Perspective

Recognition of tRNA Precursors: A Role for the Intron

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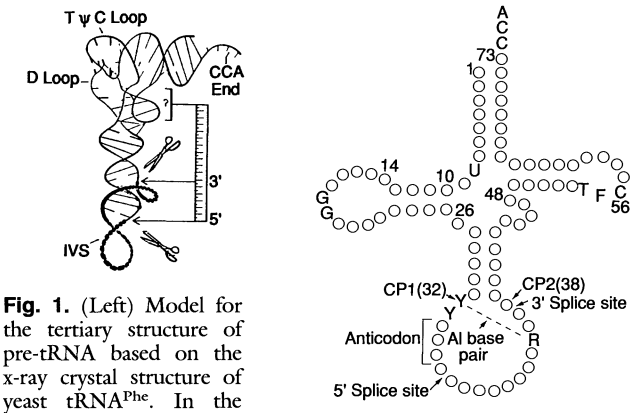

Fig. 1. (Left) Model for the tertiary structure of pre-tRNA based on the x-ray crystal structure of yeast $tRNA^{Phe}$. In the model, the enzyme recognizes some common nucleotide or structure in the mature domain of the pre-tRNA and measures the distances to the splice sites. (Right) Secondary structure of tRNA precursors illustrating the anticodon loop-intron (AI) base pair. Abbreviations: R, A or G; Y, C or U; IVS, intervening sequence; CP1 and CP2, the cardinal positions referred to in (*6*); F, pseudouridine.

MOST EUKARYOTIC GENES CONTAIN INTRONS THAT INTERRUPT the continuity of the genetic information. Introns are removed from a precursor RNA transcript by a process called splicing, whereby the intron is clipped out and the exons are rejoined to give the functional RNA molecule.

There are three distinct mechanisms of RNA splicing (*1–3*). In the self-splicing mechanism, discovered by Cech, the splicing process relies on the intron to take on a complex structure and to catalyze its own removal. In mRNA splicing in the eukaryotic nucleus, the pre-mRNA molecule is assembled into a large complex structure, the spliceosome, and components of the spliceosome, probably one or more of the small nuclear RNA's, catalyze the splicing reaction. In contrast, the small introns that interrupt the anticodon loops of eukaryotic precursor tRNA's are removed by a comparatively simple and familiar enzymatic process in which an endonuclease cleaves the two splice junctions and an ATP-dependent ligase joins the two exons.

Recognition of the intron in pre-tRNA is accomplished by the endonuclease, and just how the enzyme recognizes the splice junctions correctly in a number of different substrates has been the subject of 10 years of work on both the yeast and *Xenopus* systems. There are no absolutely conserved nucleotides in the nine yeast tRNA introns, but the position of the intron is always the same, interrupting the tRNA in the anticodon loop, one base downstream from the anticodon. The mature domain of the pre-tRNA is folded in the L-shaped tertiary structure common to all tRNA's. This result led to the early notion that the endonuclease recognizes the splice sites by binding to a site or sites in the mature domain common to all pre-tRNA's and measures the distance to the equivalently positioned intron-exon junctions (Fig. 1). This hypothesis was proved by engineering changes in the distance from mature domain to the splice sites. These alterations, insertion of base pairs in the anticodon stem, changed the size of the intron in a predictable way. Thus, the insertion of a base pair in the anticodon stem increased the size of the intron by two bases, one at each end; the insertion of two base pairs into the anticodon stem increased the size of the intron by four bases (*4, 5*). At the time, we did not believe that the intron contributed to the specificity of splice site recognition. In what seemed a convincing demonstration of this point, an intron containing mostly U residues was inserted into a pre-tRNA and it was spliced normally.

In this issue of *Science*, Baldi *et al.* (*6*) describe their elegant experiments that demonstrate the functional requirement in pre-tRNA for pairing between a base in the anticodon loop and a base in the intron. In all pre-tRNA's that they studied, a base pair must form between the pyrimidine at position 32, the first base in the anticodon loop, and a purine in the intron (the AI pair) (Fig. 1, right). In natural pre-tRNA's the purine in the AI pair is located three bases before the splice site (*7*). In some but not all cases, changing the location of this purine changed the position of the splice site. In cases where the position of the purine mattered, a counting mechanism seemed to prevail and the endonuclease cleaved the pre-tRNA three bases downstream from the purine, which pairs with the base at position 32. Whether the counting mechanism prevails depends on the identity of the first base in the 3′ exon (position 38). If this base is a C, the counting mechanism prevails, but if it is an A, changing the position of the AI base pair (still required) does not change the specificity of splicing.

These rules, at first reading, seem too arcane to be general; but to those who have studied protein recognition of RNA they should have a familiar ring. Aminoacyl tRNA synthetases (AAS's), for example, recognize tRNA by various mechanisms (*8, 9*). In some cases, they recognize specific unpaired bases in the anticodon, and in others they recognize specific base pairs in helical regions. They may also recognize general features of tRNA structure, for example, the presence or absence of the extra loop. It is not unusual for a particular AAS to recognize several of these sequence and structural features.

Endonuclease recognizes both the mature domain of the pre-tRNA and a structural feature of the intron that is mediated by the AI base pair. More research is needed to understand the function of this AI base pair. We do not yet know whether there are one or two active sites in endonuclease. We do know that there is not a preferred order of cutting the two sites. The AI base pair may be necessary to position the 3′ splice site in the active site.

These results highlight our general ignorance of tRNA tertiary structure and call for the need to know much more about the rules for RNA folding. Recent advances in the application of multidimensional nuclear magnetic resonance for the elucidation of RNA structure offer some promise for new insights (*10*).

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REFERENCES AND NOTES

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7. Ironically, the poly(U) intron, constructed to rule out involvement of intron sequences (*4*) in splice site recognition, contained a G three bases from the 3′ splice site. The G was placed there to facilitate enzymatic characterization of the intron product. Thus the AI base pair could form in that pre-tRNA.
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