Regulation of the Activity of Chloroplast Translational Initiation Factor 3 by NH$_2$- and COOH-Terminal Extensions*

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Nan-Jun Yu‡ and Linda L. Spremulli§¶

From the Department of Chemistry and Lineberger Comprehensive Cancer Research Center, University of North Carolina, Chapel Hill, North Carolina 27599-3290

The mature form of the chloroplast translational initiation factor 3 (IF3chl) from Euglena gracilis consists of an internal region homologous to prokaryotic IF3 flanked by long NH$_2$- and COOH-terminal extensions. Sequences in these extensions reduce the activity of the homology domain in promoting initiation complex formation with chloroplast mRNAs and 30 S ribosomal subunits. A series of deletions of the NH$_2$- and COOH-terminal extensions of IF3chl were constructed and tested for their effects on the activity of the homology domain. About half of the inhibitory effect arises from sequences within 9 residues of the junction between the NH$_2$-terminal extension and the homology domain. The remaining inhibitory effect is the result of sequences in the COOH-terminal extension. The equilibrium constant governing the binding of the homology domain of IF3chl to 30 S subunits is estimated to be 1.3 × 10$^7$ M$^{-1}$. Sequences close to the junction of the NH$_2$-terminal extension and the homology domain reduce this binding constant about 10-fold. Sequences in the COOH-terminal extension have a similar negative effect. The negative effects of these two regions are cumulative, resulting in a 100-fold reduction of the binding constant. The 9 residues at the NH$_2$-terminal extension effectively prevent the proofreading activity of IF3chl. The entire COOH-terminal extension reduces the proofreading ability by about half. These results are discussed in terms of the proposed three-dimensional structure of the homology domain of IF3chl.

Three translational initiation factors (IF1, IF2, and IF3) are required for the initiation of protein synthesis in Escherichia coli (1, 2). During initiation, IF3 binds to the 30 S subunit and shifts the equilibrium between the ribosome and its subunits toward dissociation (3, 4). IF1 and IF2 bind to the 30 S subunit complex. The initiation factor-30 S complex binds the mRNA and fMet-tRNA, resulting in the formation of an unstable preinitiation complex. This complex is converted into a stable initiation complex when the initiator tRNA has been selected and codon-antidcodon interaction occurs (5, 6). IF3 has three major functions: 1) it binds to the 30 S subunit, preventing the joining of 50 S subunits (1–3, 5); 2) it increases the affinity of IF1 and IF2 for the 30 S subunit and stimulates fMet-tRNA binding to the 30 S subunit by promoting the conversion of the preinitiation complex to the initiation complex (7, 8); and 3) it proofreads the selection of fMet-tRNA at an AUG initiation codon (9–14).

The chloroplast translational initiation factors are postulated to be functionally analogous to their E. coli counterparts. Only IF2chl and IF3chl from Euglena gracilis have been purified (15, 16). Both of these factors are nuclear-encoded proteins in this organism (17, 18). IF3chl has been resolved into three forms, α, β, and γ. The α form has a molecular mass of about 34 kDa, whereas the β and γ forms have molecular masses of about 45 kDa (16). In contrast, E. coli IF3 has a molecular mass of 20 kDa. IF3chl is active on E. coli ribosomes.

A complete cDNA encoding E. gracilis IF3chl has been cloned and sequenced (17). The molecular mass deduced from the nucleotide sequence is 58 kDa, including a signal peptide of 130–140 residues required for localization to the chloroplast. The mature form of this factor (IF3chlM) can be divided into three parts (Fig. 1). An NH$_2$-terminal extension termed the head (Hd) region encompasses the first 140 amino acids. This region contains a proline-rich sequence followed by a (GX)$_2$ motif and a short acidic sequence. A middle region of about 180 amino acids shows homology to prokaryotic IF3 and is referred to as the homology (H) domain (19). Structural analysis of E. coli and Bacillus stearothermophilus IF3 indicates that this region will fold into two highly compact domains separated by a lysine-rich linker (20–24). The COOH-terminal extension is referred to as the tail (T) region. This 64-amin o acid region is rich in glutamic acid residues (17).

