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CHOP/GADD153 and Methionyl-tRNA Synthetase (MetRS) Genes Overlap in a Conserved Region That Controls mRNA Stability

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The transcription factor CHOP is involved in the regulation of the cell division cycle and the control of programmed cell death in response to cellular stress. CHOP expression has been linked with several forms of cancer. A reciprocal translocation between the CHOP and TLS RNA-binding protein gene results in myxoid liposarcoma and amplifications of the CHOP gene are associated with solid tumors including several types of sarcomas. Here we report the mapping of the methionyl tRNA synthetase (MetRS) gene to the identical 12q13 locus where the CHOP gene had previously been mapped. PCR analysis demonstrates a tail-to-tail overlap of both genes over a 55-bp region. As a result the two mRNAs share a 3′ UTR complementary sequence allowing an *in vivo* interaction between the two mRNAs. An AU-rich regulatory element (ARE) known to control mRNA stability resides in the overlapping sequence. To test for functional significance of the ARE a luciferase reporter plasmid containing the 3′UTR of CHOP was constructed. Transfection experiments in NIH-3T3 cells show that CHOP 3′UTR confers a significantly lower activity than a control reporter or a reporter in which the region overlapping the MetRS mRNA is deleted. The conservation of this overlapping of the CHOP and MetRS genes and the role of their complementary sequence in the control of mRNA stability suggest the existence of a functional link between the expression of these two genes.

The C/EBP homologous protein transcription factor CHOP, also known as GADD153 (growth arrest and DNA damage inducible protein) (1), is induced by a variety of agents and conditions that produce cellular stress (1, 2). CHOP inhibits adipocyte differentiation (3), induces growth arrest at G1/S (4), and regulates programmed cell death (5–9). The cellular mechanisms responsible for these actions of CHOP are not completely understood. Upon interactions with cannonical C/EBP binding sites CHOP exerts a dominant inhibitory effect of C/EBP-mediated transcriptional activation (2). However, heterodimers of CHOP and other C/EBP family members bind to a unique class of DNA control elements in the promoters of genes induced by cellular stress (10). Additional complexity comes from the fact that CHOP transactivation is regulated by phosphorylation. The stress-activated protein kinase, p38, phosphorylates Serine 78 and Serine 81 of CHOP resulting in an enhanced transcriptional activity (11).

Separate lines of investigation implicate CHOP in the pathogenesis of certain human cancers. CHOP is mapped to human chromosome 12q13. Amplifications of this region of chromosome 12 occur in several forms of human sarcomas (12, 13), malignant fibrous histiocytoma (14), and human malignant gliomas and glioblastomas (15). Usually the corresponding amplicon contains CHOP and other neighboring genes such as MDM2, GLI, SAS, and CDK4. A chromosomal translocation (t12, 16)(q13, p11) is characteristic of myxoid and round cell liposarcomas (16, 17). The resulting fusion protein TLS-CHOP, has *in vitro* transforming activity in NIH3T3 fibroblasts and nude mice (18). Although the oncogenic activity of TLS-CHOP requires both the amino-terminal domain of TLS and the bZIP domain of CHOP (18), the precise mechanisms of the transformation remain to be elucidated. Such mechanisms may include dysregulation of the expression of CHOP or of other genes residing in the same locus. A possible role for the 3′ untranslated region (3′UTR) of the CHOP mRNA has not been excluded.

