Characterization of GTP-dependent Met-tRNA\textsubscript{f} Binding Protein*

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ALICE BARRIEUX AND MICHAEL G. ROSENFELD

From the Division of Endocrinology, Department of Medicine, University of California, San Diego, School of Medicine, La Jolla, California 92039

Purified GTP-dependent Met-tRNA\textsubscript{f}, binding protein (E\textsubscript{IF}{\textsubscript{2}}) prepared from the 0.5 M KCl eluate of reticulocyte polyribosomes was successfully resolved into its three-component subunits by isoelectric focusing in the presence of urea. The 37,000-dalton subunit focused at a pH of 5.8 and was resolved into two spots; the 18,000-dalton subunit focused at a pH of 6.6 and was resolved into three spots; and the 52,000-dalton subunit exhibited an isoelectric point of 8.9 and migrated as a single spot. When isolated 37,000- and 48,000-dalton subunits were assayed for functional activity, the 48,000-dalton subunit was found to possess Met-tRNA\textsubscript{f} and mRNA binding activities while the 37,000-dalton subunit was found to possess GDP binding activity. Phosphorylation of the 37,000- and 48,000-dalton subunits of E\textsubscript{IF}{\textsubscript{2}} by cAMP-independent protein kinases has been reported by several laboratories (20, 21, 23, 24). The present report describes the separation of the three subunits of E\textsubscript{IF}{\textsubscript{2}} via isoelectric focusing under denaturing conditions; apparent molecular weights of 52,000, 48,000, and 37,000 were calculated for these proteins. The 48,000-dalton subunit possessed Met-tRNA\textsubscript{f} and mRNA binding activities, and was preferentially phosphorylated when GTP was used as the phosphate donor. Three phosphorylated forms of this subunit could be demonstrated and represented the predominant forms associated with ribosomal subunits. The 37,000-dalton subunit possessed GDP binding activity and was preferentially phosphorylated when ATP was used as the phosphate donor.

EXPERIMENTAL PROCEDURES

Materials

Rabbit liver initiator methionine tRNA (Met-tRNA\textsubscript{f}) (95% pure) was purchased from BioGenics Research Co.; ATP, GTP, and GDP, from P-L Biochemicals; [\textsuperscript{35}S]methionine (300 to 500 Ci/mmol), [\textsuperscript{3}H]uridine (40 to 70 Ci/mmol), and \(\textsuperscript{32}P\)orthophosphoric acid (carrier-free, in water), from New England Nuclear. \([\textsuperscript{3}H]\)GDP (10 Ci/mmol) and ultrapure grade of urea were obtained from Schwarz/Mann; nucleoside diphosphate kinase, \(\gamma\)-phosphoglycerate kinase, and glyceraldehyde-3-phosphate dehydrogenase from Boehringer-Mannheim Co.; oligo(dT)-cellulose (T\textsubscript{1}) from Collaborative Research Inc.; ampholytes from LKB; acrylamide and methylenebisacrylamide from Bio-Rad Laboratories; N,N',N'-tetramethylethylenediamine, Tris base, and Triton X-100 from Sigma Chemical Co.; and sodium dodecyl sulfate from BDH Chemicals, Ltd.

