Экспрессия генов
типы сплайсинга

Н.Н. Колесников

Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

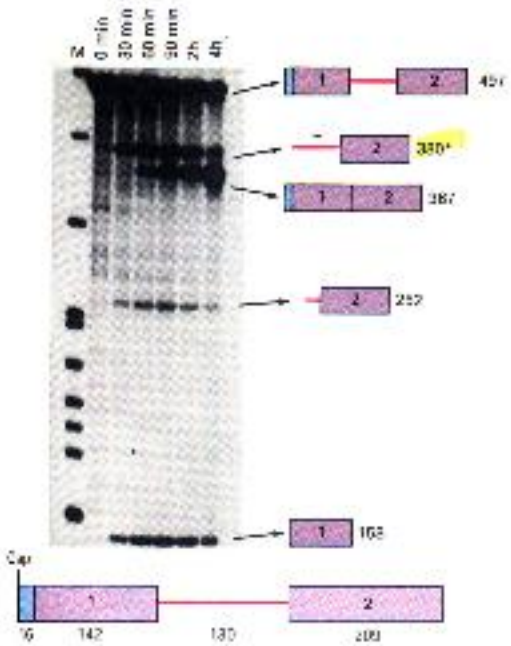


Figure 8-48 Analysis of RNA products formed in cell-free splicing system. A crude nuclear extract from HeLa cells was incubated with a 197 nucleotide long labeled RNA that had the contained portions of two exons (labeled 1 and 2) of human α-globin separated by a 130-nucleotide intron. After incubation for various times, the RNA was purified and subjected to electrophoresis and autoradiography (along with RNA markers, M). A more complete conversion of the slower-moving starting RNA (457) into the spliced form (367) occurred during the 4 h incubation. One of the intermediates in the reaction (380*) has an anomalous electrophoretic migration. It is actually 330 nucleotides long, and the retarded migration occurs because it is a lariat. This led to the discovery of the branch formation reaction diagrammed in Figure 8-49. From R. Ruskin et al., 1984, Cell 38:317. Photograph courtesy of T. Maniatis.

