The Translation Initiation Factor eIF3-p48 Subunit Is Encoded by int-6, a Site of Frequent Integration by the Mouse Mammary Tumor Virus Genome*

(Received for publication, June 30, 1997, and in revised form, July 21, 1997)

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Translation initiation factor eIF3 is a large, multisubunit protein complex that plays a central role in the pathway of initiation by promoting the binding of both methionyl-tRNA, and mRNA to the 40S ribosomal subunit. As part of a broad effort to elucidate the structure of eIF3, we have cloned and sequenced the human cDNA encoding the 48-kDa subunit, eIF3-p48. The recombiant protein comigrates with the authentic p48 subunit in purified eIF3 and coprecipitates with affinity-purified antibodies to the p170 subunit of eIF3. A search of the data base indicates that the mouse gene encoding eIF3-p48 had previously been identified and characterized by others as int-6. The int-6 gene is the site of frequent integration of mouse mammary tumor virus DNA into chromosomes, implicating the gene in the regulation of cell proliferation. In addition, it was shown elsewhere that the homologous human int-6 gene product binds to the human T-cell leukemia virus type I Tax protein, leading to the translocation of Int-6 to the cytoplasm. We discuss how the cytosolic function of eIF3-p48 (Int-6) in protein synthesis may account for oncogenesis caused by these two viruses.

The initiation phase of protein synthesis in eukaryotes is promoted by 10 or more proteins called initiation factors (reviewed in Ref. 1). Translation frequently is regulated by phosphorylation of the initiation factors, which causes either stimulation or inhibition of their activities. The levels and specific activities of these proteins are important for determining translation rates and for integrating the process of protein synthesis into the cell’s overall metabolism. Abberations in regulating initiation factor activities may result in loss of control of cell proliferation and in malignant transformation of cells (2).

To better understand the mechanism of action and regulation of initiation factors, we have sought to determine their primary structures by cloning and sequencing human cDNAs that encode them. One of the mammalian initiation factors, eIF3, is a multisubunit complex of ~600 kDa that plays a central role in the pathway of initiation (1). eIF3 binds to the 40S ribosomal subunit and acts as a ribosomal subunit antiassociation factor. It stabilizes the binding of methionyl-tRNA, to 40S ribosomal subunits and is required for mRNA binding. The 10 subunits of human eIF3 possess apparent masses of 170, 116, 110, 66, 48, 47, 44, 40, 36, and 35 kDa. We have previously reported the cloning and characterization of cDNAs encoding the p110 and p36 subunits (4), and others have reported on the p170 and p116 subunits (5, 6). This report focuses on the p48 subunit of eIF3 and provides evidence that eIF3-p48 is identical to the product of the murine int-6 gene, where the mouse mammary tumor virus (MMTV)1 genome frequently integrates.

EXPERIMENTAL PROCEDURES

Cloning and Sequencing of eIF3-p48 cDNA—Peptides from rabbit eIF3 subunits were prepared by proteolysis and sequenced as described previously (7). Internal peptide sequencing of purified HeLa eIF3 (8) was conducted in the Protein Structure Laboratory of the University of California, Davis. Clones 149207 (GenBank™ accession number R71564), 47908 (H11132 and H11044), and 71140 (T47475 and T47476) were kindly provided by the Lawrence Livermore National Laboratory (Livermore, CA) and were sequenced on both strands. Plasmid pTzP48 was constructed by subcloning the following PCR DNA fragment into the BamHI and HindIII sites of pTZ19R (9); the 1.3-kb DNA fragment was generated by PCR amplification of a heat-treated human liver cDNA library (Stratagene) with the following primers: oligo-1 (5′-CCCAAGCTTAAAGATGGCGGAGTACGACTTGAC-3′) and oligo-2 (5′-GGCGGAATTCCCCGGCAAGATGGCGG-3′), corresponding to nucleotides 4–26 in the DNA sequence with accession number U54562, tagged with a HindIII site, and oligo-2 (5′-CCCAGGATCTCCTAGTGAAGGCCGAAATCTC-3′), corresponding to nucleotides 1344–1325, tagged with a BamHI site, and was digested with BamHI and HindIII.