Methods

Preparation of GTP-dependent Met-tRNA\textsubscript{f}, Binding Protein (E\textsubscript{IF}{\textsubscript{2}}) - E\textsubscript{IF}{\textsubscript{2}} was prepared from the 0.5 M KCl eluate of reticulocyte ribosomes via ammonium sulfate fractionation, phosphocellulose chromatography, and, where indicated, DEAE-cellulose chromatography as previously described (19). Protein was quantitated by the method of Lowry et al. (35), using bovine serum albumin as standard. E\textsubscript{IF}{\textsubscript{2}} was stored at -70\(^\circ\)C in 10 mM Tris/HC1 (pH 7.6), 100 mM several laboratories and has been reported to exhibit a molecular weight of 150,000 to 160,000 (11, 14, 17, 18) and to contain either two subunits of 52,000 and 34,000 to 39,000 molecular weights (11, 19) or three subunits of 52,000 to 57,000, 47,000 to 52,000, and 32,000 to 37,000 molecular weights (14, 18, 20, 21). In addition, evidence suggesting that E\textsubscript{IF}{\textsubscript{2}} can specifically bind mRNA has been presented (19, 22). The phosphorylation of the 37,000- and 48,000-dalton subunits of E\textsubscript{IF}{\textsubscript{2}} by cAMP-independent protein kinases has been reported by several laboratories (20, 21, 23, 24). The present report describes the separation of the three subunits of E\textsubscript{IF}{\textsubscript{2}} via isoelectric focusing under denaturing conditions; apparent molecular weights of 52,000, 48,000, and 37,000 were calculated for these proteins. The 48,000-dalton subunit possessed Met-tRNA\textsubscript{f} and mRNA binding activities, and was preferentially phosphorylated when GTP was used as the phosphate donor. Three phosphorylated forms of this subunit could be demonstrated and represented the predominant forms associated with ribosomal subunits. The 37,000-dalton subunit possessed GDP binding activity and was preferentially phosphorylated when ATP was used as the phosphate donor. Analysis of E\textsubscript{IF}{\textsubscript{2}} associated with ribosomal subunits, following its purification, revealed that the 37,000-dalton subunit was predominately in the dephosphorylated form following purification.

The factors required for the initiation of eukaryotic protein synthesis are incompletely defined. One of these factors, referred to by various nomenclatures, has been characterized as a protein which forms a ternary complex with GTP and Met-tRNA\textsubscript{f}, referred to by various nomenclature, has been characterized as a GTP-dependent Met-tRNA\textsubscript{f}, binding protein, referred to by various nomenclature. The present report describes the separation of the three subunits of E\textsubscript{IF}{\textsubscript{2}} via isoelectric focusing in the presence of urea, followed by changes in the adenylate energy charge (15, 16). The GTP-dependent Met-tRNA\textsubscript{f}, binding protein has been purified in

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‡ The abbreviations used are: E\textsubscript{IF}{\textsubscript{2}}, eukaryotic initiation factor 2; Met-tRNA\textsubscript{f}, methionine tRNA; GTP, guanosine triphosphate; GDP, guanosine diphosphate; mRNA, messenger RNA; poly(A)-rich mRNA, polyadenylated messenger RNA; I\textsubscript{H}urRNA, \(\textsuperscript{[3H]}\)uridine-labeled poly(A)-rich mRNA from Ehrlich ascites tumor cells.

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KCl, 1 mM diethiothreitol, 0.1 mM EDTA, 10% glycerol (Buffer A).

Two-dimensional gel electrophoresis was performed as described by O'Farrell (26) with the following modifications. In the first dimension gel, N\textsubscript{a}N\textsubscript{b}N\textsubscript{c}N\textsubscript{d}-tetramethylenediamine was omitted and the nonionic detergent "NP-40" was replaced by 0.5% Triton X-100. Isoelectric focusing gels (1.4 x 10.5 cm) contained either 8 or 9 M urea and were electrophoresed at 600 V for 12 h. The sample in Buffer A was adjusted to 2% ampholites (pH 5.5 to 10 or pH 5 to 8), 0.5% Triton X-100, 100 mM diethiothreitol, and either 8 M or 9 M urea. Isoelectric focusing was run at 4° where the lower urea concentration was present in the system. After isoelectric focusing, the gels were shaken at 45° for 5 min in 10% sodium dodecyl sulfate, 100 mM diethiothreitol, 0.15 M Tris/sulfate (pH 6.4), the discontinuous pH 9.18 system of Neville (27) was used with modifications previously reported (28). When indicated, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed using the method of Laemmli (29).

Preparation of [\textsuperscript{35}S]Met-tRNA\textsubscript{f}, [\textsuperscript{32}P]mRNA, and [\gamma-\textsuperscript{32}P]GTP

When EIF\textsubscript{f} was tested for binding activity after isoelectric focusing, the first dimension gels were sliced in 0.8-cm fractions; each fraction was placed in a dialysis bag with 200 μl of buffer containing 10 mM Tris/HC1 (pH 7.6), 30 mM KC1, 1 mM diethiothreitol, 1 mM MgCl\textsubscript{2}, 10% glycerol (Buffer B), and dialyzed against Buffer B for 18 h at 4°.

