

CHAPTER 8

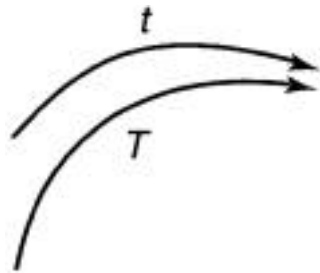
CHAMBON: DISCOVERY OF INTRONS

In 1979, Pierre Chambon, Philip Leder, and Bert O'Malley separately demonstrated that there were actual noncoding sequences embedded within eukaryotic genes. By comparing DNA with mRNA, these investigators showed that over 90 percent of a typical eukaryotic gene is not present in the mRNA produced from that gene. They called these noncoding chunks introns.

WHEN IS A DELETION NOT REALLY A DELETION?

The multiple reading frames revealed by nucleotide sequence analysis of viral genomes was an unexpected surprise, but eukaryote-infecting virus had another surprise in store, one that totally transformed ideas about the structural organization of eukaryotic genes. In studying the simian virus 40 (SV40) and adenovirus, several inexplicable results had been obtained:

1. In SV40, *t antigen* is encoded within the T antigen gene in the same reading frame, arising by late initiation of *t antigen* transcription:



The inexplicable result was that an internal section of the *t antigen* gene could be deleted, producing a *t antigen* lacking an internal methionine residue—and there was no effect on the *T antigen* at all! The expected deletion of an interior segment of the *T antigen* does not occur. If these antigen proteins reflect their genes, this doesn't make any sense at all, as both *t* and *T* are read from the same nucleotides *in the same reading frame* (see chapter 13). How can it be a deletion and not be a deletion at the same time?

2. In the human adenovirus (which causes the common cold), eight genes are transcribed late in the virus life cycle on one long RNA molecule accounting for most of the genome. This long RNA molecule is then processed to produce the eight shorter mRNA molecules that actually function in translation. The problem is that upon examination, investigators found that the resulting eight mRNA molecules were *too short*: the sum of their length was nothing like the length of the original transcript! To see if the missing RNA was due to the ends of each mRNA being “nibbled” by something, investigators hybridized the short mRNA molecules to the original DNA that made the primary transcript. If the “lost” material of the long primary transcript sequences was terminal and was “nibbled” away later, then DNA-RNA hybrids should be observed with DNA tails that could then be digested with single-strand exonuclease. What was actually observed was quite different. When investigators hybridized a gene back from the mRNA molecule, they found that the extra DNA segments were not at the *ends* of the mRNA, but *inside* it!

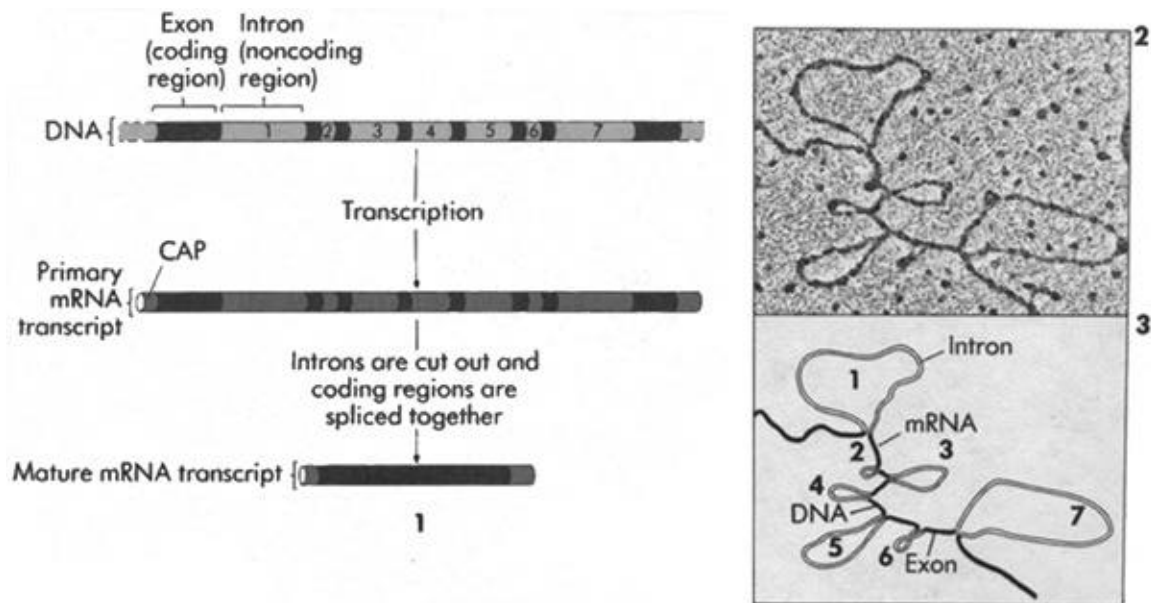
Both the SV40 and adenovirus results reflect the presence within eukaryotic genes of *intervening sequences*, soon dubbed *introns*, which are not included in transcribed mRNA. Somehow, the primary RNA transcript is cut up, the introns snipped out, and the residual *exons* (coding sequences) spliced

together to form the realized mRNA. It seems a preposterous way to go about things, and yet this pattern of *gene splicing* is a fundamental characteristic of eukaryotic DNA. It is not just another “virus trick,” easily dismissed as an evolutionary peculiarity imposed by restrictions of the virus life cycle. Introns are widespread among the genes of higher eukaryotes.

CHAMBON'S EXPERIMENT

Pierre Chambon and his colleagues set out to show that eukaryotic genes were encoded in segments excised from several locations along the transcribed mRNA. These excisions would subsequently be “stitched” together to form the mRNA that would actually be translated in the cytoplasm. To demonstrate this, they first isolated the mRNA corresponding to particular genes for the production of hemoglobin and ovalbumin in red blood cells. It was easy to extract and purify these mRNAs from the genes for those proteins, since they were so abundant in blood cells. After mRNA was isolated and purified, an enzyme called *reverse transcriptase* was used to “backtrack” and create a DNA version of the mRNA. This version is called copy DNA, or *cDNA*. The *original* gene for hemoglobin was then isolated from the nuclear DNA and cloned so that now the investigators had two versions of the gene: the original from the nucleus, and the *cDNA* “backtrack” constructed from the mRNA. Single strands of each of these gene versions were then combined to make hybrid—that is, a new duplex (double helix) was formed using one strand from the original gene and one strand from the *cDNA*.

When the resulting hybrid DNA molecules were examined by electron microscopy, Chambon found that the hybridized DNA did not appear as a single duplex. Instead, unpaired loops were observed:



As you can see, the ovalbumin gene and its primary transcript contain seven segments not present in the mRNA version that the ribosomes use to direct protein synthesis. On these data Chambon and his colleagues based their conclusion: nucleotide sequences are removed from within the gene transcript before the cytoplasmic mRNA is translated into protein. Because introns are removed from within the transcript *prior to* translation, they do not affect the structure of the protein that is encoded by the gene in which they occur.