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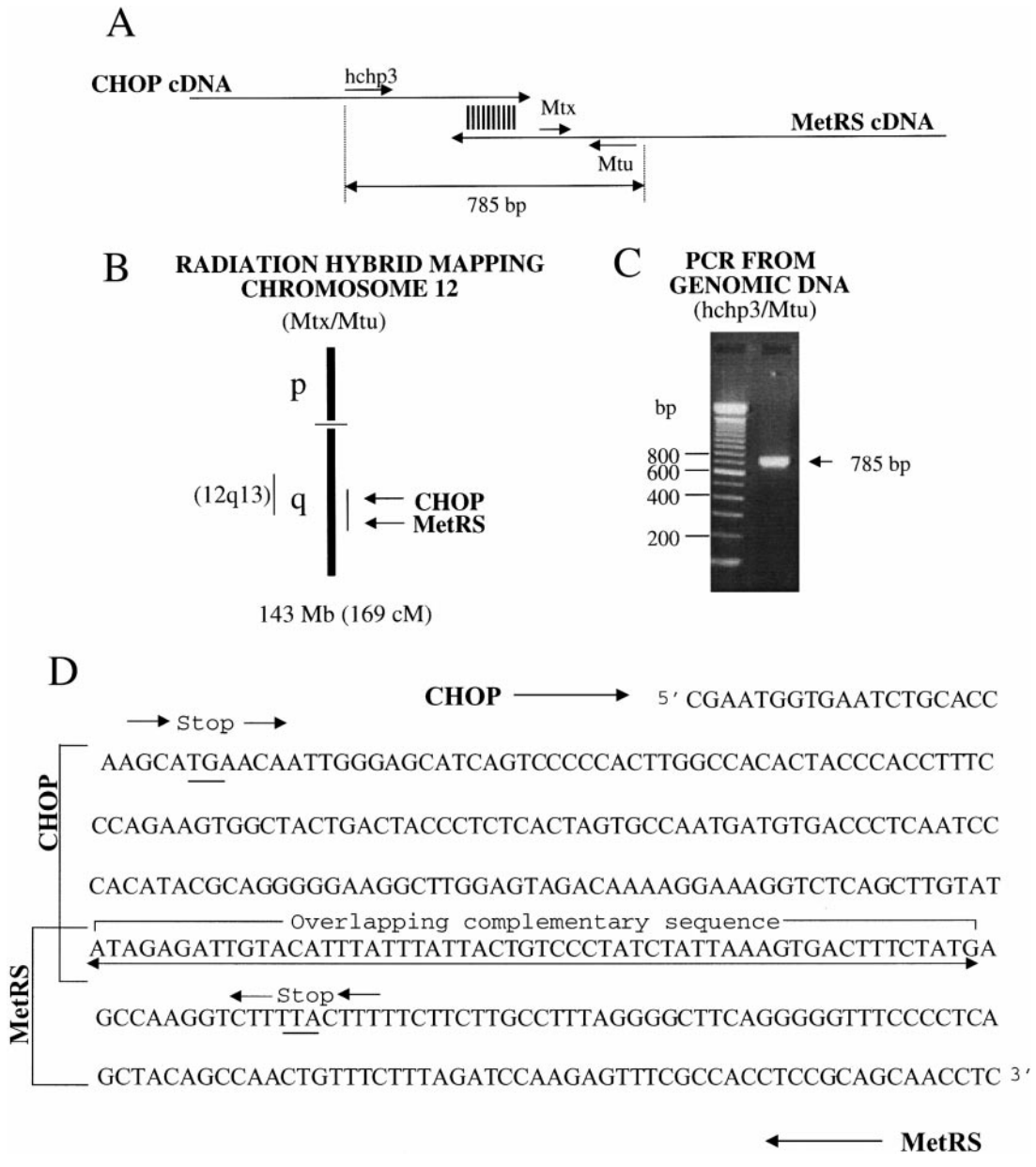

FIG. 1. CHOP and MetRS genes reside in the same locus on chromoxome 12q13 and share a common overlapping sequence in their respective 3′ ends. (**A**) A search in GenBank identifies several ESTs corresponding to the human MetRS cDNA that overlap with the most distal region of the 3′ untranslated region of CHOP cDNA. The location of the different primers used for PCR amplification is indicated. (**B**) Mapping of the human MetRS to the q arm of the human chromosome 12 was achieved by using a radiation hybrid technique (GeneBridge 4 panel). The Mtx and Mtu oligonucleotide primers were used to amplify a region of the MetRS gene from the hybrid clones. (**C**) Direct mapping was achieved by PCR amplification from human genomic DNA using a primer set that corresponds to both CHOP (hchp3, sense primer) and MetRS (Mtu, reverse primer) genes. (**D**) Sequencing of the amplified genomic segment demonstrates coding frames for both CHOP and MetRS genes as well as their full length 3′ untranslated regions. Stop codons are underlined as well as the overlapping complementary sequence. (**E**) Screening of a mouse genomic library identified two clones containing sequences pertaining to both CHOP and MetRS mouse genes and demonstrates that their corresponding last exons overlap over a 50-bp region. This complementary sequence is also present in the corresponding mRNAs.

