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Regulation of Gene Expression, DNA Sequences and Protein Factors Involved in the Initiation of Transcription, Assembly of the Transcription Apparatus

Research in Dr. Stumph's lab is aimed at understanding the molecular mechanisms of gene expression in higher organisms. Dr. Stumph's group has been characterizing and studying the expression of genes that code for the small nuclear RNAs (snRNAs) known as U1, U2, U4, U5, and U6. The snRNAs are a special class of RNA molecules that are involved in messenger RNA (mRNA) splicing (the removal of intervening sequences from mRNA precursors). The genes coding for the U-snRNAs are interesting to study not only because they code for RNA molecules with such fundamental importance to cellular metabolism, but also because their expression appears to be controlled by unique mechanisms. For example, most snRNA genes are transcribed by RNA polymerase II (the same enzyme that synthesizes mRNAs), but these Pol II-transcribed snRNA genes contain no introns and they lack the TATA sequences commonly found upstream of mRNA transcription units. Surprisingly, and in contrast, U6 snRNA genes contain TATA boxes and are transcribed by RNA polymerase III.

Despite this difference in RNA polymerase specificity, the promoter sequences of U6 genes are actually very similar to the promoters of U1-U5 genes. A 21-base-pair-long cis-acting sequence, termed the proximal sequence element, or **PSE**, is uniquely required for the transcription of both classes of snRNA genes. This PSE is recognized sequence-specifically by a multi-subunit protein termed the PSE-binding protein, **PBP**. We have shown that PBP and the TATA-binding protein (TBP) are both required for the transcription of U1 and U6 snRNA genes by RNA polymerases II and III respectively. Our goal is to understand how the same two proteins (PBP and TBP) can in one case recruit RNA polymerase II but in the other case recruit RNA polymerase III. In order to answer this question, we use the fruit fly Drosophila melanogaster as a model system, and we employ various biochemical, genetic, and molecular biological techniques.

As mentioned above, the PSEs of the Drosophila U1 and U6 genes are each 21 base pairs in length, and they are both recognized and bound sequence-specifically by the Drosophila melanogaster PSE-binding protein, **DmPBP**. The U1 and U6 PSE sequences are identical at 16 of the 21 positions. However, we have recently shown that the 5 nucleotide differences between them are sufficient to determine the RNA polymerase specificity of the U1 or U6 gene promoter. Furthermore, by site-specific protein-DNA photocrosslinking techniques, we have determined that DmPBP consists of at least three distinct polypeptide chains that contact the DNA of the PSE. *Significantly, the data also reveal that the precise contacts made between the protein and the DNA are different depending upon whether DmPBP is bound to a U1 or U6 PSE sequence.*

From these data, we have proposed that the U1 and U6 PSE sequences act as differential allosteric effectors of DmPBP. According to this model, when DmPBP binds to a U1 PSE sequence, it adopts a conformation that allows it to recruit only RNA polymerase II basal factors during subsequent steps of pre-initiation complex assembly. On the other hand, when DmPBP binds to a U6 PSE, it adopts a conformation compatible with the recruitment of only RNA polymerase III basal factors. Biochemical and biophysical experiments are in progress to further test this model.

As an alternative approach to testing the model, we are also employing the power of Drosophila genetics. Transgenic flies have been produced that contain a neomycin-resistance gene under the control of a U1 gene promoter, except that the U1 PSE has been replaced with a U6 PSE. As a result of this change, these flies do not express neomycin resistance because the gene is not transcribed by RNA polymerase II. We are currently mutagenizing flies and screening for mutants that are resistant to neomycin. The mutations are expected to alter the interaction between DmPBP and the PSE. By analyzing these mutations and the effects that they have on protein conformation, we expect to be able to uncover the molecular details involved in determining the RNA polymerase specificity of snRNA gene promoters.

Representative Publications

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