EVALUATION OF THE PH.D. THESIS OF

SAMIRA **HOZEIFI**

on the

REGULATION OF ALTERNATIVE SPLICING VIA CHROMATIN MODIFICATIONS

by Christian LANCTÔT, member of the Jury, March 12th, 2014

RECOMMENDATION **ACCEPT**

SUMMARY

This work investigates the relationships between alternative splicing of primary mRNA transcripts on the one hand and post-translational chromatin modifications/binding of chromatin remodelers on the other. It uses a vast array of techniques to do so, including exon microarrays, chromatin immunoprecipitation, advanced fluorescence microscopy, RNA FISH. The thesis is divided into 3 projects. The first project is a genome-wide analysis of the impact of a HDAC inhibitor on alternative splicing. The second project describes the link between alternative splicing and the Brd2 chromatin remodeler. The third project studies alternative splicing in the context of a myocyte differentiation model.

STRENGTHS

The main strengths of this work reside in the clear formulation of the problem that is being investigated and in the use of state-of-the-art techniques to address this problem. The three projects on which the candidate has worked during her Ph.D. studies are well tied-up together, despite either focusing on different proteins (projects 1 and 2) or using different models (project 3). The candidate is undoubtedly well trained in the most up-to-date cellular and molecular biology techniques. Her ability to handle whole genome data is a definite advantage. The experiments she presents are well-controlled and conclusive. Alternative splicing is one of the key cellular mechanisms that increase the diversity of the genomic output. The thesis reports timely advances in our understanding of the regulation of this important process. Realization of the first two projects led to the publication of 2 peer-reviewed articles.

WEAKNESSES

The main weakness of this thesis is the relative lack of critical thinking it displays. In the (too brief) literature review for instance, the candidate simply cites a series of background papers without ever challenging their results or even mentioning what are the controversies in the field. In fact, the description of these published results is often wanting, with no details being provided. More importantly, the limitations of the techniques that are used are never mentioned or discussed at the end of each chapter. In the unpublished part of the thesis (Project 3), the results are presented too uncritically. To give just one example, the fact that the alternative splicing of NCAM1 and ITGA7 concerns only a relatively small proportion of transcripts is never mentioned or critically addressed. In my opinion, the ability to critically evaluate one's results is the criteria by which the outstanding scientist is set apart. Unfortunately, it is lacking here.

MINOR POINTS

The thesis would have benefited from a thorough proof-reading. The typos are numerous, many sentences are not clear. I would have liked to see legends to the figures in the "literature review" section. Abbreviations used in the Material/Methods section are not included in the abbreviation list. The thesis could also have contained a few schematized pictures drawn by the candidate, instead of their being copied from published material.

QUESTIONS

1. The splicing of 683 genes out of 17.771 was changed upon HDAC inhibition.

   Considering that histone acetylation is presumably increased throughout the genome or throughout the gene (Fig 5.1.3B), what are the main determinants of the specificity that is observed, both at the gene level and at the exon level?

2. The main findings of this work concern the interplay between alternative splicing and chromatin. Two models are presented, kinetic and recruitment. In the former case (kinetic):

   How do histone post-translational modifiations affect the processivity of the RNA polymerase holoenzyme?

   In the latter case (recruitment):

   How is the nascent RNA associated with chromatin? What are the molecular players involved and how are their activities influenced by post-translational modifications of the chromatin?

3. Sodium butyrate was used to increase histone acetylation levels throughout this work (e.g. Fig. 5.1.1).

   What is/are the mechanisms of action of this drug? What is its specificity? What is its influence on cellular metabolism? How does it compare to other HDAC inhibitors?

4. Brd2 was shown to preferentially bind at the promoters of genes (Fig 5.2.2). Yet, almost half of the transcripts that are alternatively spliced upon Brd2 knockdown do not show changes in expression.

   Do you expect Brd2 to act at the promoter also in these cases? Assuming it does, which mechanism of action can you suggest?

5. It was shown that sodium butyrate increases the retention time of Brd2 onto the chromatin (Table 5.2.2).

   How does the splicing of the genes that are affected by Brd2 knockdown respond to sodium butyrate? In other words, what functional link is there between Brd2 dynamics and the regulation of alternative splicing?