In this study we discovered that the human 3′ untranslated region (3′UTR) of the CHOP gene overlaps and is complementary to the gene encoding methionyl tRNA synthesis (MetRS), essential for the initiation of the translation of mRNAs. Analyses of ESTs show close homology to two other sequences deposited in GenBank. One corresponds to a mitoxantrone resistance partial cDNA, and the other to a human homolog of the yeast methionyl-tRNA synthetase (MetRS) cDNA (19). We report that both

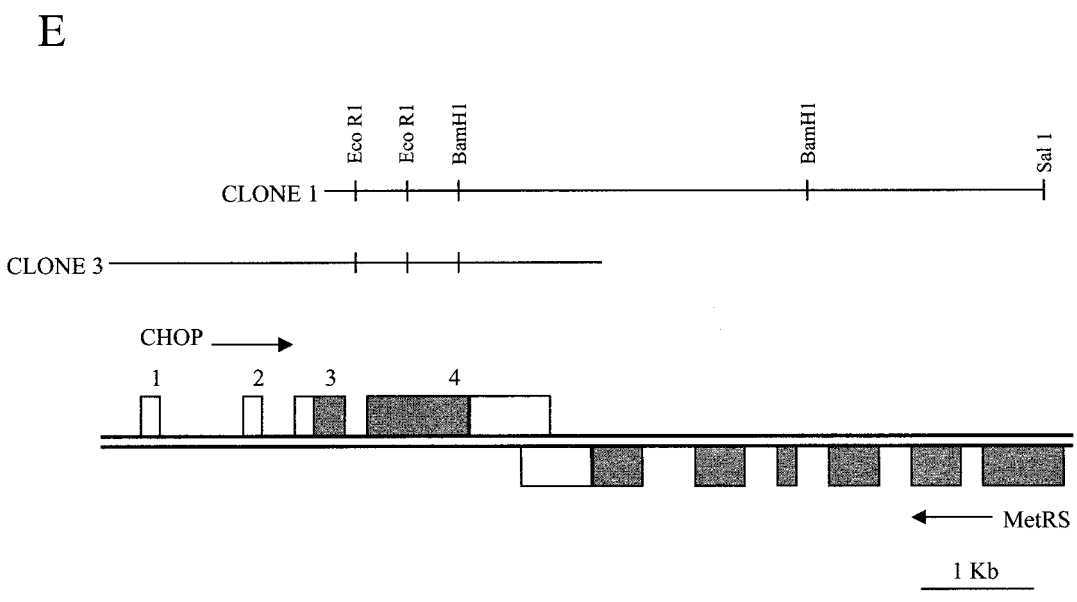

FIG. 1—*Continued*

sequences are part of the same cDNA coding for the human methionyl-tRNA synthetase (MetRS). CHOP and MetRS genes are arranged in an overlapping tail-to-tail configuration sharing a 55 bp segment of complementary sequence in their corresponding 3′ ends containing a classical AU-rich mRNA destabilization sequence. We propose a model in which the interaction between the overlapping CHOP and MetRS mRNAs would have important implications for their functional regulation in cellular response to environmental stress, and oncogenic transformation in which amplifications or translocations of the CHOP gene occur.

MATERIALS AND METHODS

Materials. DNA modifying enzymes were obtained from New England Biolabs (Beverly, MA) or Boehringer-Mannheim Biochemicals (Indianapolis, IN), radioactive compounds from DuPont-New England Nuclear (Boston, MA), nucleotides from Pharmacia-LKB (Piscataway, NJ), and tissue culture media and reagents from Gibco-BRL (Grand Island, NY). A panel of 93 Radiation Hybrid clones representing the entire human genome (GeneBridge 4 Radiation Hybrid Panel) was obtained from Research Genetics, Inc. (Huntsville, AL) (20).

Cell culture and transfections. NIH3T3 fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum and antibiotics (penicillin, 100 U/ml and streptomycin, 100 μg/ml). Transfections were performed by the calcium phosphate method using a reagent kit (5 prime 3 prime, Boulder, CO). Cells were collected 36 h after transfection and extracts were assayed for luciferase activity using a commercial kit (Promega, Madison, WI).