Preparation of [\textsuperscript{35}S]Met-tRNA\textsubscript{f}, [\textsuperscript{32}P]mRNA, and [\gamma-\textsuperscript{32}P]GTP

Triphosphate Nucleotides - Initiator Met-tRNA\textsubscript{f}, was charged in the presence of a 2-fold excess of [\textsuperscript{35}S]methionine (540 Ci/mM) as previously described (30). [\textsuperscript{35}S]Uridine-labeled poly(A)-rich RNA was prepared from Ehrlich ascites tumor cells using sodium dodecyl sulfate, phenol, chloroform extraction, and oligo(dT)-cellulose chromatography (31); the specific activity was 10,000 to 18,000 cpm/μg of RNA. [\gamma-\textsuperscript{32}P]ATP and [\gamma-\textsuperscript{32}P]GTP were prepared by the method of Glynn and Chappell (32) with minor modifications.

Assay of Ternary (Met-tRNA\textsubscript{f},-GTP-EIF\textsubscript{f}) Complex Formation - Assay for GTP-dependent Met-tRNA\textsubscript{f}, binding activity was performed in 50 μl of reaction mixture containing 20 mM Tris/HC1 (pH 7.5), 60 mM KC1, 1 mM MgCl\textsubscript{2}, 1 mM dithiothreitol, 1 mM ATP, 0.1 mM GTP, 2 μg of beef liver nucleoside diphosphate kinase, and 3 to 10 pmol of [\textsuperscript{35}S]Met-tRNA\textsubscript{f} (40,000 to 120,000 cpm/pmol). Following incubation for 10 min at 30°, the reaction mixture was applied to a nitrocellulose filter (Millipore, 0.45 μm) which was washed with 30 ml of Tris/HC1 (pH 7.5), 90 mM KC1, dried, and counted as previously described (15, 19).

Assay for mRNA and GDP Binding - EIF\textsubscript{f}, was incubated at 30° for 10 min in the presence of 20 mM Tris/HC1 (pH 7.5), 60 mM KC1, 1 mM diethiothreitol (Buffer C), and 1 to 5 μg of [\textsuperscript{35}S]poly(A)-rich RNA or 30 to 55 pmol of [\textsuperscript{32}P]GDP. Reactions were terminated by the addition of 1 ml of ice-cold Buffer C and filtration through nitrocellulose filters (Millipore, 0.45 μm). Filters were washed with 40 ml of Buffer C, dried, and counted in 5 ml of toluene/Liquifluor. Binding of [\textsuperscript{32}P]GDP to the added protein represented less than 0.5% of added radioactivity and was subtracted from each experimental result.

Phosphorylation of EIF\textsubscript{f} - Reticulocytes obtained from anemic rabbits were lysed in 2 mM MgCl\textsubscript{2}, 1 mM dithiothreitol, adjusted to 0.25 M sucrose, and centrifuged at 10,000 × g for 15 min. The supernatant was further centrifuged at 150,000 × g for 3 h in order to pellet the ribosomal fraction. The pellet (1800 A\textsubscript{260} units) was resuspended in 10 ml of reticulocyte postribosomal supernatant which had been extensively dialyzed against 20 mM Tris/HC1 (pH 7.6), 30 mM KC1, 1 mM MgCl\textsubscript{2}, 1 mM dithiothreitol, and 0.25 M sucrose. The suspension was divided into two aliquots and either 5 μCi of [\gamma-\textsuperscript{32}P]ATP (6.9 Ci/mM) or 5 μCi of [\gamma-\textsuperscript{32}P]GTP (5.1 Ci/mM) were added; the aliquots were incubated at 25° for 30 min. Polysomal and subribosomal fractions were prepared and EIF\textsubscript{f}, was purified from the 0.5 M KC1 eluate of each fraction as previously described (28).