Previous studies have shown that both IF3chlM and the homology domain, IF3chlH, are active in promoting the dissociation of ribosomal subunits and in promoting initiation complex formation on E. coli ribosomes using poly(A,U,G) as an mRNA (19). However, IF3chlM is only 10–20% as active as IF3chlH in promoting initiation complex formation on chloroplast 30 S ribosomal subunits using mRNAs carrying natural translational start sites for chloroplast mRNAs (19). These observations suggest that sequences in the head and tail regions of IF3chl down-regulate the activity of this factor in initiation. In the present work, the roles of sequences in the head and tail regions in affecting the activity of IF3chl have been examined in more detail.

EXPERIMENTAL PROCEDURES

Materials—[$^{35}$S]fMet-tRNA and [14C]AcPhe-tRNA were prepared as described (25, 26). A plasmid carrying the 5’ untranslated leader region and the translational start site of the E. gracilis chloroplast rbcL gene fused in-frame to an internal coding region of the neomycin phosphotransferase gene was transfected in vitro providing the mRNA, mRbcN (27). E. coli ribosomes, initiation factors, E. gracilis chloroplast 30 S subunits, IF3chl, IF3chlH, and IF3chlH antisem were prepared as described (16, 19, 28–31).

Induction and Purification of Various Derivatives of IF3chl—Qiagen
pQE vectors were used to express IF3chl or its derivatives carrying a His tag at the COOH terminus. The regions of IF3chl to be expressed were amplified by polymerase chain reaction using the cDNA clone described previously (17) or a derivative of this plasmid as template. Cells were grown and IF3chl derivatives were induced as described previously (19). Induction times were as follows: 45 min for IF3chlM, 20 min for IF3chlHT, and 2–3 h for the remaining constructs. IF3chlHT was purified as described for IF3chlM (19). The other forms of IF3chl were purified using the two-step purification procedure developed for IF3chlH (19).

Binding of IF3chl to 30 S Subunits—The indicated concentrations of IF3chl and chloroplast 30 S subunits were incubated in a total volume of 250 μl in 50 mM Tris-HCl, pH 7.8, 10 mM dithiothreitol (DTT), 50 mM NH4Cl, and 10 mM MgCl2 at room temperature for 5 min. The mixture was applied to a 5-ml 10–30% linear sucrose gradient prepared in the same buffer except that the concentration of Tris-HCl was reduced to 10 mM. Samples were subjected to centrifugation at 48,000 rpm for 2 h in a Beckman SW50.1 rotor. Gradients were fractionated at a flow rate of 1 ml/min. Fractions (100 μl) were collected from the region of the gradient containing the 30 S subunits. Aliquots (50 μl) of appropriate fractions were analyzed for the amount of IF3chl present using an ELISA (32). A standard curve for each derivative tested was determined following dilution with 1 ml of prewarmed dilution buffer (50 mM Tris-HCl, pH 7.8, and 50 mM NH4Cl). The amount of IF3chl in each fraction was determined using a nitrocellulose filter binding assay (19).

RESULTS

Inhibitory Effects of the Head and the Tail Regions on the Activity of the Homology Domain of IF3chl—Previous studies have shown that the mature form of IF3chlM is almost as active as the homology domain (IF3chlH) in an assay that measures the ability of IF3chl to promote the binding of fMet-tRNA to E. coli 70 S ribosomes using poly(A,U,G). However, IF3chlM shows very poor activity in promoting the binding of fMet-tRNA to chloroplast 30 S subunits in the presence of an mRNA carrying the translational initiation region of a natural mRNA (19). This observation indicates that the head, the tail, or both have a negative effect on the activity of the homology domain of IF3chl. To investigate which region or regions of IF3chlM are responsible for this inhibitory effect, the activities of a series of derivatives of IF3chl containing different parts of the homology domain of IF3chl were determined as indicated (19).