Chromosomal mapping. Chromosomal localization of the human MetRS gene to a particular chromosome was achieved by two different methods. **1**) PCR screening of a radiation hybrid panel of 93 clones, representing the entire human genome (GeneBridge 4, Research Genetics, Inc., Huntsville, AL). Results from PCR amplification in each one of the clones were scored as 1: positive; 0: negative; and 2: equivocal interpretation. Data were submitted to the World-Wide-Web at the Whitehead Institute-MIT Center for Genome Research, for chromosomal localization. The sequence of the PCR oligonucleotide primers that were used is the following: Mtu: 5′ TGAAAGCACAAAAGGCAGACAA 3′, and Mtx: 5′ CTTTAGGGGCTTCAGGGGGTTTC 3′. **2**) Direct mapping of the MetRS gene to the CHOP locus was performed by PCR amplification and sequencing of a genomic DNA fragment, amplified with a sense primer located in the exon 4 of CHOP (hchp3): 5′ GAAGAATCAAAAATCTTCACC 3′, and a reverse primer, Mtx, complementary to the MetRS cDNA. Both restriction analysis and direct sequencing were used to confirm the identity of the predicted 785 bp fragment.

Genomic cloning. A mouse (129 Sv) genomic library (Stratagene) made of partially Sau 3A digested DNA was screened with a mouse CHOP cDNA probe encompassing exon 2, exon 3 and part of exon 4 sequences.The probe was the product of a PCR reaction using forward primer TGACGTGTTCCAGAAGGAAGT and reverse primer GAGCAGTTCTTCCTTGCTC and was P^{32} labeled by random priming reaction. Approximately 10^6 plaques were screened on nitrocellulose filters. Four positive clones were obtained and purified. The inserts were characterized and two overlapping clones spending the biggest genomic area were selected for further analysis. DNA fragments of interest were subcloned into bluescript vector for mapping and sequencing.

Reporter constructs and expression vectors. To examine the functional role of the overlapping 3′UTR regions we engineered a luciferase reporter construct (pGL3 control from Promega, Madison, WI) to contain the entire 3′UTR of the human CHOP mRNA including the region that overlaps with the MetRS (pLUC-CHOP 3′UTR). The activity of the reporter construct was compared with a modified construct in which the 55 bp overlapping region was deleted (pLUC-CHOP 3D). Both constructs and the corresponding control were transfected in NIH3T3 fibroblasts under the control of a constitutively active SV40 promoter. The relative transcriptional activities were determined by normalization to the expression of a co-transfected control expression plasmid encoding β-actin.

RESULTS

EST sequences overlapping the 3′ end of CHOP correspond to the 3′ end of the methionyl-tRNA synthetase cDNA (MetRS). A search of the National Center of Biotechnology Information (NCBI) Expressed Sequence Tag database (dbEST) for transcripts related to the CHOP cDNA sequence revealed several EST clones with a 3′ end that corresponded to the complementary sequence of the 3′ end of the CHOP cDNA. The overlapping sequence consisted of a 55 bp segment adjacent to their corresponding 3′ poly (A) tracts of the mRNAs. Further analyses of these clones were performed by generating a "contig" from the sequences available for these clones in the NCBI data base. Several of the EST sequences, in which both 3′ and 5′ sequences were available, were identical to the corresponding 3′ and 5′ sequences of the human methionyl-tRNA synthetase (MetRS) (19) (Fig. 1A). This circumstance suggested that both CHOP and MetRS genes reside in the same chromosomal locus, and are coded by opposite strands of DNA. To experimentally corroborate these findings a radiation hybrid panel of 93 clones (GeneBridge 4) was screened by using two primers directed against the human MetRS cDNA (see Methods). Submission of the data to the Whitehead Institute-MIT Center for Genome Research for analysis indicated that MetRS gene is localized to chromosome 12q13, and it placed 87.23 cR from the marker: D12S367, with a LOD score $>$ 9 (Fig. 1B). The finding that this marker resides at the 12q13 region where CHOP was already mapped indicated that both genes localize to the same locus in the human genome. A more precise mapping was achieved by direct PCR amplification of human genomic DNA using a sense primer directed against the CHOP sequence and a reverse primer directed against the MetRS sequence (Fig. 1C). Both restriction analysis and sequencing (Fig. 1D) of the amplified 785 bp product confirmed that both CHOP and MetRS genes reside in the same locus of chromosome 12 (12q13), and that they share a 55 bp overlapping complementary region in their corresponding 3′UTR. Our data are consistent with a previous report showing complementation of a temperature-sensitive MetRS CHO cell mutant with a cell-hybrid containing the human chromosome 12 (21).