RESULTS

The purified GTP-dependent Met-tRNA\textsubscript{f}, binding protein was found to have a binding capacity of 1 to 2.5 pmol of Met-tRNA\textsubscript{f},/μg of EIF\textsubscript{f}, protein, comparable to values reported by others for this protein (11, 13-15, 18, 19). When the protein was dialyzed against water, lyophilized, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously reported (19), only two bands of approximately 48,000 and 37,000 apparent molecular weights were observed (Fig. 1). Under other conditions, such as subjecting EIF\textsubscript{f}, to isoelectric focusing under nondenaturing (Fig. 1) or denaturing conditions prior to sodium dodecyl sulfate-polyacrylamide gel electrophoresis or electrophoresis using the method of Laemmli (29), the same EIF\textsubscript{f}, preparation was resolved into three bands of 51,000 to 52,000, 48,000 to 49,000, and 37,000 to 38,000 apparent molecular weights. Isoelectric focusing the protein in the presence of 9 M urea revealed apparent isoelectric points of 5.4 to 5.8 for the 37,000-dalton subunit, and 6.2 to 6.6 for the 48,000-dalton subunit (Fig. 2). The pH gradient generated in the presence of urea did not exceed pH 7.8 even when amphotiles ranging from pH 3.5 to 10 were used; however, a basic pH gradient could be generated in the presence of amphotiles ranging from pH 9 to pH 11. Under these conditions, the two lighter subunits migrated out of the first dimension gel, while the 52,000-dalton subunit exhibited an apparent isoelectric point of 8.9 (data not shown).

Binding of Met-tRNA\textsubscript{f},, mRNA, and GDP to EIF\textsubscript{f}, Subunits - Because isoelectric focusing under denaturing conditions allowed separation of EIF\textsubscript{f}, subunits, the ability of each subunit to bind Met-tRNA\textsubscript{f},, GDP, and mRNA was tested. As shown in Fig. 3, following treatment with 9 M urea and isoelectric focusing, most of the recovered Met-tRNA\textsubscript{f}, binding activity was present in fractions containing the 48,000-dalton subunit. A small amount of binding remained at the basic end of the gel where the sample was applied. The total Met-tRNA\textsubscript{f}, binding activity recovered under these experimental conditions represented virtually 100% of the activity of EIF\textsubscript{f}, subjected to identical manipulations except isoelectric focusing, but only 15 to 20% of the initial activity. Since it is well recognized that EIF\textsubscript{f}, activity is very labile, the relative effects
of urea denaturation, dialysis, and dilution on activity were evaluated. As shown in Table I, the dilution and dialysis alone resulted in >70% loss of Met-tRNA, binding activity; this was largely prevented by addition of carrier protein during dialysis, resulting in a 80 to 90% recovery of activity. Even when EIF, was placed in 5 M urea for 6 h, dialysis in the presence of carrier protein resulted in recovery of 60 to 65% of the original Met-tRNA, binding capacity (Table I); however, the quantitative separation of the 48,000- and 37,000-dalton subunits during isoelectric focusing was inconsistent when 5 M urea was used, reflecting an incomplete denaturation of the protein. In contrast, complete denaturation was consistently achieved using 8 M urea at 4°; even dialysis in the presence of carrier protein resulted in no more than a 15 to 20% recovery of initial activity. Analysis of the subunits of EIF, separated by isoelectric focusing at 4° in 8 M urea revealed that the GDP binding activity and the Met-tRNA, binding activity could be separated (Fig. 4). GDP binding activity was present predominantly in fractions containing the 37,000-dalton subunit, while Met-tRNA, binding activity was present in fractions containing the 48,000-dalton subunit. The GDP binding activity recovered represented 25 to 35% of the binding capacity of the untreated EIF,.

Subunits to bind mRNA was examined. Two peaks of mRNA binding activity were reproducibly demonstrated; one migrating at the acidic end of the isoelectric focusing gels, and the other with the 48,000-dalton subunit (Fig. 3). Usually, no protein band was demonstrated by protein staining at the acidic end of the gel, although a 34,000-dalton phosphoprotein was present in this fraction (Fig. 5).