Sequencing the 5′ End of Mouse int-6 cDNA—Poly(A)+ mRNA purified from mouse FM3A cells was employed for an reverse transcription-PCR reaction (Gene Amp RNA PCR kit, Perkin-Elmer) using the primers 5′-CCGGAGATCCCCGGCAAGATGGCGG-3′ (corresponding to nucleotides 1–17 in accession number U54563, tagged with an EcoRI site) and 5′-CCCCAAGCTTCCCATGTTTGTCTGCCAGG-3′ (corresponding to nucleotides 1090–1109 in U54563, tagged with an EcoRI site) and 5′-CCCAAGCTTCCCATGTTTGTCTGCCAGG-3′ (corresponding to nucleotides 376–358, tagged with a HindIII site). The resulting 0.4-kb DNA fragment was sequenced and subcloned into Bluescript SKII (Stratagene), followed by sequencing again. The human and corresponding mouse sequences are deposited in GenBank™ as U54562 and U54563, respectively.

PCR Analysis of eIF3-p48 cDNA Derived from Different Tissues—PCR reactions were conducted with 16 human libraries from a QUICKSCREW cDNA library panel (CLONTECH) and Taq DNA polymerase (Stratagene). For the first amplification, the primers used were oligo 3 (5′-CCCAAGCTTACATAGAGAAGGCTGAA-3′, corresponding to nucleotides 1090–1109 in U54563, tagged with EcoRI) and oligo 2 (described above). For the second amplification, oligo 3 and oligo 7 (5′-ACGCCTAAAAGGAAACACACGGAAATATAA-3′, corresponding to nucleotides 1188–1174, with the antisense sequence of the insertion underlined) were used with 0.2% of the first amplification reaction mixture as template. The conditions were 35 cycles (30 s at 94 °C, 30 s...
and the human homolog. The entire two human peptide sequences match portions of mouse Int-6.

...5 human ESTs homologous to murine int-6. This surprising result suggests that eIF3-p48 corresponds to the nonredundant protein and expressed sequence tag (EST) data bases, and a perfect match of the first 14 amino acid residues was found to the mouse EST clone; the latter lacks 28 bp at the 5′ terminus that includes the correct initiation codon.

In Vitro Synthesis and Immunoprecipitation of eIF3-p48—In vitro translation of eIF3-p48 mRNA employed the TnT translation kit (Promega) with CaCl₂-purified covalently closed circular pTZp48 as template. Immunoprecipitation was conducted essentially as described (4). The immune complexes were isolated with GammaBind G Sepharose beads (Pharmacia Biotech Inc.), washed, and eluted with SDS. Only a small fraction (<5%) of the de novo synthesized p48 was precipitated with anti-eIF3 (or with anti-p170 antiserum), because p48 alone is not recognized by these antibodies.

RESULTS

Cloning and Characterization of a Human cDNA Encoding eIF3-p48—eIF3 purified from both rabbit reticulocytes and the p48 band was excised. After proteolysis and high pressure liquid chromatography fractionation as described under “Experimental Procedures,” the following peptide sequences were obtained: LGHVVMGNNAVSPYXQX(VIEK) from the rabbit protein, and VIQQESSYTYK and NQN(S)R(P/I)(EAPN) from the data base (results not shown). That the upstream AUG is the correct initiation site is suggested by the size of the protein product expressed in the reticulocyte lysate (see Fig. 3b).

The derived amino acid sequence of human eIF3-p48 is identical to the corrected mouse Int-6 sequence; both proteins contain 445 amino acid residues with a calculated mass of 52,187 Da. Northern blot analysis of HeLa poly(A)+ mRNAs gave a single 1.6-kb band when probed with DNA from the coding region of p48 (results not shown). Therefore, the 1507-bp DNA reported here as U54562 represents nearly full-length cDNA. eIF3-p48 (Int-6) sequences are found with a frequency of about 0.02% in the human EST data base, with essentially all tissues represented, suggesting that p48 is a ubiquitous, moderately abundant protein, as expected for a subunit of eIF3. Although homologs in many animal and plant species were identified in the data base (results not shown), no homolog of eIF3-p48 was found in the complete Saccharomyces cerevisiae genome data base.