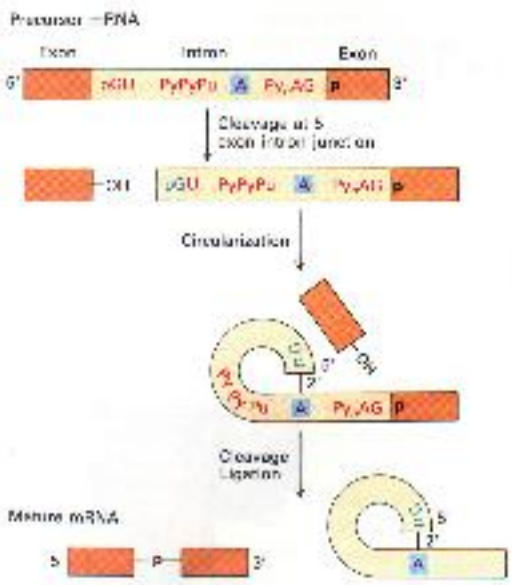
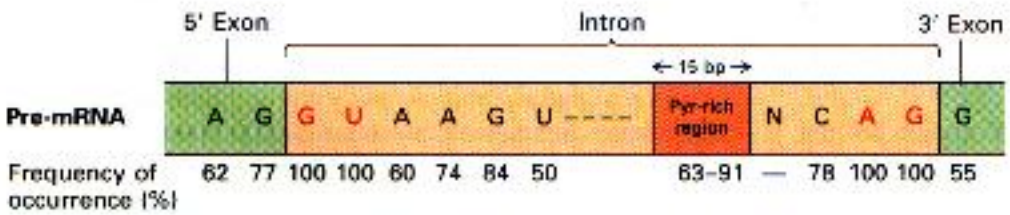
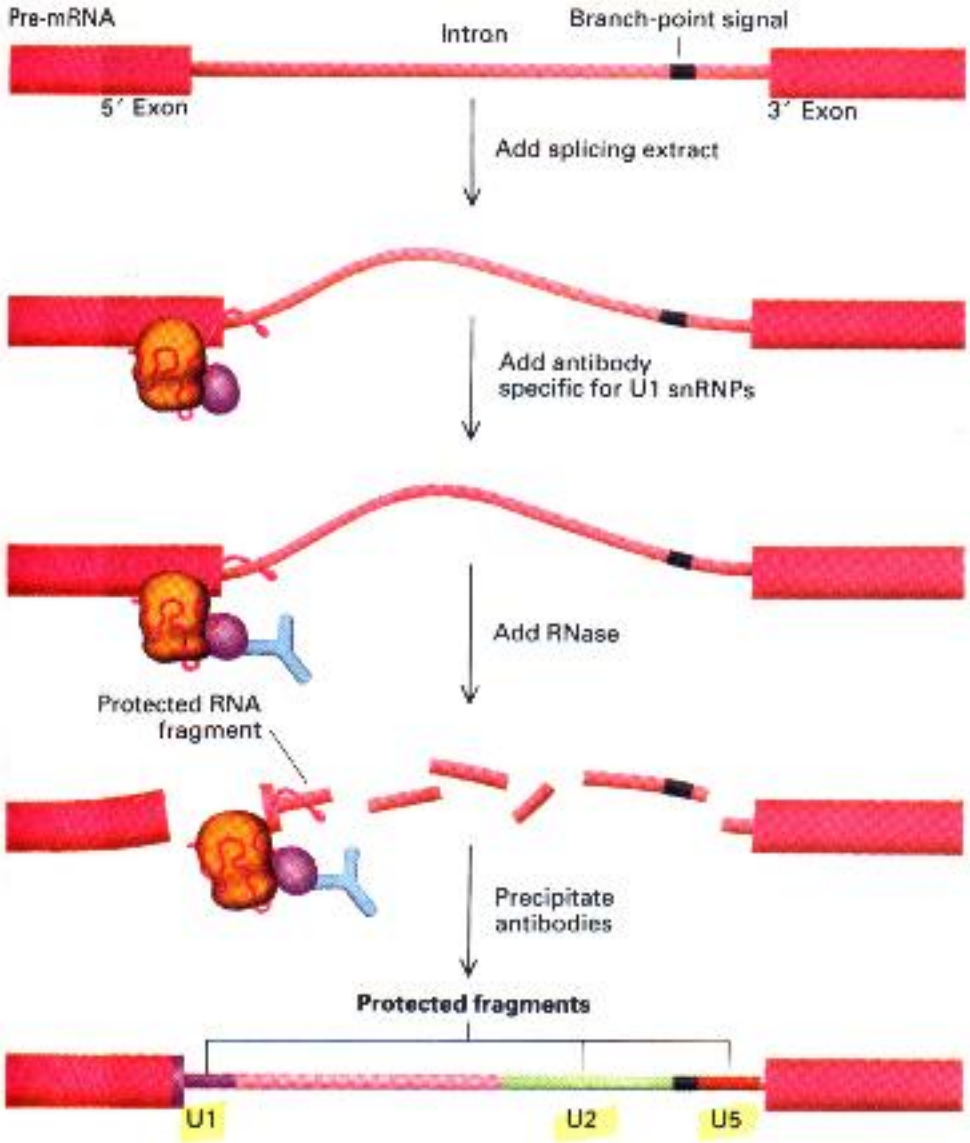


Figure 8-49 Pathway of splicing of pre mRNA. The steps illustrated have been shown to occur during in vitro splicing of adenovirus and globin hnRNA sequences by cell extracts. The consensus nucleotides GU and AG that begin and end the intron are indicated, as is the pyrimidine rich stretch (Py_n) near the 3' end. After the first cleavage at the 5' exon-intron junction, a circularization occurs to create a branched structure in which the guanylate at the cut 5' end of the intron forms an unusual linkage (5' → 2') with an adenylate located near the 3' end of the intron. The adenylate retains its normal 3' → 5' and 5 → 3' linkages to adjacent nucleotides. The pyrimidine rich sequence at the branch point shown in the diagram is characteristic of vertebrate and invertebrate hnRNAs. The corresponding yeast sequence is UACUAAC and is highly conserved. How the cleavage and ligation occur at the 3' junction is not yet known, but the phosphate linking the two exons is derived from the first nucleotide of the 3' exon. (See R. Ruskin et al., 1984, Cell 38:317, and R. A. Padgett et al., 1984, Science 225:898.)