**Phosphorylation of EIF, in Reticulocyte Lysates** – As shown in Fig. 2, the 37,000-dalton subunit could be resolved into one major and one minor spot, while the 48,000-dalton subunit appeared as three discrete spots. Autoradiographic analysis of EIF, prepared from 32P-labeled ribosomal wash indicated that the two subunits could be phosphorylated. The phosphorylated 37,000-dalton subunit migrated with the minor spot of protein staining (Fig. 2b) of the subunit on isoelectric focusing gels, while the major spot of protein staining (Fig. 2b) contained little or no radioactivity. In addition, a protein of 34,000 apparent molecular weight was observed at the acidic end of the isoelectric focusing gel; this protein appeared to be responsible for the mRNA binding activity.
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placed in 200 μl of Buffer B containing 0.2 mg/ml of trypsin inhibitor and assayed for binding activity with addition of 5 pmol of [35S]Met-tRNA<sub>i</sub> (40,000 cpm/pmol) or 30 pmol of [3H]GDP (10,000 cpm/pmol) (see "Methods"). A third aliquot of each fraction was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis; the 37,000-dalton subunit was present in Fractions 4 and 5 (peak in 5), the 48,000-dalton subunit in Fractions 7 and 8 (peak in 8). Similar results were obtained in eight experiments of identical design utilizing five separate preparations of EIF<sub>i</sub>.

![FIG. 4. Met-tRNA<sub>i</sub>, and GDP binding by isolated EIF<sub>i</sub> subunits. EIF<sub>i</sub> (15 μg; GDP binding activity, 12.5 pmol) was subjected to isoelectric focusing at 4° in the presence of 8 M urea (see "Methods"). The fractions were numbered from the acidic end. The gel slices were placed in 200 μl of Buffer B containing 0.2 mg/ml of trypsin inhibitor, and dialyzed for 12 h at 4° against excess Buffer B. A 40-μl aliquot of each fraction was assayed for binding activity with addition of 5 pmol of [35S]Met-tRNA<sub>i</sub> (40,000 cpm/pmol) or 30 pmol of [3H]GDP (10,000 cpm/pmol) (see "Methods"). A third aliquot of each fraction was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis; the 37,000-dalton subunit was present in Fractions 4 and 5 (peak in 5), the 48,000-dalton subunit in Fractions 7 and 8 (peak in 8). Similar results were obtained in eight experiments of identical design utilizing five separate preparations of EIF<sub>i</sub>.

activity of this fraction. In contrast, the three forms of the 48,000-dalton subunit appeared to be phosphorylated. Comparison of GTP-dependent and ATP-dependent phosphorylation of EIF<sub>i</sub> by endogenous protein kinases is shown in Fig. 5. The 48,000-dalton subunit was preferentially phosphorylated when [γ-32P]GTP was used as the phosphate donor, while the 37,000-dalton subunit was preferentially phosphorylated with [γ-32P]ATP used as the phosphate donor. After autoradiography, the 48,000- and 37,000-dalton spots were cut and counted in toluene/Liquifluor. The ratio of 32P incorporated into the 48,000-dalton subunit to that found in the 37,000-dalton subunit was 0.25 when [γ-32P]ATP was used as the phosphate donor and 3.26 when [γ-32P]GTP was used as the phosphate donor.

![FIG. 5. ATP- and GTP-dependent phosphorylation of EIF<sub>i</sub>. Ribosomes were phosphorylated in the presence of [32P]ATP or [32P]GTP. EIF<sub>i</sub> was purified through the phosphocellulose chromatography step and subjected to two-dimensional gel electrophoresis as described under "Methods." 9 M urea was present during isoelectric focusing. Radioautographs of EIF<sub>i</sub> purified from the subribosomal fraction are shown above; a similar amount of protein was applied to each gel. 1. [32P]GTP was used as phosphate donor; 2. [32P]ATP was used as phosphate donor.

D I S C U S S I O N

The molecular weight of the GTP-dependent Met-tRNA<sub>i</sub>, binding protein has been estimated to be 150,000 to 160,000 using different analytic techniques (11, 14, 17, 18, 21). Analysis of the purified proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis has revealed either two subunits of 52,000 and 34,000 to 39,000 daltons (11, 19) or three subunits of 50,000 to 57,000, 48,000 to 52,000, and 32,000 to 39,000 daltons (14, 17, 18, 21). Using an EIF<sub>i</sub>, preparation which demonstrated only two subunits on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, it has been possible to modify conditions and resolve the protein into three subunits of 51,000 to 52,000, 49,000 to 49,000, and 37,000 to 39,000 apparent molecular weights. Apparent isoelectric points of 5.8, 6.6, and 8.9 for the 37,000-, 48,000-, and the 52,000-dalton subunits, respectively, provided additional evidence that the 52,000 and 48,000 subunits represented distinct molecular species rather than an artifact of method of analysis.

