Toward Full 3D Structural Investigation of Intron Splicing in Human Cytochrome P450 2D6 pre-mRNA

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1 Introduction

Critical features in intron sequences were investigated using molecular dynamics simulation techniques to help understand the initial conditions of the intron splicing mechanism in pre-mRNA sequences associated with constitutive splicing.

2 Methods

Intron sequences from the human cytochrome P4502D6 mRNA (2D6) were examined based upon previous evidence of unusually robust structural features in these sequences [2, 3]. Molecular dynamics simulation techniques were used to identify significant 3D framework features in the intron superstructure which may potentially assist in the promotion of this splicing. In particular, known critical structural segments of the introns sequences were studied in the context of the folded pre-mRNA architecture: *viz*, the $5'$ end, the branch point, the poly-pyrimidine tract, and the $3'$ end of the introns. Calculations were carried out under *in vacuo* conditions for 1 ns time intervals using the Discover 97 package at the Institute of Medical Science Human Genome Center computer facility.

3 Results

It was found that the framework of the fully analysable intron sequences from the 2D6 mRNA family appear to fold into 3D structures in which the critical segments are in close proximity ($15 \sim 30$ Å) to each other. There was some evidence of flexing in the superstructure; nevertheless, the structure forms a roughly triangular pattern with a fairly rigid framework to support this degree of localization. The structures appear to be an evolutionary balance between rigidity and structural flexibility. For 2D6 pre-mRNA, such stable structural features may provide an important signal to the splicing factors.

4 Discussion

These results are in strong agreement with experimental evidence indicating the close proximity of the branchpoint to the $5'$ end and the probable necessity of structure in distinguishing the $3'$ end [1]. The structural framework is expected to provide a template for attachment of splicing factors which show little sequence specificity yet appear to localize first at these critical junction points of the majority of pre-mRNA sequences [4]. Proximity of critical splicing features coupled with a comparative structural rigidity in the framework has the potential to enhance the rate of attachment of splicing factors and

to increase the probability of connecting vast segments of pre-mRNA intronic regions at the critical junction points. However, whether this characteristic applies only to human 2D6 introns or whether it represents a more general phenomena in constitutive splicing is still under investigation.

In the current evaluations, the pre-mRNA is considered in the context of the initial recognition which precedes the formation of complex E or exon/intron definition [5]. After attachment of splicing factors [4], These initial conditions in the pre-mRNA structure are likely to be significantly altered as the splicing machinery assembles and progresses toward the commitment complex.

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