Sequence and expression of the murine iron-responsive element binding protein

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The iron-responsive element binding protein (IRE-BP) binds to specific stem-loop structures known as iron-responsive elements (IREs) originally identified in the mRNAs of the ferritin and transferrin receptor genes (1). An incomplete cDNA clone of the human IRE-BP that has previously been isolated lacks a portion of the amino terminal coding sequence (2). We have cloned murine cDNAs which encode a functional IRE-BP. Transfection of the reconstructed murine IRE-BP cDNA into HeLa cells results in the expression of an IRE-BP which binds radiolabeled IREs with characteristic affinity and specificity.

We screened a murine liver λgt11 cDNA library (Clontech) by nucleic acid hybridization of human IRE-BP sequences. The reconstructed cDNA sequence was compiled from overlapping cDNA clones. The resulting sequence is 3412 bp in length with an open reading frame of 2667 bp. The predicted protein product contains 889 amino acids with a molecular weight of 98.1 kD. That we had obtained the entire open reading frame is supported by the finding of in-frame stop codons 5′ of the presumed initiating ATG, confirming that this region is the 5′ UTR. Human and murine IRE-BP clones are 87% identical at the nucleotide level. A comparison of the predicted amino acid sequences of the murine and human IRE-BP shows them to be 94% identical over 817 residues with the mouse encoding an additional 72 residues at the amino terminus. We have recently discussed the striking level of sequence conservation between human IRE-BP and porcine mitochondrial aconitase (3). The crystal structure of aconitase (4) is composed of four structural domains which form an active site cleft. All 18 active site residues previously identified as shared between porcine aconitase and human IRE-BP are present in murine IRE-BP as well (3). We have recently demonstrated that a reconstructed mouse-human chimeric IRE-BP has aconitase activity (5) and likely represents a cytosolic aconitase, an enzyme which has previously been described, but not purified or cloned (6, 7).

We reconstructed the murine IRE-BP in the expression vector pCDLSRα, transfected this construct into HeLa cells, and assayed for IRE-BP expression by IRE gel-retardation. Murine IRE-BP/IRE complexes migrate more slowly than human IRE-BP/IRE complexes in nondenaturing gels. Figure 1 demonstrates the presence of murine IRE-BP in lysates of HeLa cells transfected with the reconstructed cDNA clone. The transfected murine IRE-BP/IRE complex has the same electrophoretic mobility as a native murine IRE-BP/IRE complex (data not shown). In summary, we have cloned and reconstructed a murine IRE-BP cDNA that, when transfected into human cells, is expressed as a protein that binds to IREs with characteristic specificity.

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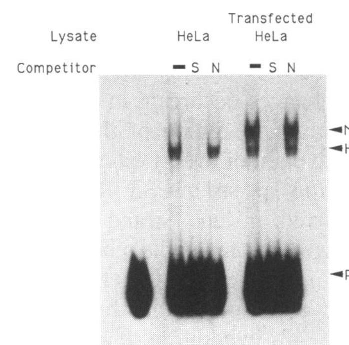

Figure 1. Expression of murine IRE-BP in human cells. Lysates (5 μg) from control-transfected HeLa cells (HeLa) or HeLa cells transfected with the murine IRE-BP (Transfected HeLa) were mixed with ^{32}P-labeled IRE and subjected to electrophoresis on a non-denaturing polyacrylamide gel. Arrows mark the bands corresponding to endogenous human IRE-BP/IRE complex (H), transfected murine IRE-BP/IRE complex (M), and uncomplexed IRE probe (P). The specificity of the IRE-BP/IRE interaction is demonstrated by the addition of 200-fold molar excess of unlabelled IRE as a specific competitor (S) or an equivalent molar excess of tRNA as a non-specific competitor (N).

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