. The figure shows the subsequent separations of each of these pools rechromatographed on a C18 reverse-phase column developed using an acetoni trile gradient, as described under "Experimental Procedures." Fractions containing 3H-label are indicated by the shaded boxes, and the cpm values represent background subtracted counts per fraction. Single radioactive peptides were collected from these traces (S1, S2, S3, S4, and S5) and submitted for N-terminal sequencing. The traces were run at 18 °C while the absorbance was monitored at \( \lambda_{220\text{nm}} \).

**Table I**

| Sample | Cycle number | Primary sequence | Secondary sequence | cpm *b |
|--------|--------------|------------------|--------------------|-------|
|        |              | Residue        | Yield *a          |       |
| S1     | 1            | \[^{27}\text{Y}^c\] | 101                | 42    |
| 2      | R            | 67               | 110                | 40    |
| 3      | P            | 95               | 74                 | 44    |
| 4      | Q            | 74               | 101                | 39    |
| 5      | F            | 110              | 130                | 37    |
| 6      | Y            | 130              |                    |       |
| 7      | \[^{27}\text{P}^d\] | 81 | 27               |
| 8      | T            | 85               | 26                 |
| S3     | 1            | \[^{25}\text{S}^e\] | 81                |
| 2      | T            | 85               | 26                 |
| 3      | G            | 102              | 28                 |
| 4      | V            | 91               | 26                 |
| 5      | E            | 132              | 27                 |
| 6      | \[^{29}\text{M}^f\] | 104 | 26               |
| S4     | 1            | \[^{27}\text{A}^g\] | 54              |
| 2      | G            | 81               |                    |
| 3      | M            | 68               |                    |
| 4      | N            | 121              | 45                 |
| 5      | V            | 108              | 50                 |
| 6      | G            | 59               | 51                 |
| 7      | V            | 161              | 46                 |
| 8      | \[^{28}\text{L}^h\] | 32 | 33               |

* Yield of each phenylthiohydantoin-derivative.

**T4 Gol Peptide Binding to EF-Tu Targets the Cell for Lit-mediated Proteolysis**

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**DISCUSSION**

**T4 Gol Peptide Binding to EF-Tu Targets the Cell for Lit-mediated Proteolysis**—The function of the Gol peptide from gp23 in the T4-directed phage exclusion response of *E. coli* could be to activate proteolysis either by contributing essential catalytic groups to Lit, activating the substrate EF-Tu, or both. Circumstantial evidence suggests that the peptide does not activate the protease directly. For example, if the peptide furnished essential catalytic groups for Lit, then it might be expected that some of the originally identified *gol* mutations in...
the gp23 protein would not support cleavage of EF-Tu, as is the case for active site mutations of essential catalytic groups in enzymes. However, this is not the case, because overexpression of Lit renders bacteria susceptible to T4 gol mutants (17) and equivalent Gol mutant peptides in the in vitro assay can activate the cleavage of EF-Tu at elevated concentrations (12).

The present work shows for the first time that the Gol peptide binds to EF-Tu, to a site distinct to that cleaved by the enzyme, to form the specific substrate for Lit. There is still the formal possibility that the peptide furnishes amino acids for catalysis in the ternary complex, but this does not seem likely based on the effects of gol mutations discussed above. It is also possible that the peptide might bind to Lit in the absence of EF-Tu, although it has not been possible to detect stable binary complexes between the Gol peptide and Lit using column chromatography or chemical cross-linking experiments. The most plausible mechanism at the present time is that Lit only forms a stable complex with EF-Tu when the Gol peptide is bound, implying that the binary, Gol peptide-EF-Tu complex is an "activated" substrate for Lit. This type of substrate activation mechanism has similarities to the staphylokinase cofactor in the action of plasmin (29). The protein cofactor does not affect the geometry of the plasmin-active site but instead forms an additional docking site for the enhanced presentation of the plasminogen substrate to the enzyme. In this case, both the enzyme and the substrate are in contact with the activating...
EF-Tu in both its nucleotide-bound conformations (EF-Tu
formation, both the Lit cleavage site (Gly59–Ile60) and the Gol
crystal structures, it is apparent that, in the GDP-bound con-
I and II increases by
EF-Tu involves a large intramolecular movement of domain I
preferential binding of the peptide and the faster rate of
binding sites are more solvent accessible. This could explain
that this association must occur in bacteria whether or not they
contain Lit implies that it has a physiological role. The inter-
action as measured in vitro with the 29-residue peptide is weak
(Ki ~ 0.3 nM) by comparison to other macromolecular associ-
as such as antibody-antigen interactions (micromolar to
nanomolar), although this affinity may well be higher for intact
gp23. In this context it is interesting to note the relative con-
centrations of EF-Tu and gp23 in an infected E. coli cell. EF-Tu
represents 5–10% of total cell protein (~120,000–240,000 cop-
equals equivalent to a concentration of ~0.1–0.2 mM (30)) and
that a similar number of gp23 molecules appear late in T4 infection,
with 960 self-associating to form a single phage head particle
(31, 32). Because binding of gp23 to EF-Tu is localized to a
small, 29-residue region of the major coat protein (as defined in
vivo by the original gol mutants and in vitro by peptide bind-
ing), this association could occur as gp23 is being synthesized,
suggesting a possible role in the assembly of the phage head
itself. T4 bacteriophage head assembly is highly complex, in-
volving up to 11 different proteins in the prohead and requiring
both host and phage-encoded chaperones (GroEL and gp31,
respectively) as well as scaffolding proteins (for a review see
Black et al. (33)). With its high cellular concentration and its
ability to bind a specific sequence in gp23, EF-Tu might be
acting as an early chaperone or ancillary scaffolding protein in
the assembly process.