Proofreading Assay—This assay has been modified from the method described in Ref. 33 for E. coli IF3. A complex carrying AcPhe-tRNA bound to chloroplast 30 S subunits (AcPhe-tRNA-poly(U)-30 S) was formed by incubation of chloroplast 30 S subunits (10 pmol) with poly(U) (2.5 μg) and AcPhe-tRNA (4 pmol) in a reaction mixture (50 μl) containing 50 mM Tris-HCl, pH 7.8, 10 mM dithiothreitol, 50 mM NH4Cl, and 15 mM MgCl2. After incubation at 37 °C for 30 min, the mixture was diluted 2-fold with 50 mM Tris-HCl, pH 7.8, and 50 mM NH4Cl in the presence of different concentrations of IF3chl or its derivatives. Mixtures were incubated for an additional 5 min at 37 °C. The destabilization of the complex by IF3chl was monitored following dilution with 1 ml of prewarmed dilution buffer (50 mM Tris-HCl, pH 7.8, 10 mM dithiothreitol, 50 mM NH4Cl, and 7.5 mM MgCl2). This reaction mixture was incubated at 37 °C for 5 min. The amount of initiation complex remaining was determined by a nitrocellulose filter binding assay (19).

A similar assay was also carried out using a complex formed with 30 S subunits (10 pmol), poly(A,U,G) (2.5 μg) and fMet-tRNA (4 pmol).

FIG. 1. Derivatives of IF3chl. A, the overall structures of E. coli IF3, B. stearothermophilus IF3, and IF3chlM are shown. The open area represents the homology domain. For E. coli IF3, the striped area represents the 9 residues at the NH2 terminus. For IF3chlM, the black area represents the head, and the cross-hatched area represents the tail. B, the regions of IF3chl present in each of the derivatives. The residues encompassed in each construct are IF3chl, 130–538; IF3chlM, 130–489; IF3chlHT, 278–538; IF3chlHdH, 278–489; IF3chlH, 284–476; IF3sHT, 293–498; IF3srH, 293–498; IF3srH, 293–498; IF3srH, 293–498; IF3hrH, 293–498; IF3chH, 293–498; and IF3chlHT, 293–498. The numbering is based on the initiator Met as residue 1. The transit peptide is predicted to be 130–140 amino acids in length.
is 9 residues shorter than *E. coli* IF3 at the NH₂ terminus.

Chloroplast homologues of both these prokaryotic IF3s were prepared (Fig. 1). IF3<sub>chl</sub>rH is the homologue of *B. stearothermophilus* IF3, whereas IF3<sub>chl</sub>rH is the homologue of *E. coli* IF3. These two forms of IF3<sub>chl</sub> differ by 9 residues at the NH₂ terminus. To test the effects of sequences in the NH₂-terminal extension, a derivative, IF3<sub>chl</sub>rHdH, encompassing the homology domain and the entire head region was prepared. To test the effects of sequences in the COOH-terminal extension, a derivative, IF3<sub>chl</sub>sHT, covering the homology domain and the entire tail region was prepared. Note that this derivative of IF3<sub>chl</sub>rH begins at the position corresponding to the start of the *B. stearothermophilus* factor.

The induction of IF3<sub>chl</sub>sHT, like IF3<sub>chl</sub>M, results in a significant decrease in cell growth, indicating that the tail of IF3<sub>chl</sub>rH is quite toxic to the cell. The induction of IF3<sub>chl</sub>rHdH has less effect on cell growth, whereas the expression of IF3<sub>chl</sub>rHrH does not affect cell growth to an appreciable extent (data not shown). Each derivative of IF3<sub>chl</sub>rH was purified; the derivatives were estimated to be 90–95% pure in all cases (Fig. 2).