The separation of subunits by isoelectric focusing in urea followed by renaturation permitted partial definition of subunit function. The 48,000 dalton subunit was shown to possess Met-tRNA<sub>i</sub>, binding activity, while the 37,000-dalton subunit was shown to possess GDP binding activity. Since the treatment of the protein involved in this method of analysis allowed no more than 20% to 30% recovery of activity, a direct or indirect role for other subunits in GDP or Met-tRNA<sub>i</sub>, binding cannot be absolutely excluded. Although the present model for ternary complex formation predicts 1 pmol of Met-tRNA<sub>i</sub>, bound/pmol of pure EIF<sub>i</sub>, the observed molar ratio has generally been 0.15 to 0.3 (11, 13-15, 18, 19); while this low binding has been ascribed to lability of the factor during purification, a requirement for additional factors permitting maximal binding cannot be excluded. The significance of the reported mRNA binding by EIF<sub>i</sub> (19, 22) is unclear. Competition experiments suggested that mRNA and Met-tRNA<sub>i</sub>, were both specifically bound by EIF<sub>i</sub> (19). A portion of mRNA binding activity in EIF<sub>i</sub> preparations has been shown to reside in the 48,000-dalton subunit.

Phosphorylation of EIF<sub>i</sub> subunits depends upon the protein kinase used (21-24). Using a pure preparation of EIF<sub>i</sub> and protein kinases, Issinger et al. (20) have shown that the subunit of intermediate size, which they calculated to be 52,000 daltons, was phosphorylated specifically by a CAMP-independent kinase specific for acidic proteins; although both ATP and GTP could be used as phosphate donors, ATP afforded a greater phosphorylation. The acidic nature of the subunit of intermediate size, estimated as 48,000 daltons in this report, suggests that it is probably phosphorylated by the "casein" kinase present in the reticulocyte lysate used in the present experiment. However, the incorporation of [32P]phosphate into the 48,000-dalton subunit was 3-fold greater when GTP rather than ATP was used as a phosphate donor. In addition, the
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37,000-dalton subunit, also an acidic protein, was found to be phosphorylated although it is not a substrate for the purified "casein" kinase (20, 21). The phosphorylated form of this subunit is clearly separated from the dephosphorylated form by isoelectric focusing. A cAMP-independent protein kinase postulated to serve as the hemin-controlled repressor in rabbit reticulocyte has been demonstrated to catalyze the ATP-dependent phosphorylation of the 37,000-dalton subunit of EIF\textsubscript{2} (23, 24); this phosphorylation of EIF\textsubscript{2} is proposed to relate to the inhibitory activity on initiation of protein synthesis. This kinase is probably responsible for the phosphorylation of the 37,000-dalton subunit observed in the present experiment where a preferential phosphorylation by ATP is observed. The hemin-controlled repressor apparently does not inhibit (EIF\textsubscript{2}:GTP:Met-tRNA\textsubscript{A}) ternary complex formation (33), but appears to ultimately inhibit formation of the (40 S subunit:Met-tRNA\textsubscript{A}:EIF\textsubscript{2}) initiation complex (33–37). The very small amount of the phosphorylated form of the 37,000-dalton subunit found on the ribosomes is consistent with this postulate. Since the 37,000-dalton subunit appears to be involved with binding GTP but not Met-tRNA\textsubscript{A}, it is possible that the dephosphorylated form of the 37,000-dalton subunit exhibits a modified affinity for GTP, GDP, the 40 S ribosomal subunit, or other subunits of EIF\textsubscript{2}.

REFERENCES
Characterization of GTP-dependent Met-tRNAf binding protein.
A Barrieux and M G Rosenfeld


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