EF-1α, the human equivalent of EF-Tu, has been reported to
have a chaperone-like function by binding proteins and target-
them for degradation by the ubiquitin system, a role for
which EF-Tu can substitute (34). The potential for a chaperone-
like role for EF-Tu has been given further credence by recent in
vitro work. EF-Tu can aid the renaturation of denatured pro-
teins such as citrate synthase, α-glucosidase, and rhodanase (3,
35); forms stable complexes with several unfolded proteins
such as bovine pancreatic trypsin inhibitor and carboxymethyl
α-lactalbumin; and protects some proteins from irreversible
aggregation (3). We speculate that this chaperone activity may
have been put to use by infecting bacteriophage in vivo to bind
the gol region of gp23.

The fact that EF-Tu is a GTPase is also consistent with a
putative chaperone role in phage head assembly, because the
translation factor can switch between two distinct conforma-
tional states. Importantly, the folding properties of EF-Tu are
enhanced in the open GDP-bound conformation (3), the confor-
mation favored for Gol binding, and the binding of unfolded
proteins inhibit its intrinsic GTPase activity (35), as does Gol
peptide. Therefore, it seems possible that EF-Tu could have a
chaperone-like role early in the synthesis/assembly of T4 sim-
ply by binding to the Gol sequence of gp23 in a nucleotide-de-
pendent manner prior to it being shuttled to GroEL and gp31
(33).

Viral Protein Interactions with Host Translation Factors—
The association of EF-1α and the human immunodeficiency virus
(HIV) type 1 gag polyprotein was reported recently by
Cimarelli and Luban (36), but although this interaction seems to
be essential for viral replication, its role remains unclear.
The HIV-1 gag polyprotein is processed by an activated viral
protease and so directs the formation and release of nascent
virions. These proteolyzed products exhibit important func-
tions during virion assembly, forming the capsid, the nucleo-
capsid, and the matrix of the virion while others, such as p6,
play key roles in the early phases of the viral life cycle (for a
review see Swanstrom and Wills (37)). Two of these domains
(nucleocapsid and matrix) have been shown to interact with

3 R. Bingham and C. Kleanthous, unpublished results.
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EF-1α in an RNA-dependent manner (36). In sharp contrast to the interaction between gp23 and bacterial EF-Tu, which occurs at the domain II/III interface, the interaction between matrix and EF-1α has been shown to take place with domain I at the N terminus of EF-1α (residues 1–14). Furthermore, this interaction not only inhibits host protein translation but also directs the incorporation of a truncated form of EF-1α into the viral membrane. Translational inhibition has also been reported upon the interaction of the herpes simplex virus 1-infected cell protein 0 and EF-1α (38). In addition to these interactions, it has been shown that RNA polymerase from vesicular stomatitis virus specifically associates with EF-1α (39), which is analogous to the association of bacterial EF-Tu with the Qβ RNA polymerase (2). Whatever their relationship to Gol binding, these other studies indicate that peptides other than Gol peptide can bind to translation factors such as EF-Tu. In view of our data on the binding of a bacteriophage head protein to EF-Tu and that of Cimarelli and Luban (36) on the complex formed between HIV-1 capsid protein 0 and EF-1α, it has been shown that RNA polymerase from vesicular stomatitis virus specifically associates with EF-1α for its activity (39), which is analogous to the association of bacterial EF-Tu with the Qβ RNA polymerase (2). Whatever their relationship to Gol binding, these other studies indicate that peptides other than Gol peptide can bind to translation factors such as EF-Tu. In view of our data on the binding of a bacteriophage head protein to EF-Tu and that of Cimarelli and Luban (36) on the complex formed between HIV-1 capsid proteins with EF-1α, it is tempting to speculate that viral capsid protein association with host translation factors is the norm rather than the exception and that this association plays a pivotal role in viral maturation in prokaryotes and eukaryotes.