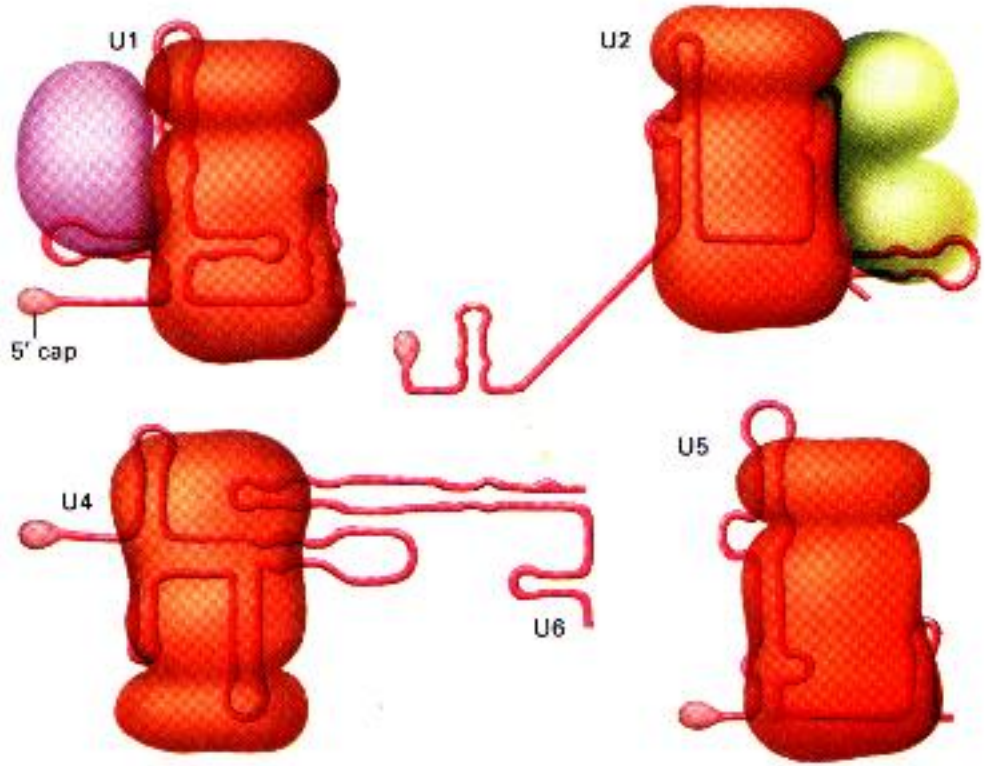


◀ **Figure 8-47** Frequency of occurrence of nucleotides around intron-exon junctions in eukaryotic pre-mRNAs. The consistent features are the (5')GU and (3')AG, which are universal, and a pyrimidine-rich region close to the 3' end, which is quite common. [See R. A. Padgett et al., 1986, *Ann. Rev. Biochem.* 55:1119.]



◀ **Figure 8-51** Identification of the site on pre-mRNA to which U1 snRNP binds. A labeled pre-mRNA substrate containing an intron was mixed with a nuclear extract capable of splicing (the extract contains all the necessary snRNPs). Addition of antibodies (blue) specific for U1 snRNP protected the binding region from subsequent attack by RNase. Analysis of the pre-mRNA in the antibody precipitate showed that it corresponds to a 15- to 20-nucleotide segment (purple) containing the 5′ exon-intron junction. Similar experiments with antibodies to U2 and U5 snRNPs have identified the binding regions shown at the bottom of the diagram. *After T. A. Steitz, 1988,* Sci. Am. *258:56.*

▶ **Figure 8-52** Electron micrograph of a spliceosome. Extracts of HeLa cells were mixed with a β-globin pre-mRNA substrate, and the reaction was interrupted before splicing was completed. This allowed purification of spliceosomes containing snRNPs and the substrate RNA. *From R. Reid, J. Griffith, and T. Maniatis, 1988,* Cell *53:949. Photograph courtesy of J. Griffith.*



▲ **Figure 8-50** Components of four snRNPs that take part in splicing of hnRNA. The five snRNAs (red)—U1, U2, U4, U5, and U6—share a complex of proteins (orange); the U1 and U2 snRNAs also bind with unique proteins (purple, green). The secondary structures of the snRNAs shown are based on maximum base pairing of the known nucleotide sequence. *After T. A. Steitz, 1988,* Sci. Am. *258:56.*