A second, more minor difference between the human and mouse cDNAs is the presence in the human int-6 clones (47098) of a 21-nucleotide insertion following nucleotide 1170, which lies between the Leu388 and Gly389 codons (Fig. 1). The insertion occurs at the junction of exons 11 and 12, conforms to the 3′ intron consensus sequence (12), and therefore appears to be due to alternative splicing. The insertion encodes FPCVSF-stop; thus the mRNA is predicted to generate a C-terminal truncation. Given the possible oncogenic properties of truncated eIF3-p48 (Int-6) (see below), the frequency of occurrence of this insertion was determined. The 21-bp insertion was found in only 1 of 26 ESTs encoding that region of human eIF3-p48 and therefore may be a unique or very rare event. To investigate the occurrence of the alternative splice/insertion event in human cells, two independent cDNA libraries from 8 different tissues were analyzed by PCR as described under “Experimental Procedures.” Whereas no larger PCR product indicative of the 21-bp insertion was detected in any of the primary amplification reactions (Fig. 2, upper panel), when a nested PCR analysis was performed by using a primer corresponding to the insert sequence, 7 of the 16 libraries representing 5 different tissues generated a band indicative of the insert (Fig. 2, lower panel). Thus the putative alternative splice/insertion event occurs widely but at such a low frequency that the resulting truncated protein likely would not affect cell physiology.

eIF3-p48 (int-6) Is a Subunit of eIF3—To demonstrate that the cloned human int-6 DNA sequence actually encodes the p48 subunit of eIF3, a full-length cDNA was generated by PCR amplification of a human liver cDNA library (Stratagene), and the product was cloned under control of the T7 promoter to generate pTZp48, as described under “Experimental Procedures.” The pTZp48 insert was sequenced to confirm the sequence of the gene product and therefore may be involved in oncogenes.
sequence reported here (accession number U54562), and eIF3-p48 was expressed in an in vitro coupled transcription/translation system (Promega). The largest and most abundant of the three $^{35}$S-labeled products comigrates precisely with the p48 subunit of purified eIF3 when analyzed by SDS-PAGE (Fig. 3b). The minor, lower molecular weight products presumably are partially degraded forms of p48. Further evidence that pTz4p8 encodes a subunit of eIF3 was obtained by immunoprecipitation of the eIF3 complex present in the in vitro translation reaction. Labeled p48 and its degraded forms are detected in precipitates obtained with crude anti-eIF3 antiserum (Fig. 3c), even though eIF3-p48 is not directly recognized by this antiserum (13, 14). Immunoprecipitates formed with affinity-purified antibodies specific for the p170 subunit of eIF3 also contain the recombinant eIF3-p48, but that with preimmune serum does not (Fig. 3c). Apparently, newly synthesized p48 exchanges inefficiently into endogenous eIF3 complexes, leading to its coprecipitation.

**DISCUSSION**

Evidence that the cDNA described here encodes eIF3-p48 includes matches to three peptide sequences derived from a 48-kDa protein in purified eIF3, co-migration of the recombinant protein with the 48-kDa subunit, and co-immunoprecipitation with anti-eIF3 and anti-p170 antibodies. eIF3-p48 itself appears not to be very antigenic, because the eIF3 antisera does not recognize the p48 subunit. This lack of antigenicity and the comigration of the p48 and p47 subunits of eIF3 during routine SDS-PAGE resulted in a failure in earlier work to distinguish the two subunits, which together were called eIF3-p47 (13, 14). However, the p48 and p47 subunits are distinctly different proteins, as shown conclusively by the cloning of the p48 cDNA described here, as well as the cloning and sequencing of a cDNA encoding eIF3-p47. The situation is similar to that of the p116 and p110 subunits, which also were not separated during SDS-PAGE and were identified earlier as a single 115-kDa band (14). Thus the cloning and sequencing of cDNAs for eIF3 subunits has led to a refinement of our knowledge of the composition of this complex factor.