Sequence analysis and conservation of the overlapping 3′UTR domain reveal a possible new form of genetic regulation. To further explore whether the overlapping arrangement of the CHOP and MetRS genes may have a functional significance, we extended our studies to the mouse genome and found the same type of overlapping arrangement between the corresponding mouse CHOP and MetRS genes (Fig. 1E). In the mouse a 50 bp region is shared by both genes in their corresponding 3′ends (Fig. 2B). As a result of the overlapping arrangement the corresponding mRNAs of CHOP and MetRS genes contain a complementary domain capable of *in vivo* hybridization (Fig. 2A). Sequence analysis showed that this region of the human genome is 82% identical to the mouse and rat sequences (Fig. 2B). The high degree of conservation across different species suggests the presence of important functional domains in the CHOP/MetRS overlapping region. A few examples of a similar tail-to tail overlapping arrangement between a pair genes have been reported (22, 23). The first example involves a 133 bp region of the mouse genome shared by two different genes T3C and T5B, the functions of which have remained unknown (23). A more recent report demonstrates the existence of a similar overlap between the Nab2 and Stat6 genes in both human and mouse genes (22). The overlapping domain of the Nab2 and Stat6 genes encompasses a 78 bp sequence that is also highly conserved between the mouse and human genes. A sequence comparison between the CHOP/MetRS, T3C/T5B, and Nab2/Stat6 overlapping sequences show a high degree of similarity (Fig. 2C), suggesting the possibility of a new form of genetic control conferred by the tail-to-tail overlapping of the corresponding complementary sequences in their respective mRNAs. Sequence analysis of these regions identifies the presence of AU rich elements (ARE), previously described as mRNA destabilization elements in early response genes (24).

The overlapping region of the CHOP and MetRS genes regulates the stability of CHOP mRNA. Additional experiments were designed to determine a functional role for the overlapping domain between CHOP and MetRS genes. We investigated whether the ARE identified in the 3′UTR of the CHOP mRNA is a functional element. If so an interaction between the two mRNAs could prevent the formation of the destabilization complex and result in stabilization of the CHOP mRNA. A reporter construct expressing the luciferase gene was engineered in such a way that its activity after transfection into NIH-3T3 cells depends only on the stability of its mRNA (see Methods section). By replacing the 3′UTR of the luciferase gene with 3′UTR corresponding of CHOP (Luc-3′UTR CHOP) gene or alternatively by one in which the overlapping domain was deleted (Luc-3′UTR-D CHOP) (Fig. 3A), the existence of an mRNA destabilization element was identified. A significant loss of activity of the reporter containing the entire CHOP 3′UTR (Fig. 3B) and recovery of its activity to the control level in the absence of the overlapping domain clearly indicated that the overlapping domain plays a role in the CHOP mRNA stability. It also suggests that an interaction between the CHOP and the MetRS transcripts would prevent the formation of the destabilization complex and therefore increase the stability of the CHOP mRNA.

