Prof I C Eperon - Analysis of mechanisms of pre-mRNA splicing by single molecule fluorescence

Pre-mRNA splicing is highly complex. Over a hundred proteins and several RNA species assemble into dynamic complexes, in a pathway defined by several stable partial complexes. However, the relevance of these has been challenged and very little is known about transient or unstable components. Even less is known about the mechanisms by which many proteins affect the selection of splice sites. Splicing reactions in vitro cannot be reconstituted from pure components but require nuclear extracts. We will use single molecule fluorescence to follow the order, stoichiometry and rates of assembly and disassembly of components during splicing reactions. In effect, we will watch individual protein molecules as they join the splicing complex. This methodology may have widespread uses for other complex processes, such as transcription.

We have recently made a breakthrough in studies on the mechanisms by which splicing is regulated. PTB is a major splicing regulatory protein, implicated in the control of tissue-specific splicing. In collaboration with Prof. C.W.J. Smith and co-workers in Cambridge, we have used single molecule methods to determine the number of molecules of PTB bound to a regulated exon in complexes assembled in nuclear extracts. This has allowed us to identify where PTB molecules are bound on the pre-mRNA and to formulate models for their mode of action. The project offered is to use the same methods to study the processes during which splice sites are selected in pre-mRNA. A strong background in mathematics or physics is not essential but would be helpful.

Prof I C Eperon - Mechanisms of splice site selection

Splicing requires extraordinary precision and fidelity, as introns that comprise as much as 98% of an initial transcript are identified and removed accurately. However, over 90% of protein-coding genes also undergo alternative reactions that allow other mRNA isoforms and, often, proteins to be produced. These reactions can be tissue-specific, or connected to signalling pathways. This is the principal means by which 21,000 genes can encode many more proteins.

Our aim is to investigate the mechanisms by which splice sites are selected, and the ways in which this selection is modulated or controlled in some genes in different tissues. This involves the analysis of RNA-protein interactions in vitro, isolation of complexes and mass spectrometry, cross-linking and the mapping of sites of protein binding. Part of the work may also involve analysis of the coupling between transcription and splicing. The roles of some sequences and proteins will be tested in cell culture by mutagenesis and expression, as well as RNAi and protein over-expression.

References

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Prof I C Eperon - Correcting splicing: development of therapies for cancer based on targeted oligonucleotide enhancers of splicing

Many genetic diseases result from point mutations that prevent an exon being incorporated during pre-mRNA splicing. Changes in splicing patterns are beginning to be implicated also in acquired disorders, which is unsurprising since alternative splicing patterns account for three-quarters of the proteins expressed in mammals, including those that control cell life or differentiation. Hence, it was an important advance when we developed a method recently for rescuing the incorporation of defective or silent exons. In this method, RNA-binding splicing factors are recruited to a target exon using a bipartite oligoribonucleotide adaptor that contains a binding site for the factors and is complementary to the target RNA. The principle was tested with exon 7 of the SMN2 gene, which is defective in splicing but incorporation of which would rescue spinal muscular atrophy (SMA), one of the most common lethal autosomal recessive diseases. A bipartite oligoribonucleotide adaptor complementary to exon 7 stimulated splicing in vitro and rescued the incorporation of the exon and hence SMN protein expression in fibroblasts from patients. The method would have widespread applications if it could be developed to the point of clinical use.

The aim of the project is to optimise use of the method to control splicing reactions implicated in cancer development. You will optimise the design of the oligoribonucleotide measuring changes in RNA and protein levels in cultured cells, and you will join collaborative efforts with other labs to investigate ways of improving the uptake of oligos into cells and tissues.

References.

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