The activity of each construct in promoting the binding of fMet-tRNA to *E. coli* 70 S ribosomes was examined. As indicated in Fig. 3A, all of these forms of IF3<sub>chl</sub>rH were quite active in this assay. IF3<sub>chl</sub>rHdH and IF3<sub>chl</sub>sHT had slightly less activity than IF3<sub>chl</sub>rH but slightly more activity than IF3<sub>chl</sub>M. These results indicate that the head and tail had little effect on the activity of IF3<sub>chl</sub>rH when *E. coli* 70 S ribosomes and a synthetic mRNA, poly(A,U,G), were used. These derivatives of IF3<sub>chl</sub>rH were then tested for the ability to promote the binding of fMet-tRNA to chloroplast 30 S subunits using an mRNA carrying the translational initiation region of the *rbcL* gene. As shown previously and as indicated in Fig. 3B, IF3<sub>chl</sub>rH had only 15–20% of the activity of the homology domain of IF3<sub>chl</sub>rH in this assay. The effect of sequences in the head was assessed by comparing the activity of IF3<sub>chl</sub>rH with IF3<sub>chl</sub>sHT (Fig. 3B). The head region reduced the activity of the homology domain by 2-fold, indicating that the head accounts for about half of the reduction in activity seen with IF3<sub>chl</sub>M. To assess the effect of sequences in the tail, the activity of IF3<sub>chl</sub>sHT was tested. IF3<sub>chl</sub>sHT had about 30% of the activity seen with IF3<sub>chl</sub>rH (Fig. 3B), indicating that sequences in the tail account for a little over half of the inhibitory effect seen in IF3<sub>chl</sub>rH.

**A Small Region of the Head Is Sufficient to Confer Its Full Inhibitory Effect**—The results presented above indicate that sequences in both the head and the tail of IF3<sub>chl</sub>rH have a negative effect on the ability of the homology domain to promote initiation complex formation. Additional constructs were then prepared to narrow down the inhibitory region in the head. IF3<sub>chl</sub>rH, the *E. coli* homologue, is 9 residues longer than IF3<sub>chl</sub>rHrH at the NH₂ terminus (Fig. 1). The induction of either IF3<sub>chl</sub>rH or IF3<sub>chl</sub>rHrH retards cell growth indicating that their expression is toxic to *E. coli* (data not shown). Both IF3<sub>chl</sub>rH and IF3<sub>chl</sub>rHrH were purified (Fig. 2, lanes 3 and 6). IF3<sub>chl</sub>rH and IF3<sub>chl</sub>rHrH were as active as IF3<sub>chl</sub>sHT/3 when tested on *E. coli* 70 S ribosomes (Fig. 4A). However, IF3<sub>chl</sub>rH and IF3<sub>chl</sub>rHrH, like IF3<sub>chl</sub>rHdH, had half the activity of IF3<sub>chl</sub>sHT when tested on chloroplast 30 S subunits (Fig. 4B). These results indicate that only 9 residues in the NH₂-terminal extension measured from the *B. stearothermophilus* homologue to the edge of (GX)<sub>12</sub>-acidic motif (19). IF3<sub>chl</sub>rH, the *E. coli* homologue, is 9 residues longer than IF3<sub>chl</sub>rHrH at the NH₂ terminus (Fig. 1). The induction of either IF3<sub>chl</sub>rH or IF3<sub>chl</sub>rHrH retards cell growth indicating that their expression is toxic to *E. coli* (data not shown). Both IF3<sub>chl</sub>rH and IF3<sub>chl</sub>rHrH were purified (Fig. 2, lanes 3 and 6). IF3<sub>chl</sub>rH and IF3<sub>chl</sub>rHrH were as active as IF3<sub>chl</sub>sHT/3 when tested on *E. coli* 70 S ribosomes (Fig. 4A). However, IF3<sub>chl</sub>rH and IF3<sub>chl</sub>rHrH, like IF3<sub>chl</sub>rHdH, had half the activity of IF3<sub>chl</sub>sHT when tested on chloroplast 30 S subunits (Fig. 4B). These results indicate that only 9 residues in the NH₂-terminal extension measured from the *B. stearothermophilus* factor are required to give the inhibitory effect of the entire head region. This observation is quite surprising because IF3<sub>chl</sub>rH is the same length at the NH₂ terminus as *E. coli* IF3. The activity of *E. coli* IF3 decreases markedly without the NH₂-terminal hexapeptide (34).