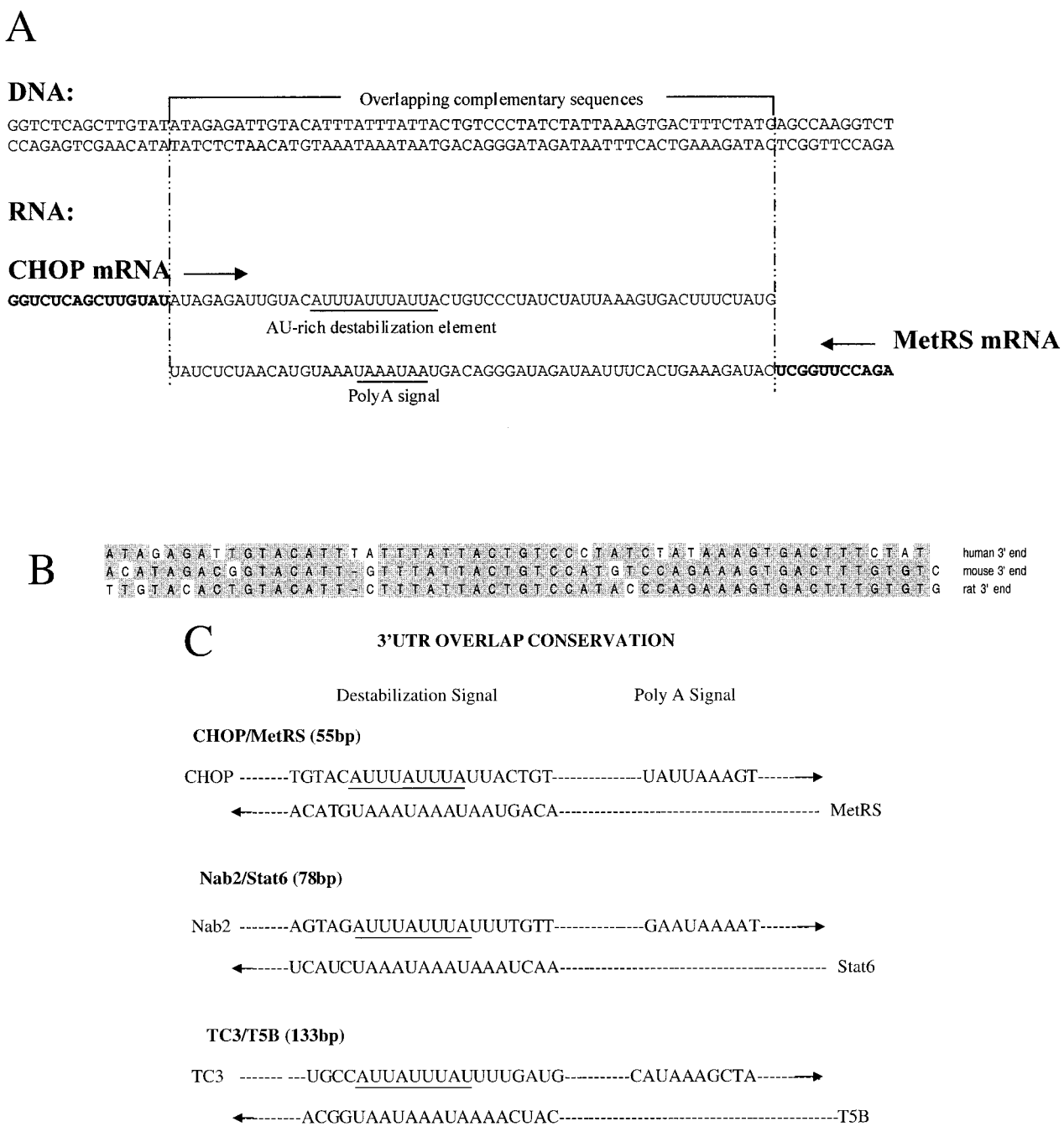

FIG. 2. CHOP and MetRS transcripts overlap in a region containing mRNA polyadenylation and destabilization elements. (**A**) Transcription of the CHOP and MetRS genes results in the appearance of two mRNAs with overlapping 3′ UTR sequences. (**B**) Comparisons of human, mouse, and rat 3′ overlapping sequences of the CHOP and MetRS genes show significant sequence conservation not observed on their nonoverlapping 3′UTR domains. (**C**) Comparisons of nucleotide sequences in the 3′UTRs of three tail-to-tail overlapping pairs of genes identify maximal conservation in functional domains involved in mRNA stability, AUUUA motifs (ARE), and in polyadenylation signals.

