Supplementary Material and Methods

In vitro Mutagenesis

Extension of P15. Mutations at U110 were introduced by PCR using Pfu DNA polymerase of a wild-type GIR1 template (pDi162G1; (Decatur et al., 1995)) and oligos C377: 5'- GGG AAG TAT CAT GGC TAA TCA CCA TGA TGC AAT CGG GAT GAA C (U110A) or C378: 5'- GGG AAG TAT CAT GGC TAA TCA CCA TGA TGC AAT CGG GCT GAA C (U110C) as the 5'-oligo and OP12: 5'- TCA CCA TGG TTG TTG AAG TGC ACA GAT TG as the 3'-oligo. The PCR-product was subsequently re-amplified to make templates for in vitro transcription (see below). Mutations of A206 were introduced by in vitro mutagenesis using the Quick Change site-directed mutagenesis kit (Stratagene) and oligos C405: 5'- CCT CTT AGG TGT GTT CAT TGA ACA GTC GTG TCC CCG/ C406: 5'- CGG AAC GAC TGT TCA ATG AAG (A206T) and C407: 5'- CCT CTT AGG TGT GTT CAG TGA ACA GTC GTT CGG/ C408: 5'- CGG AAC GAC TGT TCA CTG AAG AGG (A206G). In order to make double mutants, the U110 mutations were introduced into the A206 mutated templates.

GU pair. Construction of G109A and U232C were previously published (Johansen et al., 2002). Mutations at U207 were made as described above using oligos C477: 5'- GCC TCT TAG GTG TGT TCA ACG AAC AGT CGT TCC GAA AGG/ C478: 5'- CCT TTC GGA ACG ACT GTT CGT TGA ACA CAC CTA AGA GGC (U207C) or C479: 5'- GCC TCT TAG GTG TGT TCA ACG AAC AGT CGT TCC GAA AGG/ C480: 5'- CCT TTC GGA ACG ACT GTT CTT TGA ACA CAC CTA AGA GGC.

J5/4. Mutations were made in a wild-type GIR1 template as described above and oligos C415: 5'- CGG TGG GGG AAC ATC CCA TAA CAT CCG TCC TAA CGG/ C416: 5'- CGG TTA GGA CGG ATG TTA TGG GAT CGT CCC CCA CCG (G150A), C424: 5'- CGG TGG GGG AAC ATC CCA CAA CAT CCG TCC TAA CGG/ C425: 5'- CGG TTA GGA CGG ATG TTA GAT CGT CCC CCA CCG (U151C), C270: 5'- GAC GAT CCC GTG ACA TCC GTC CTA AGC/ C271: 5'- CGT TAG GAC GGA TGT CAC GGG ATC GTC (A152G), and C417: 5'- CGG TGG GGG AAC ATC CCG TAG CAT CCG TCC TAA CGG/ C418: 5'- CGG TTA GGA CGG ATG CTA CGG GAT CGT CCC CCA CCG.
Supplementary figure legends

**Supplementary Figure S1**: Mutational analysis of the P15 extension. (A) Secondary structure diagram of DiGIR1 showing the proposed extension of P15 (boxed). The nucleotides are numbered according to their position within the Dir.S956-1 intron. BP: Branch Point. IPS: Internal Processing Site (the GIR1 cleavage site). The initiation codon of the HE ORF is also boxed. (B) Kinetic cleavage analysis of substitution of the central base pair of the P15 extension (U110-A206) into an A-U (top panel) or a C-G (bottom panel) pair. In both cases, the disruptive mutations result in poor cleavage, whereas the compensatory mutations restore the activity to close to wild-type activity ( ● U110A/C, ○ A206U/G, ▼ U110A:A206U/U110C:A206G, ▽ wild-type GIR1).

**Supplementary Figure S2**: Mutational analysis of J5/4. (A) Comparison of the base pairing schemes at the P4-P5 interface in the DiGIR1 branching ribozyme and the *Azoarcus* group I ribozyme. (B) Kinetic cleavage analysis of individual mutations of each of the four nucleotides in the J5/4 junction. All of the mutations result in decreased cleavage rate. A153 is identified as a critical residue for DiGIR1 activity (● G150A, ○ U151C, ▼ A152G, ▽ A153G, ■ wild-type GIR1).

**Supplementary Figure S3**: The recognition of the G109oU207 pair from P15 is mediated by two A residues belonging to J5/4 that make A-minor interactions. Since A153 is critical for the branching reaction, it seems likely that this residue plays the same role as A residues from *Azo J4/5* for docking of the substrate helix.

**Supplementary Figure S4**: Transposition of the 5’-GUGUUC (grey box) from the 3’ strand of P15 (P15'' in GIR1) in the middle of J15/3 (shuffled GIR1-P1) accounts for restoring a topology of group I ribozymes. A prerequisite to this event is to provide an upstream
sequence capable of forming a new P1 segment so as to mimic the second step of self-splicing intron catalysis. This situation is opposite to the one described in Figure 6.
Supplementary Figure S1 (Beckert et al.)
Supplementary Figure S3 (Beckert et al)
Supplementary Figure S4 (Beckert et al.)