**Effect of the Tail on IF3<sub>chl</sub>rH and Additive Effects of Sequences in the Head and Tail Regions**—As indicated in Fig. 3B, the tail region contributed about half of the negative regulatory effect seen with the mature form of IF3<sub>chl</sub>rH. Secondary structure analysis indicated that the tail probably contains two long helices that have a high probability of forming a coiled-coil. To gain further insight into which sequences in the tail might be responsible for this result, a derivative was prepared (IF3<sub>chl</sub>sHT/3) that contained about ⅛ of the sequences in the tail encompassing residues through the first helix (Fig. 1). The
induction of IF3chlHT/3 had little effect on the growth of *E. coli*. IF3chlHT/3 was purified to greater than 95% purity (Fig. 2, lane 8). The induction of chloroplast 30 S subunits and mRNA were used, IF3chlHT/3 was as active as IF3chlsrH (Fig. 5B). This result indicates that the last 2/3 of the tail region are essential for the inhibitory effect of the tail.

To test the effects from the short NH2- and the entire COOH-terminal extension, IF3chlHT, consisting of the homology domain surrounded by the tail and 15 residues of the head (Fig. 1), was prepared and purified (Fig. 2, lane 8). The induction of IF3chlHT resulted in a significant decrease in cell growth and eventually appeared to cause cell lysis. IF3chlHT had activity slightly lower than that of IF3chlsrH but the same as that of IF3chlHT and IF3chlM when tested on *E. coli* 70 ribosomes with poly(A,U,G) (Fig. 5A). When chloroplast 30 S subunits and mRNA were used, IF3chlHT had the same low activity observed with IF3chlM (Fig. 5B). These results indicate that IF3chlHT contains all the negative regulatory elements present in IF3chlM and that the negative effects due to sequences in the head and tail are additive.

**Basis for the Inhibitory Effect of Sequences in the Head and Tail on the Activity of IF3chl**—In an attempt to understand whether the low activity of IF3chlHT could be overcome by raising the concentrations of 30 S subunits, mRNA, or IF2, assays were carried out using different amounts of each component, separately. As indicated in Fig. 6, increasing the concentration of chloroplast 30 S subunits, mRNA, or IF2 did not allow IF3chlHT to increase its activity relative to the activity of IF3chlsrH. Similar results were obtained when the levels of either IF2chl or *E. coli* IF2 were varied. These observations suggest that the low activity of IF3chlHT is a complex phenomenon involving the interplay of IF3chlHT with multiple components of the initiation machinery.

The activities of several derivatives of IF3chl in promoting initiation complex formation on chloroplast 30 S subunits were tested in the presence of either the α or the β form of IF2chl (data not shown) and *E. coli* IF2. The results of this study indicated that all of the negative regulatory effects from the head and tail regions are seen in the presence of either form of IF2chl or *E. coli* IF2. The natural mRNA used above (mRbcN) carries the initiation region of the *rbcL* gene. This region does not have a Shine/Dalgarno sequence. Indeed, about half of the chloroplast mRNAs in *E. gracilis* lack a Shine/Dalgarno sequence (35, 36). The negative effects of the head and tail were also tested with an mRNA carrying the translational start site for the *atpH* gene, which has a Shine/Dalgarno sequence just upstream of the start codon. The head and tail also inhibited the activity of the homology domain when this mRNA was used (data not shown).

