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Internal Loop/Bulge and Hairpin Loop of the Iron-Responsive Element of Ferritin mRNA Contribute to Maximal Iron Regulatory Protein 2 Binding and Translational Regulation in the Iso-iron-responsive Element/Iso-iron Regulatory Protein Family

Yeohuang Ke, Hanna Sierzputowska-Gracz, Zofia Gdaniec, and Elizabeth C. Theil

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Internal Loop/Bulge and Hairpin Loop of the Iron-Responsive Element mRNA Contribute to Maximal Iron Regulatory Protein 2 Binding and Translation in the Iso-iron-responsive Element/Iso-iron Regulatory Protein Family

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ABSTRACT. Iron-responsive elements (IREs), a natural group of mRNA-specific sequences, bind iron regulatory proteins (IRPs) differentially and fold into hairpins [with a hexaloop (HL) CAGUGX] with helical distortions: an internal loop bulge (IL/B) (UGC/C) or C-bulge. C-bulge iso-IREs bind IRP2 more poorly, as oligomers (n = 28–30), and have a weaker signal response in vivo. Two trans-loop GC base pairs occur in the ferritin IRE (IL/B and HL) but only one in C-bulge iso-IREs (HL); metal ions and protons perturb the IL/B [Gdaniec et al. (1998) Biochemistry 37, 1505–1512]. IRE function (translation and physical properties (Tm and accessibility to nuclease)) is now compared for IL/B and C-bulge IREs and for HL mutants. Conversion of the IL/B into a C-bulge by a single deletion in the IL/B or by substituting the HL GC base pair with UA both derepressed ferritin synthesis 4-fold in rabbit reticulocyte lysates (IRP1 + IRP2), confirming differences in IRP2 binding observed for the oligomers. Since the engineered C-bulge IRE was more helical near the IL/B [Cu(phen)] resistant and more stable (Tm increased) and the HL mutant was less helical near the IL/B (ribonuclease T1 sensitive) and less stable (Tm decreased), both GC trans-loop base pairs contribute to maximum IRP2 binding and translational regulation. The 1H NMR spectrum of the Mg-IRE complex revealed, in contrast to the localized IL/B effects of Cu(II) hexammina...