DISCUSSION

Here we report that the human gene coding for methionyl-tRNA synthetase maps to the chromosome 12, precisely the location where the CHOP/GADD153 gene had been mapped previously on 12q13. CHOP and MetRS genes are coded by opposite strands of DNA and overlap in a 55 bp domain corresponding to their 3′ ends. This region of the chromosome 12 has been implicated in different forms of human cancer in at least two different

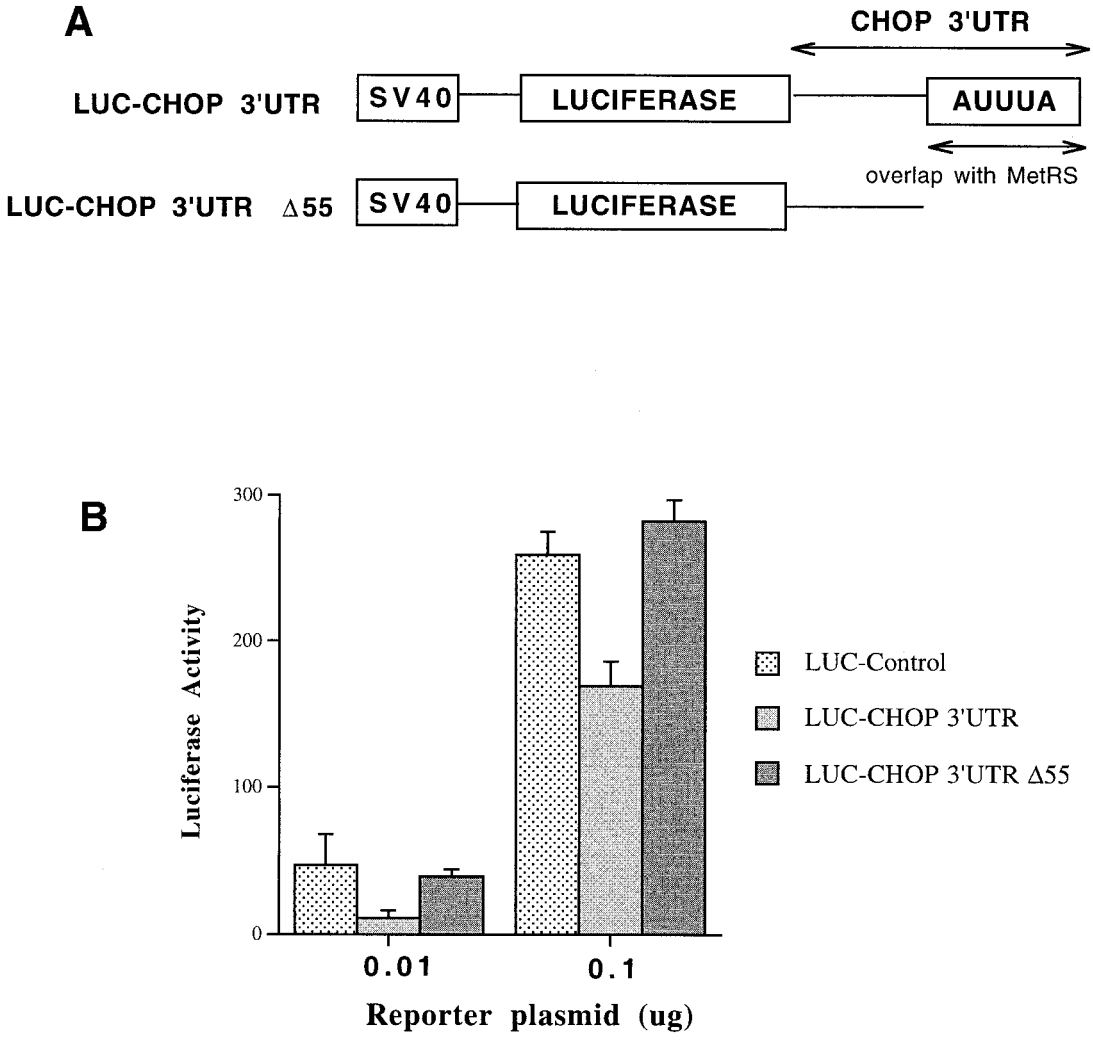

FIG. 3. The CHOP/MetRS overlapping domain contains a functional CHOP mRNA destabilization element. (**A**) Map of the luciferase reporter constructs engineered to contain the 3′UTR of CHOP and a deletion mutant that lacks the 55-bp overlapping region. (**B**) Transient transfections of a luciferase reporter plasmid containing the entire sequence of CHOP 3′UTR (LUC-CHOP 3′UTR) has significantly lower activity compared to the reporter control (LUC-Control). This effect is rescued if the CHOP/MetRS overlapping region is removed (LUC-CHOP 3′UTR Δ55).