Direct measurements of the abilities of various derivatives of IF3chl to bind to chloroplast 30 S subunits were carried out using sucrose density gradient centrifugation. For these experiments, the appropriate derivatives of IF3chl were incubated with chloroplast 30 S subunits. The bound factor was separated from the free factor by sucrose gradient centrifugation. The amount of IF3chl bound to the 30 S subunit was quantified...
using an enzyme-linked immunosorbent assay. The amount of IF3chl bound was calculated based on a standard curve providing a measure of the response of each IF3 chl derivative to the antibody. The standard curves for each of the derivatives are quite similar (Table I). This observation was expected because the antibodies were raised against the homology domain. The total amount of each derivative of IF3chl bound to 30 S subunits and the estimated $K_{\text{obs}}$ are indicated in Table I. IF3srH had the highest affinity for 30 S subunits, with a $K_{\text{obs}} = 1.3 \times 10^7 \text{ M}^{-1}$. This value is similar to the affinity of E. coli IF3 for E. coli 30 S subunits ($K = 2.5 \times 10^7 \text{ M}^{-1}$) (37). IF3chlerH and IF3chlsHT bound to 30 S subunits with about 10-fold lower affinity than IF3chlsrH. IF3chlsHT showed the lowest ability to bind, with a $K_{\text{obs}}$ approximately 100-fold lower than that of IF3chlsrH. These results suggest that the small NH$_2$-terminal extension region and the full tail interfere with the ability of IF3chl to bind to 30 S subunits. IF3chlsHT, which contains both regions, showed the lowest affinity for chloroplast 30 S subunits. Because the low activity of IF3chlHT was not overcome by raising the concentration of 30 S subunits (Fig. 6), the head and tail must still down-regulate the activity of IF3chl after this factor binds to 30 S subunits.

### Table I

| IF3 type | [IF3]$_0$ M$^a$ | [30 S$_{chl}$]$_0$ M$^a$ | IF3-bound, pmol$^b$ | $K_{\text{obs}}$ M$^{-1}$ |
|----------|----------------|----------------|----------------|----------------|
| IF3srH   | 1x10$^{-7}$    | 1x10$^{-7}$    | 10.0           | 1.1x10$^7$     |
| IF3srH   | 4x10$^{-4}$    | 1x10$^{-7}$    | 5.4            | 1.5x10$^7$     |
| IF3erH   | 1x10$^{-7}$    | 1x10$^{-7}$    | 5.2            | 3.2x10$^6$     |
| IF3erH   | 1x10$^{-7}$    | 1x10$^{-7}$    | 4.8            | 2.9x10$^6$     |
| IF3sHT   | 1x10$^{-7}$    | 1x10$^{-7}$    | 4.8            | 2.9x10$^6$     |
| IF3sHT   | 1x10$^{-7}$    | 1x10$^{-7}$    | 4.3            | 2.5x10$^6$     |
| IF3HT    | 1x10$^{-7}$    | 1x10$^{-7}$    | 0.9            | 3.8x10$^5$     |
| IF3-3HT  | 2x10$^{-7}$    | 2x10$^{-7}$    | 4.8            | 5.9x10$^5$     |

$^a$ [IF3]$_0$ and [30 S$_{chl}$]$_0$ indicate the initial concentrations of the indicated derivatives of IF3chl and of 30 S subunits.

$^b$ Quantitated from the standard curve shown to the left.

$^c$ Calculated from the equation 30 S + IF3$_{chl}$ $\rightleftharpoons$ [30 S IF3$_{chl}$].

**Prooﬁng Ability of IF3chl and Its Derivatives**—In E. coli, IF3 is believed to proofread the selection of the initiator tRNA and the AUG start codon (10–12, 38). One procedure for monitoring this function is to examine the ability of IF3chl to bind to 30 S subunits. IF3chl, which contains both regions, showed the lowest affinity for chloroplast 30 S subunits. Because the low activity of IF3chlHT was not overcome by raising the concentration of 30 S subunits (Fig. 6), the head and tail must still down-regulate the activity of IF3chl after this factor binds to 30 S subunits.
codon, which remains resistant to the destabilization induced by IF3 (39).

