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Phylogeny of conserved adenines in linkers of Group-I introns

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Abstract:

We have analyzed the linkers in group-I introns, a characteristic region that is crucial to the folding and splicing process of the folded RNA, in seventy sequences spreading across r-RNA, t-RNA and organelle genes from various organisms including algae, fungi and protozoa. The study revealed a high degree of consensus of specific adenine residues in J3/4, J6/7 and J8/7 stems of the linker regions that were required to stabilize the local orientation, either as single residue or by forming unusual base pairs along with divalent metal ions. Conservation of these residues in the Group-I intron linkers suggests their significant contribution to the folded structure whose bonding and geometry recruit metal ions to interact in stabilizing the folded nature of RNA.

Key words: Riboyzme, Group-I intron, Adenine, Conservation and RNA

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Introduction

Group-I intron ribozymes belong to a class of unique RNA molecules that act as enzymes, store information and undergo autocatalytic processes (Cech 2002; Scott 2007). These are present in diverse organisms and are increasingly found in human pathogens such as *Acanthameoba sp.*, *Pnemocystisis sp.*, *Candida sp.* (Disney and Turner 2002). However, their absence in the human genome and the ease in assaying their activities make them ideal targets for design of inhibitors with potentially less side effects. Here we show the salient features of their evolutionarily conserved residues whose interactions with the metal binding domains might form putative sites for design of metal-based drugs.

Catalytic RNAs of the group-I type share a common secondary and tertiary structure , and unlike other RNAs they require a divalent metal ion and an exogenous guanosine co-factor to splice out the introns (Michel and Westhof 1990). During the catalytic activity, the well organized domains consisting of stems and loops (P1-P9), are brought closer without any helix distortion, to build up an active core in the ribozyme through sequences referred to as "linkers" (Golden et al. 1997). Linkers are very crucial in stabilizing the core region of the RNA folds through the adenosine bases (J8/7) and Mg^{2+} ion and assist in the formation of unusual base pairs whose bonding and geometry would enable flexibility in the stem folding (Golden et al. 1997; Rangan and Woodson 2003; Znosko et al. 2004) . All these observations led us to hypothesize that if adenyl residues in the linkers of the group-I intron structure are very important for autocatalytic process, we should expect a high degree of their conservation from diverse species.

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Materials and methods

Gene sequences of group-I intron ribozymes were obtained from the *Comparative RNA page* (Cannone et al. 2002) and conservation of residues in linkers was quantitated. As many as 70 sequences of the group I intron including rRNA, tRNA and organelle genes from various organisms representing bacteria, fungi, chlorophyta, protozoa etc., were used in the analysis. We classified linkers as inter-domain types (J3/4, J6/7) and intra-domain types (J4/5, J8/7) (Figure 1).

Results and Discussion

All the four linkers showed variable base sequences (Figure 1) but were highly conserved in the position of certain adenines, from diverse species. About 99% of these conserved residues were observed in J3/4 and J6/7 except in the case of chlorophyta and ascomycota. In linkers J8/9 we found only 90% conservation in the adenine residues and its position. A possible explanation for this could be divergence in evolution. In J4/5, the adenines varied in position but were nevertheless present everywhere. Conservation of these adenine residues in the four linkers of group-I intron sequences in diverse species, from blue green algae to lower eukaryotes, demonstrated a few points 1) Reaffirmed the importance of adenines in these four linkers in forming the crucial quadrate structures (Golden et al. 1997). 2) These adenines may have been present in the primordial stage of catalytic RNA and that's why they have been conserved to the present gene sequence, reflecting their evolutionary and functional importance. 3) During the folding of catalytic RNA, the quadrate structure formed by these conserved adenines, might form a possible

target for predominantly purine preferring drugs to compete for Mg^{2+} to interact and inhibit autocatalytic process.

Some examples could include the use of metal coordination complexes e.g. Pt^{2+} (from cisplatin) in combination with natural biopolymers such as ß-poly-L-malate (PMLA) - a polymer of L-malate produced naturally by a slime mold *Physarum polycephalum* (Karl et al. 2004). Earlier studies by us on platinum based complexes with PMLA showed interesting DNA binding kinetics (Malathi et al. 1998). Further molecular studies with such metal complexes could reveal if linkers can form better blocking sites of self-splicing process, thus forming potential strategies in controlling the pathogens.

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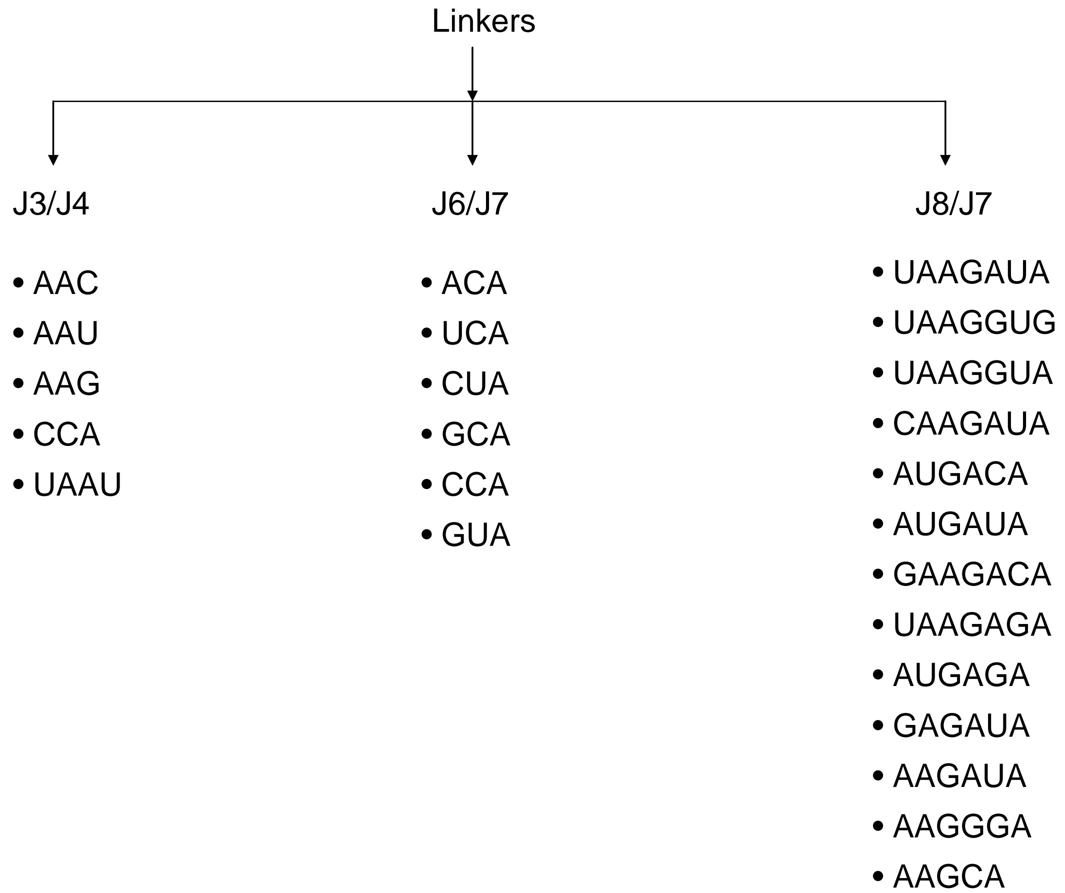

Figure 1: Different types of bases sequences observed for each of the linkers of group-I intron ribozyme from different species.

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