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REFERENCES
1. Abel, K., and Jurnak, F. (1996) Structure 4, 229–238
2. Blumenthal, T., Landers, T. A., and Weber, K. (1972) Proc. Natl. Acad. Sci. U. S. A. 228, 748–751
3. Caldas, T. D., El Yaagoubi, A., and Richarme, G. (1998) J. Biol. Chem. 273, 11478–11482
4. Van Noort, J. M., Kraal, B., Sinjorgo, K. M., Peroon, N. L. M., Johanns, E. S. D., and Bosch, L. (1998) Eur. J. Biochem. 160, 557–561
5. Lippsmann, C., Lindechau, C., Vigenboom, E., Schroder, W., Bosch, L., and Erdmann, V. A. (1993) J. Biol. Chem. 268, 601–607
6. Yu, Y.-T. N., and Snyder, L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 802–806
7. Snyder, L. (1999) Mol. Microbiol. 15, 415–420
8. Yarmolinsky, M. (1995) Science 267, 836–837
9. Vaux, D. L., and Korsmeyer, S. J. (1999) Cell 96, 245–254
10. Wittinghofer, A., Frank, R., and Leberman, R. (1980) Eur. J. Biochem. 94, 245–250
11. Sprinzl, M. (1994) Trends Biochem. Sci. 19, 245–250
12. Georgiou, T., Yu, Y.-T. N., Ekunwe, S., Buttmn, M. J., Zuromnd, A. M., Kraal, B., Kleanthous, C., and Snyder, L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2891–2895
13. Champness, W. C., and Snyder, L. (1982) J. Mol. Biol. 155, 395–407
14. Monod, C., Repola, F., Kutateladze, M., Tetart, F., and Krisch, H. M. (1997) J. Mol. Biol. 267, 237–249
15. Webster, A., Hay, R. T., and Kemp, G. (1993) Cell 72, 97–104
16. Boon, K., Vigenboom, E., Madsen, L. V., Talens, A., Kraal, B., and Bosch, L. (1992) Eur. J. Biochem. 210, 177–183
17. Bergland, K. J., Kao, C., Yu, Y.-T. N., Gulati, R., and Snyder, L. (1990) J. Mol. Biol. 213, 477–494
18. Cetin, R., Anborgh, P. H., Cool, R. H., and Parmeggiani, A. (1998) Biochemistry 37, 486–495
19. Riddles, P. W., Blackeley, R. L., and Zerner, B. (1979) Anal. Biochem. 94, 75–81
20. Wittinghofer, A., Frank, R., and Leberman, R. (1980) Eur. J. Biochem. 108, 423–431
21. Arai, K.-I., Nakamura, S., Arai, T., Kawakita, M., and Kaziro, Y. (1975) J. Biochem. 79, 69–83
22. Fasano, O., Bruns, W., Cretchet, J. B., Sander, G., and Parmeggiani, A. (1978) Eur. J. Biochem. 86, 557–565
23. Mesters, J. R., Zeef, L. A. H., Hilgenfeld, R., de Graaf, J. M., Kraal, B., and Bosch, L. (1994) EMBO J. 13, 4877–4885
24. Shacter, E. (1984) Anal. Biochem. 138, 416–420
25. Wolf, H., Chiniali, G., and Parmeggiani, A. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4910–4914
26. Pingoud, A., Block, W., Urbanke, C., and Wolf, H. (1982) Eur. J. Biochem. 123, 261–265
27. Berchtold, H., Reshetnikova, L., Reiser, C. O. A., Schirmer, N. K., Sprinzl, M., and Hilgenfeld, R. (1993) Nature 365, 126–132
28. Polekhina, G., Thirup, S., Kjeldgaard, M., Nissen, P., Lippmann, C., and Nyborg, J. (1999) Structure 4, 1141–1151
29. Parry, M. A. A., Fernandez-Catalan, C., Bergner, A., Huber, R., Hopfner, K.-P., Scholott, B., Ghrs, K.-H., and Bode, W. (1998) Nat. Struct. Biol. 5, 917–923
30. Neidhardt, F. C., Ingraham, J. L., and Schaefer, M. (1990) molec. Biology of Bacteria 4, 205–212
31. Black, L. W., Showe, M. K., and Steven, A. C. (1994) in Molecular Biology of Bacteriophage T4 (Karam, J. D., ed) pp. 213–218, ASM Press, Washington, D. C.
32. Black, L. W., Showe, M. K., and Steven, A. C. (1994) in Molecular Biology of Bacteriophage T4 (Karam, J. D., ed) pp. 218–258, ASM Press, Washington, D. C.
33. Kudlicki, W., Kramer, G., and Harbes, B. (1997) J. Biol. Chem. 272, 32206–32210
34. Georgopoulos, C. P., and Linder, C. H. (1994) in Molecular Biology of Bacteriophage T4 (Karam, J. D., ed) pp. 213–218, ASM Press, Washington, D. C.
35. Kudlicki, W., Kramer, G., and Harbes, B. (1997) J. Biol. Chem. 272, 32206–32210
36. Cimarelli, A., and Luban, J. (1999) J. Virol. 73, 5388–5401
37. Swanstrom, R., and Wills, J. W. (1997) in Retroviruses (Coffin, J. M., Hughes, S. H., and Varmus, H. E., eds) pp. 263–334, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
38. Kawaguchi, Y., Bruni, R., and Roizman, B. (1997) J. Virol. 71, 1019–1024
39. Das, T., Mathur, M., Gupta, A. K., Jain, M. C., and Banerjee, A. K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95 1449–1454
40. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950