

## In Search of the U1 snRNA from *Entamoeba histolytica*

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Most early branching eukaryotes have few or no intron-interrupted genes. In the almost ten thousand genes of the protozoan parasite *Entamoeba histolytica* a little less than 2 thousand introns have been reported [1]. From these, just a few have been validated by comparison of the genomic and cDNA sequences and or RT-PCR [2-9]. Strikingly, most of the identified *Entamoeba* introns possess well-conserved 5' (GUUUGU) and 3' (UAG) splice sites (ss) although the Branch point Sequences (BS) are less conserved or not identifiable at all [7]. Also, the *Entamoeba* introns share a strong intron mean size bias of 50 nucleotides. Due to their size it has been proposed that the splicing mechanism operates through intron definition [10].

Data mining of amoebic proteins led to infer that *Entamoeba* sp and their common ancestor as well possess the components of the major spliceosome [11], which might be responsible for the splicing events performed by this parasite, as suggested by their data. Furthermore, the U2, U4, U5 [9] and U6 snRNA [12] have been cloned and identified.

Two pieces of information are still required to approach the problem of snRNP functions in the amoebic spliceosome. The first is the identification of the identification of the U1 snRNA; the second is to gain functional insights of these snRNPs.

We wish to put forward a strategy for the recovery and identification of the *Entamoeba* U1 snRNA. To this end, it has been customary to perform immunoprecipitation experiments using antibodies targeted to one of the U1 snRNP components assembled therein during its biogenesis, for example U1 70K [13]. This approach has proven to be useful to disclose the snRNA sequence and the snRNP structure, and using in vitro splicing assays it also provided mechanistic insights of the U1 snRNP.

In a recent paper, Shao et al. [14] reported a variation to this approach, allowing not only the precipitation of the U1 snRNP but also evidencing its functional relationships with other spliceosomal components during intron definition in the fission yeast *Schizosaccharomyces pombe*. They had previously shown that the DExD/H RNA-dependent ATPase Prp5 interacts with both U1 and U2 snRNP during intron definition in [15]. They expressed a tagged Prp5 in the fission yeast, and co-immunoprecipitated the proteins interacting with this splicing factor. This way Shao and coworkers found that SF3b mediates U2 snRNP/Prp5 recognition as much as Rsd1 mediates U1A (a component of the U1 snRNP)/Prp5 recognition.

Since all of the aforementioned factors have been annotated in the *Entamoeba* genome, it is possible that using a similar approach the U1 snRNA could be isolated. Albeit two DExD/H ATPases are readily identified in the *Entamoeba* databases, no information is available regarding which of them performs the role Prp5 exerts during spliceosome assembly. Therefore, to carry out this approach in two well conserved proteins can be used, SF3b or U2AF65, both of them key factors in U2 snRNP recognition of the 5' ss. In addition, the method itself provides the appropriate co-immunoprecipitation controls, namely the U2 snRNA, and provides insights in U1 snRNP functions.

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