That eIF3-p48 is encoded by the int-6 gene is intriguing and requires further examination. MMTV genomes integrate into mouse genomes at a number of different sites, thereby causing deregulation of the expression of adjacent genes in mammary tumors (15). One of these sites, called int-6, was identified in a mammary hyperplastic outgrowth cell line obtained from a feral mouse as well as in two other independent mammary tumors (10). Murine int-6 comprises 13 exons that encode a protein known to be highly conserved and ubiquitously expressed. Insertion of the MMTV genome into int-6 may result in the expression of a truncated protein that either is activated or functions as a dominant-negative mutant form (10). Thus truncated eIF3-p48 (Int-6) may be a dominant-negative oncoprotein. However, a truncated Int-6 protein has not yet been demonstrated experimentally in the MMTV tumor cells, and other possible mechanisms of mutagenesis are possible, such as altered expression of other genes near to int-6. Int-6 also has been identified recently as binding to the human T-cell leukemia virus type I Tax protein (11). It is proposed that binding of Tax relocates Int-6 from the nucleus to the cytosol.

If truncated eIF3-p48 indeed is generated and functions as an oncoprotein, how might it be involved in malignancy? A number of components of the translational apparatus have been implicated in regulation of cell proliferation (2). Overexpression of the cDNA encoding eIF4E causes malignant transformation of NIH 3T3 and rat L1 cells (16) and aberrant growth in HeLa cells (17). The increased level of eIF4E apparently exceeds the level of the 4E-BPs that are responsible for inhibiting the activity of this initiation factor, resulting in

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$k$ A. Asano, H.-P. Vornlocher, N. J. Richter-Cook, W. C. Merrick, A. G. Hinnebusch, and J. W. B. Hershey, submitted for publication.
uncontrolled activation of protein synthesis (2). Expression of a
dominant-negative form of the eIF2α kinase, PKR, also causes
malignant transformation (18), presumably by preventing the
down-regulation of protein synthesis through phosphorylation
of eIF2α. This view is reinforced by the demonstration that
overexpression of the Ser51→Ala mutant form of eIF2α, which
cannot be phosphorylated by PKR, also deregulates cell growth
(19). In each of these cases, loss of proliferation control appears
to involve the cell’s failure to repress translation. It is therefore
perplexing how truncated forms of eIF3-p48 might lead to
deregulation of cell growth. Large C-terminal deletions often
lead to loss of function, which in the case of an eIF3 subunit
would be expected to result in an inhibition of protein synthe-
sis. However, it is possible that p48 is not part of the active core
of mammalian eIF3, inasmuch as no homolog is found in yeast
eIF3. Rather, we speculate that it might function as a regula-
tory subunit involved in down-regulating eIF3 activity. There-
fore, disruption of eIF3-p48 could result in loss of repression of
eIF3 activity and thus of protein synthesis. Similarly, Tax
localized in the cytosol of human T-cell leukemia virus type
I-infected cells (11) might bind to eIF3-p48 (Int-6) and remove
it from eIF3, resulting in deregulation of protein synthesis.

A second interesting possibility is that eIF3-p48 plays two
roles in cells, one as a subunit of eIF3 and another as a regula-
tor of cell growth together with the promyelocytic leukemia
protein as previously proposed (11). If this is the case, the fate
of a cell might be determined by the dynamic distribution of the
bifunctional protein, stimulating either differentiation (nucle-
us) or cell growth (cytosol). The nuclear localization of eIF3-p48
(Int-6) is surprising (11). However, nuclear localization of an
initiation factor has a precedence in eIF4E whose antibodies
predominantly immunostain the nucleus (20). Experiments are
in progress to investigate the function of the truncated or
Tax-bound p48 subunit of eIF3 in protein synthesis and cell
growth.

Acknowledgments—We thank Joachim Schnier for providing purified
FM3A mRNA, Nancy Richter-Cook for assistance in peptide sequencing
and Jim Anderson, Tom Dever, and Alan Hinnebusch for critical read-
ing of the manuscript.

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\textit{J. Biol. Chem.} 1997, 272:23477-23480.
doi: 10.1074/jbc.272.38.23477

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