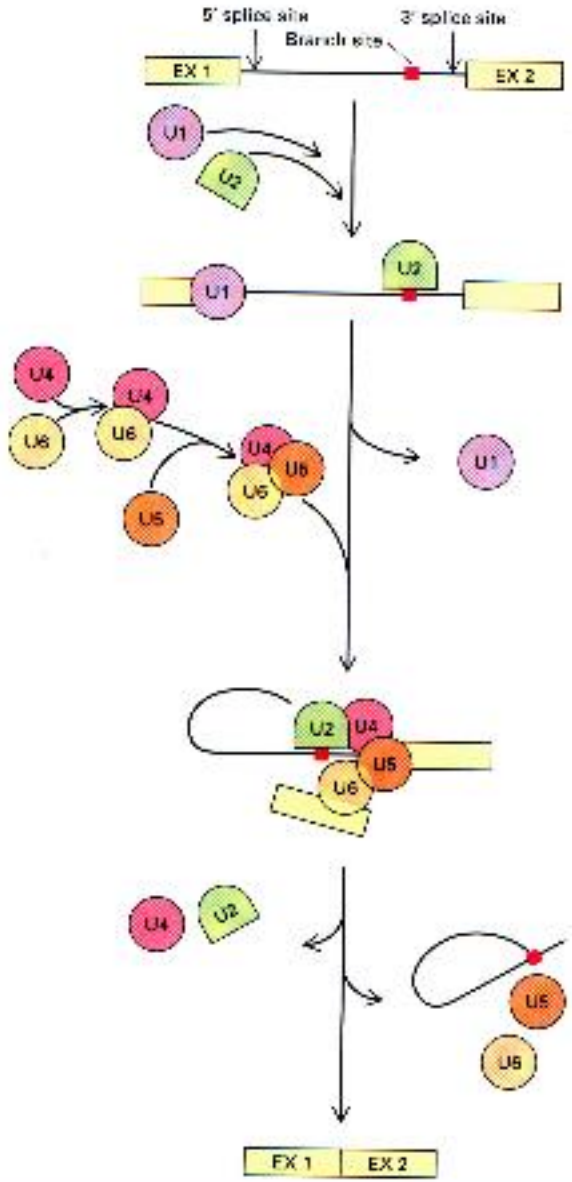


Figure 8-53 Possible pathway of spliceosome assembly and function. *After M. M. Konarska and P. A. Sharp, 1987,* Cell *49:763.*

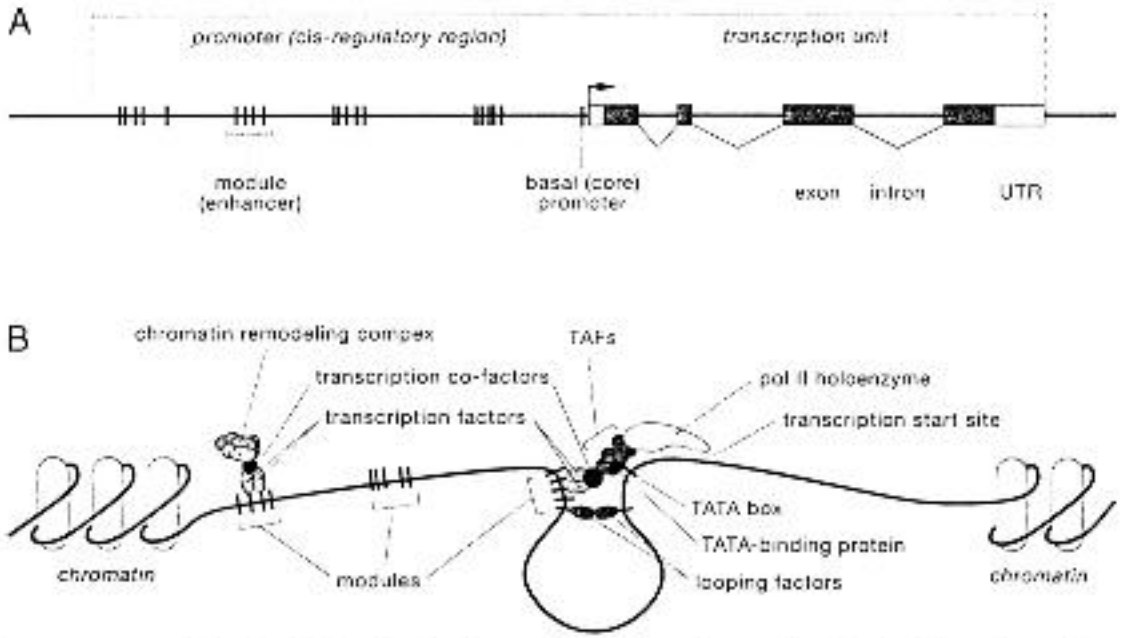


FIG. 1.—Promoter structure and function. (*A*) Organization of a generalized eukaryotic gene, showing the relative position of the transcription unit, basal promoter region (black box with bent arrow), and transcription factor binding sites (vertical bars). The position of transcription factor binding sites differs enormously between loci: although they often reside within a few kb 5′ of the start site of transcription (as shown here), many other configurations are possible (fig. 2). (*B*) Idealized promoter in operation. Initiating transcription requires several dozen different proteins which interact with each other in specific ways. These include the RNA polymerase II holoenzyme complex (~15 proteins); TATA-binding protein (TBP; 1 protein); TAFs (TBP associated factors, also known as general transcription factors; ~8 proteins); transcription factors (precise composition and number bound differs among loci and varies in space and time and according to environmental conditions, but several to many any time transcription is active); transcription cofactors (again, precise composition and number will vary); and chromatin remodeling complexes (which can contain a dozen or more proteins).