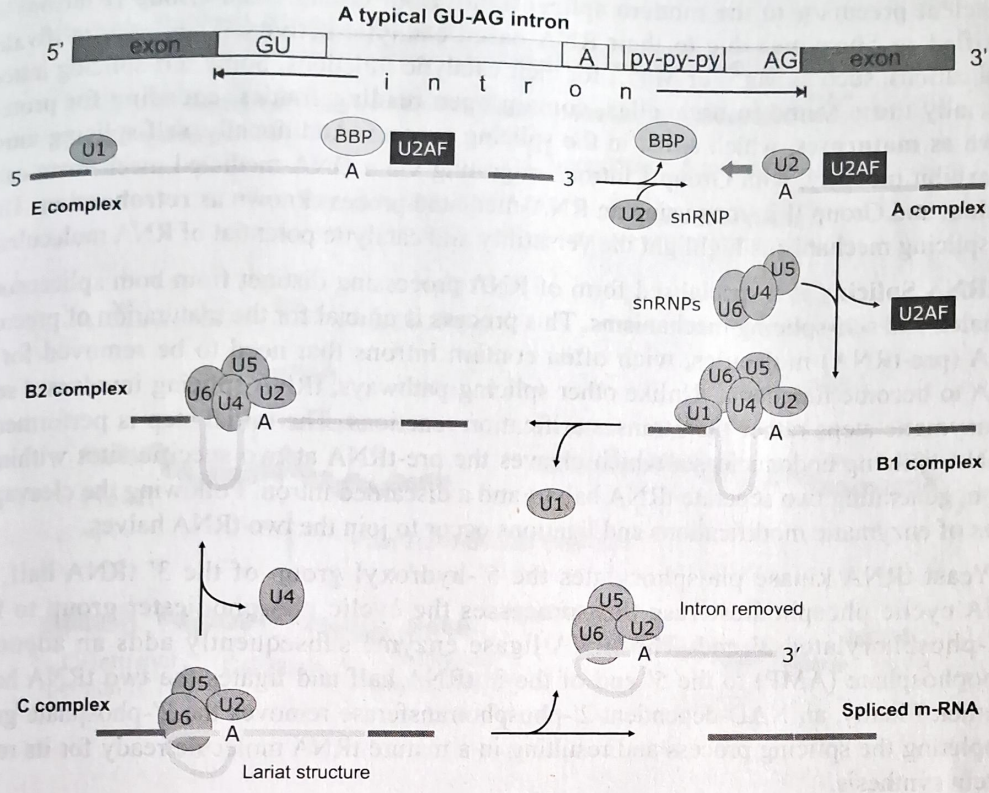
Canonical RNA Splicing (2)

The canonical RNA splicing can be illustrated by three different biochemical pathways namely, spliceosome mediated, self-splicing and t-RNA splicing which different in their mechanisms. **Spliceosome-mediated splicing** is a critical process for the maturation of pre-mRNA in eukaryotic cells. This complex mechanism predominantly removes introns that adhere to the GU-AG rule, where the 5′ end of the intron contains a GU sequence and the 3′ end an AG sequence. The process is orchestrated by the spliceosome, a large ribonucleoprotein complex composed of small nuclear RNAs (snRNAs) and various protein factors. The core snRNAs involved are U1, U2, U4, U5, and U6, which form small nuclear ribonucleoproteins (snRNPs). The assembly of the spliceosome begins with the binding of U1 snRNP to the 5′ splice site and SF1 to the branch point, followed by the binding of U2AF1 and U2AF2 to the 3′ splice site and polypyrimidine tract, respectively, forming the E complex. The transition from the E complex to the A complex involves the displacement of SF1 by U2 snRNP, which binds to the branch point sequence. This is followed by the binding of the U4/U6-U5 tri-snRNP to form the B complex. The B complex undergoes several rearrangements, releasing U1 snRNP and facilitating the interaction between U6 and the 5′ splice site. The final catalytic steps occur in the C complex, where U4 snRNP is released, and U6 snRNA pairs with U2 snRNA to form the catalytic core. This complex mediates the two transesterification reactions: the first one cuts the 5′ splice site and forms a lariat structure, and the second one cleaves the 3′ splice site and joins the exons. The spliced mRNA is then released, and the intron lariat is degraded. Throughout the splicing process, numerous ATP molecules are hydrolysed, primarily for the assembly and rearrangement of the spliceosome, rather than the splicing chemistry itself. Spliceosome-mediated splicing is vital for gene expression regulation and generating protein diversity through alternative splicing. The accurate recognition of splice sites and the efficiency of the splicing process are influenced by various cis-acting elements and trans-acting splicing factors, which help to ensure the fidelity of mRNA splicing in eukaryotic cells.