ways. First, a translocation between chromosome 12 and chromosome 16 in human myxoid liposarcoma (16, 17) result in the expression of a fusion protein encompassing the amino-terminal domain of the RNA binding protein, TLS, and the complete coding region of CHOP (16, 17). The TLS-CHOP transcript also includes the 3′ untranslated region of the CHOP mRNA and its 55 bp of complementary sequence with MetRS mRNA has the potential to hybridize with the MetRS transcript. Our studies on the functional role of the overlapping domain suggest that the stability of the TLS-CHOP transcript may be increased as a consequence of its overlap with the endogenous MetRS mRNA. In addition, the regulation of MetRS expression could be altered in myxoid liposarcoma as a result of the chromosomal translocation and an increased level of MetRS could provide liposarcoma cells with a significant growth advantage compared to normal cells. This possibility is supported by previous reports-demonstrating an increased methionyl-tRNA synthetase activity in some forms of human cancer (25). Second, as a consequence of the overlap between the CHOP and the MetRS genes, amplification of the 12q13 locus in sarcomas (12, 13) and other forms of human tumors (14, 15) likely results not only in overexpression of the CHOP gene but also in overexpression of MetRS. This circumstance may result in a growth advantage of cells that could contribute to tumor progression. We are currently testing these possibilities.

Conservation of the CHOP/MetRS overlapping sequence in different species and its similarity to the overlapping sequences shared by other pairs of genes arranged in the same way, suggests a new form of genetic regulation. This idea is supported by our experiments showing that the overlapping domain plays

a role in mRNA stability. Because amino acid deprivation both induces the expression of CHOP (26, 27) and regulates the activity of the methionyl-tRNA synthetase (28) we speculate that a functional interaction between the two genes is highly probable. This interaction may consist of a functional or a physical interaction that could be established at one or more levels involving DNA, RNA or protein. MetRS catalyzes the specific attachment of methionine to its specific $tRNA^{Met}$ to generate methionyl-$tRNA^{Met}$ required for protein synthesis. A ternary complex composed of eIF2, GTP and Met-$tRNA^{Met}$ is also required for the initiation of translation. The availability of amino acids changes the concentration of the ternary complex to regulate the process of translation (29). Because CHOP expression is strongly regulated by amino acid deprivation (26, 27), we anticipate that the level of MetRS through its role in protein synthesis would also affect the expression CHOP. Another attractive possibility to explain the inter-species conservation of the tail to tail arrangement of the CHOP and MetRS genes and its role in translation control is the formation of a double-stranded RNA hybrid between the two transcripts in conditions of cellular stress. An *in vivo* double-stranded RNA duplex of more than 30 bp was shown to activate a double-stranded RNA-dependent signal pathway that controls initiation of protein synthesis. It has been shown previously that PKR (double-stranded RNA-dependent protein kinase), binds to segments of double-stranded RNA and phosphorylates the translational initiation factor eIF2a at Ser-51 to inhibit translation initiation. Phosphorylation of eIF2a by PKR is the major mechanism by which both animal and plant cells regulate protein synthesis when exposed to cellular stress (30).

Several pairs of genes are arranged in an overlapping manner similar to that of the CHOP and MetRS genes. A tail-to-tail arrangement was originally described in the mouse (23) and *Drosophila* (31) and more recently an overlap of the 3′UTRs of the Stat6 and Nab2 genes has been described (22). In this latter instance the 78 bp overlapping sequence is highly conserved between mouse and human, as occurs for CHOP and MetRS. So far no functional role for such overlapping arrangements of pairs of genes has been demonstrated in any of these cases. In *C. elegans* generation of a temporal gradient of LIN-14 that control stage-specific patterns during development has been shown to be dependent on an interaction of its 3′ untranslated region (3′UTR) with another RNA, lin-4 which bears sequences complementary to the lin-14 3′UTR (32). More recently, a similar RNA-RNA interaction has been suggested to play a role in Drosophila neurogenesis (33) An effect on mRNA stability and translation control of the RNA-RNA duplexes has been suggested (32, 33). Our data demonstrate that the CHOP/MetRS overlapping region also controls mRNA stability and therefore we propose a model in which in vivo formation of RNA-RNA duplexes is a new mechanism of regulation of gene expression.

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