The ability of IF3chl to proofread in the chloroplast system was examined by testing its ability to promote the dissociation of a preformed 30S poly(U)-AcPhe-tRNA complex. IF3chl has the greatest ability to destabilize the 30S poly(U)-AcPhe-tRNA complex (Fig. 7A). This observation is in agreement with its greater ability to bind to 30S subunits and to promote initiation complex formation. IF3chl shows the least activity in this assay, however, it still has some ability to proofread. Surprisingly, all of the reduced proofreading ability seen with IF3chl is also observed with IF3chlsrH. IF3chl has more than half of the proofreading ability of IF3chl. These observations suggest that sequences in the head region interfere with proofreading to a greater extent than those in the tail region. The ability of derivatives of IF3chl to discriminate between initiation complexes containing fMet-tRNA was also tested (Fig. 7B). None of the derivatives that were examined destabilized the binding of fMet-tRNA to 30S subunits. Indeed, some stimulation of fMet-tRNA binding was observed with IF3chl even under the dilute conditions used in this assay. This stimulation presumably reflects the high activity of this derivative in promoting initiation complex formation.

**DISCUSSION**

IF3chloroplast from *E. gracilis* is the first organellar IF3 that has been cloned and over-expressed. The results presented here indicate that a 9-residue sequence in the head region of IF3chloroplast and sequences in the tail play a negative regulatory role in promoting initiation complex formation on chloroplast ribosomes. Structural studies on *E. coli* and *B. stearothermophilus* IF3 (20–22) indicate that both factors fold into two compact domains separated by a lysine-rich linker (Fig. 8). These two domains are formed by the independent folding of sequences in the NH2-terminal and COOH-terminal halves of the protein. The center of mass of the two domains are separated by about 45 Å (22). The crystal structures of the NH2-domain and COOH-domain of *B. stearothermophilus* IF3 taken from the x-ray coordinates. The two domains were crystallized separately, and their exact orientation relative to one another is not well understood. The centers of mass of the two domains are about 45 Å apart (22). Both the NH2 and COOH termini are oriented toward the central linker region.

One attractive hypothesis is that these regions down-regulate the intrinsic activity of IF3chloroplast and that other factors in the chloroplast alleviate this inhibition under appropriate conditions. This idea is based on numerous observations that indicate that chloroplast protein synthesis is regulated in response...
to light and mRNA-specific trans-acting factors (40–47). Because IF3chl is required for the translation of all mRNAs, it could play a key role in modulating the activity of the chloroplast translational system as a whole, for example, in response to light or developmental signals. In addition, trans-acting factors bound to specific chloroplast mRNAs could interact with IF3chl to recruit this factor for the translation of a specific mRNA. The most logical region of IF3chl to interact with such putative regulatory proteins is the head. The rationale for this idea is as follows. The head has an unusual amino acid sequence and, presumably, structure. It contains a Pro-rich region reminiscent of many protein-protein interaction sites and their flanking regions (48–54). Prominent examples of such sites include proteins recognized by SH3 domains or the WW motif found in many proteins participating in regulatory cascades. The (GX)n motif (glycine-X motif, where X indicates a large basic hydrophobic residue) following the Pro-rich region would be expected to have significant structural flexibility and could function as a flexible hinge region.

In a working model (Fig. 9), IF3chl is visualized as being in a low activity state due to the negative effects from the extensions on the homology domain (Fig. 9). In this low activity state, the activity of IF3chl would limit the rate of translation in the chloroplast to some basal amount. This level would, presumably, allow the chloroplast to maintain the amounts of critical proteins at minimum required levels. In the presence of appropriate environmental signals (for example, in conditions promoting photosynthesis), a regulatory factor interacts with the head on IF3chl relieving the inhibitory effects and allowing the homology domain to become fully active. A protein affecting the activity of IF3chl could potentially act either in general, increasing the overall rate of chloroplast protein synthesis, or more specifically, promoting the translation of specific mRNAs. In the latter case, IF3chl can be envisioned as playing a role in tying mRNA-specific trans-acting factors to the general translational machinery. Current efforts are designed to gain insight into the factors that modulate the activity of IF3chl and, thus, the rate of chloroplast protein synthesis.

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