Thesis: "The role of human RECQ5 helicase in the maintenance of genomic stability"

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The thesis "The role of human RECQ5 helicase in the maintenance of genomic stability" by Vaclav Urban investigates the role of RECQ5 in the maintenance of genomic stability, particularly in situation when transcriptional and replication machineries collide. The process is well recognized to be a significant source of genome instability, however the mechanistic bases and molecular mechanism(s) leading to genome instability are poorly understood.

The thesis consists of five major sections: 1) General introduction to the topic; 2) Clearly defined aims of the study; 3) List of used methods and publications; 4) Participation of the author in the performed studies; 5) Discussion; 6) References and 7) Enclosed three published papers including one paper under submission. Sections 1-6 are very well-written and provide informative and well-selected overview of the studied field without burdening a reader with unnecessary details. Enclosed papers, one first- author and three co-author, are published in excellent peer-reviewed journals.

Overall I am very positive about the work presented in the thesis which is without any doubt outstanding. It is clear that Vaclav Urban obtained hands-on expertise with many biochemistry and molecular biology techniques and gained an insight into the fields of transcription and DNA damage repair. Notably, he performed all the experiments (seven full figures and five supplementary figures) in his first-author paper (The Journal of Cell Biology) by himself. The experiments involve many non-trivial molecular biology and biochemistry methods including chromatin immunoprecipitation (ChIP) and fluorescence recovery after photobleaching (FRAP) and it was clearly a lot of lab work to successfully establish/apply those techniques. He can also summarize scientific text in the form of review as clearly evident from the thesis. His experimental work was published in three prestigious peer-review journals, including the paper in the Journal of Cell Biology where he is the first author.

In conclusion I can recommend the thesis for successful defense without any reservation.

Dalibor Blazek 

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Student prokázal tvůrčí schopnosti: ANO

Práce splnuje požadavky kladené na PhD disertační práci v daném oboru: ANO

Questions:

1) It was shown (by using Actinomycin D or Cordycepin) that BRCA1 activity in formation of RAD51 foci is dependent on active transcription. Did you test other inhibitors of transcription, such as flavopiridol, that inhibits transcription elongation of most protein coding genes? If yes, did you observe decrease of the number of BRCA1 foci? If not what phenotype, if any, would you expect in your system if such experiment would be performed?

2) Numerous earlier papers suggested that BRCA1 plays an active role in regulation of transcription via interaction with RNAPII and various transcription factors. What is your opinion about the possibility that BRCA1 gets preloaded on RNAPII and travels with the RNAPII on genes to be "ready to use" when collision with replication fork occurs? What is your favorite mechanistic hypothesis about "the BRCA1 activity dependence on active transcription" in your system?

3) Is there any prove that local chromatin/histone modification changes contribute to the recruitment of BRCA1/RECQ5 to the sites of replication/transcription interference?

4) Does lack of helicase activity of RECQ5 affect transcription by RNAPII or RNAPI? Aberrations in RECQ5 were shown to be connected with cancer development. Are those aberrations (mutations, deletions) mapped into helicase or other domains of RECQ5?

5) Where is the major source of the DNA damage when replication/transcription interference occurs? Is it on the side of transcription or replication?