

P027 Analysis of the conserved cleavage sequence 5'-CAU in the GIR1 ribozyme

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GIR1 is a cleavage ribozyme found as part of the group I introns of the twin-ribozyme organization that in addition contain a conventional splicing ribozyme (GIR2) and a gene (HEG) encoding a homing endonuclease. GIR1 has been reported to perform two sequential cleavages, three nucleotides apart, at internal positions (IPS1 and IPS2) close to the 3'-end. The function of GIR1 is thought to be the generation of a proper 5'-end of the homing endonuclease mRNA. Current examples of twin-ribozyme introns comprise the DISSU1 intron in the myxomycete *Didymium iridis* and the NASSU1 intron found in several species of the amoebaflagellate *Naegleria*. The sequence between the two processing sites in the GIR1 part of these introns, 5'-CAU, is conserved. We have performed a mutation analysis of DiGIR1 in two different length contexts (166.65 and 162.65). We find that A231 is critical for cleavage, whereas the requirement for C230 and U232 is relaxed and dependent on the context. Some of the mutants were found to cleave at incorrect sites.