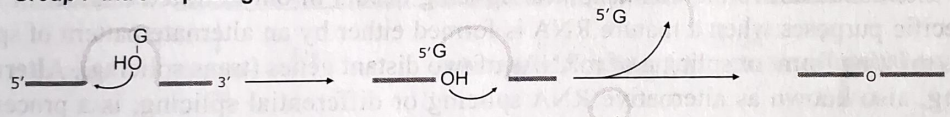
Self-splicing is characteristic of introns with unique RNA sequences capable of catalysing their own excision from precursor RNA molecules without the need for protein enzymes (generally called ribozymes). These introns are divided into two main groups: Group I and Group II introns. **Group I introns** typically begin splicing by binding a guanine nucleotide or nucleoside, which acts as the attacking group that cleaves the 5′ splice site. This initial cleavage is followed by a second transesterification reaction, where the 3′-OH of the 5′ exon attacks the 3′ splice site, joining the two exons and releasing the intron. **Group II introns**, on the other hand, initiate splicing through the action of a specific adenosine within the intron itself. The 2′-OH group of this adenosine attacks the 5′ splice site, forming a **lariat structure** similar to the one seen in spliceosome-mediated splicing. The subsequent transesterification reaction involves the 3′-OH of the 5′ exon attacking the 3′ splice site, thereby joining the exons and releasing the intron as a lariat. The structural and mechanistic similarities between Group II introns and the

Spliceosome Mediated Splicing

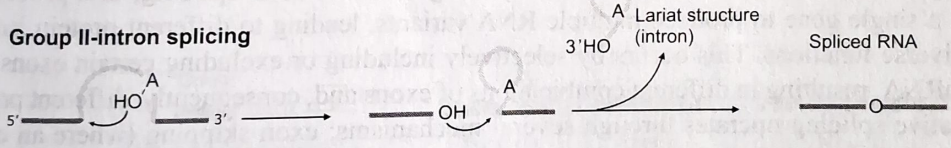


Self-Splicing

Group I- intron splicing



Group II-intron splicing



t-RNA splicing

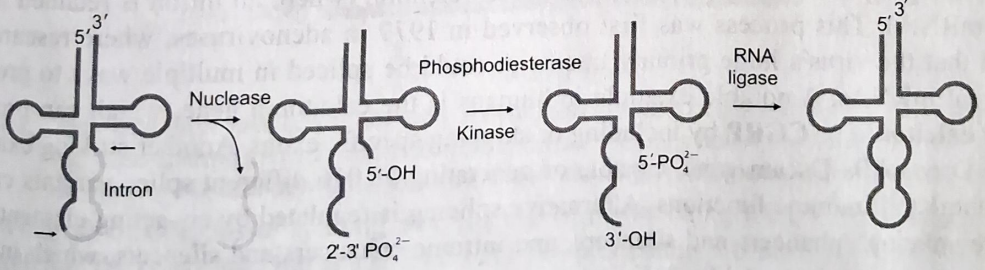


Fig. 7.2. Three commonly known mechanisms of canonical splicing (a) spliceosome mediated (b) self-splicing and (c) tRNA splicing.

spliceosome suggest an evolutionary relationship, with Group II introns possibly represent an ancient precursor to the modern spliceosome. Both Group I and Group II introns a classified as ribozymes due to their RNA-based catalytic activity. They require divale metal cations, such as Mg^{2+} or Mn^{2+}, for their catalytic functions. Some self-splicing intron especially those found in organelles, contain open reading frames, encoding for protei known as **maturases**, which assist in the splicing process. Additionally, self-splicing intron can exhibit mobility, with Group I introns migrating via a DNA-mediated mechanism calle **homing**, and Group II introns using an RNA-mediated process known as **retrohoming**. Thes self-splicing mechanisms highlight the versatility and catalytic potential of RNA molecules.

tRNA Splicing is a specialised form of RNA processing distinct from both spliceosome mediated and self-splicing mechanisms. This process is crucial for the maturation of precurso tRNA (pre-tRNA) molecules, wich often contain introns that need to be removed for the tRNA to become functional. Unlike other splicing pathways, tRNA splicing involves a series of enzymatic steps rather than transesterification reactions. The initial step is performed by a tRNA splicing endonuclease, which cleaves the pre-tRNA at two specific sites within the intron, generating two separate tRNA halves and a discarded intron. Following the cleavage, a series of enzymatic modifications and ligations occur to join the two tRNA halves.

Yeast tRNA kinase phosphorylates the 5′-hydroxyl group of the 3′ tRNA half, and tRNA cyclic phosphodiesterase then processes the cyclic phosphodiester group to form a 2′-phosphorylated 3′ end. The tRNA ligase enzyme subsequently adds an adenosine monophosphate (AMP) to the 5′ end of the 3′ tRNA half and ligates the two tRNA halves together. Finally, an NAD-dependent 2′-phosphotransferase removes the 2′-phosphate group, completing the splicing process and resulting in a mature tRNA molecule ready for its role in protein synthesis.