MASARYKOVA UNIVERZITA

Annex No. 11 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

Habilitation Thesis Reviewer's Report

Masaryk University Faculty	Faculty of Science
Procedure field	Genomics and proteomics
Applicant	RNDr. Martin Falk, Ph.D.
Applicant's home unit, institution	Institute of Biophysics of the Czech Academy of Sciences
Habilitation thesis	DNA Damage and Repair upon Cell Exposure to Different Types of Ionizing Radiation ? the Importance of Chromatin Context and New Perspectives of Cancer Radiotherapy
Reviewer	Penelope A Jeggo
Reviewer's home unit, institution	Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton UK…

This habilitation thesis discusses a range of studies examining the responses of normal or cancer cells to ionizing radiation with a strong focus on how chromatin structure can influence the response. The work covers a very nice and appropriate mix of basic, mechanistic research on DNA damage induction and repair after IR exposure coupled with more translational questions, such as procedures to radiosensitise cancer cells (or protect normal cells). I consider that understanding how chromatin structure influences processes such as DNA repair is an important subject area that is currently highly relevant. Although focused, the thesis encompasses a good range of questions addressed and indeed represents a very substantial amount of work. The thesis is extremely well written and presented, and I found it a delight to read. I also found the multiple figures to be of outstanding quality. I will review specific sections of the thesis first and then overview some of the papers included.

The thesis commences with an introduction reviewing the literature relevant to the ensuing work. I found this section to be outstanding. The literature was discussed intelligently, in depth and raised important questions that need addressing. This section demonstrated an in-depth knowledge of the field and literature that, indeed, is evident throughout the thesis. I particularly appreciated the literature review and analysis in section 1.5 and its subsections (DNA damage and repair in the Context of Chromatin). This section, but also the other sections, set the scene for the papers subsequently discussed – but was great to read as I felt I learned a lot.

The introduction is followed by the presentation and discussion of selected papers that Martin Falk has published, either collaboratively or as a senior author. This exposes the breadth and extent of his work, as well as his intellect. A strength is that there is breadth in the overall scope of the work yet a focus is maintained – for example, the approaches for radiosensitisation of cancer cells has breadth but the focus remains on DNA damage and the chromatin impact.

Another important quality of Martin's work, is his ability to exploit state of the art technology and approaches. His earlier work included novel approaches, such as the analysis of Ridge domains, to assess chromatin impacts. He has evaluated the analysis of damage response foci when such work was only just being undertaken as well as Omic approaches, and more recently his use of high resolution microscopy, including SMLM, is outstanding. This ability to exploit emerging and complex technology is a strength and must be a useful asset to colleagues within his research environment and will be excellent training for PhD students. Although slightly distinct, I also like his ability and willingness to study the effects of high LET radiation, which is a complex research area. Martin has achieved good collaborations to progress this work and gained an excellent understanding of the complex issues. His papers show an excellent understanding, for example in how to exploit both perpendicular and tangential irradiation to address the specific question being asked.

An emerging theme in many of Martin's papers is the ability to identify a key or interesting question and then address the question in a focused manner, often using sophisticated technology. An early example of this, is the paper Kozubek, 2002, where the goal is to examine the spatial organization of higher-order chromatin structure. The question of significance at the time was whether chromosomes had specific territories and how this was influenced by gene expression or repression. The question, in part, was whether chromosomes moved or occupied distinct regions – and whether it was random or predetermined. Martin used some wonderful techniques to address this question including 3D measurements (ie confocal microsopy), FISH and transcriptome mapping. It was determined that there is "order" to nuclear positioning. The figures based on the data obtained are exquisite, the arguments, reasoning and discussion are superb and the paper made an important contribution to the more accepted current view that chromosomes have specific territories both at the whole chromosome but more regional level. The work described in Lukasova et al, 2002 was similar, focusing more on the transcriptionally activity of specific chromosomes or regions. This importantly established that RIDGE domains are more oriented towards the nuclear centre compared to antiRIDGE domains.
Two further examples of papers addressing an important question in the field were Falk 2007 and Falk 2008. Having established that undamaged chromosomes exist in relatively defined territories, the question addressed by Martin in 2007, was whether the presence of a DSB made the chromosome or DNA region more mobile. This was recognized as an important question to address because it was critical to ongoing discussions about whether translocations originated from a DSB forming first, having mobility and rejoining to another DSB (the breakage first model) versus the position first model, in which translocations form when DSBs arise in two regions that are spatially together in the undamaged cell. This elegant study revealed several important findings including the fact that there is chromatin decondensation at the DSB site, and that gH2AX foci move from condensed regions to interchromatin spaces. Despite this the majority of DSBs do not move extensively. Hence a novel, hybrid model for translocation formation was proposed. In Falk 2008, Martin addressed another very important question that was being considered by many – that is - do DSBs form equally in euchromatic and heterochromatic regions. This very important study showed that compacted heterochromatin can protect DNA from ROS damage and hence from X or gamma-ray damage. As mentioned above, these were all studies of high technical quality but also addressing an important question in the field at the time.
In more recent studies, Martin has exploited super resolution microscopy to gain insight into the structure of damage response foci, especially following high LET radiation. This work is still at an early stage and I am certain has the potential to reveal and address some important

further questions. Additionally, Martin has started to address translational questions including how to radiosensitive cancer cells and the effect of freeze-thawing on cells. Again, these questions are addressed using excellent technology and are always carried out to a high technical standard.

I will finish by making some summary comments. Martin has revealed himself to be an extremely intelligent scientist able to address significant questions in the field. These questions are addressed using state of the art technology and high technical skills. This represents the major strength of Martin's work. Considering this, it is perhaps surprising that Martin's papers tend to be in lower impact journals, which is a monitor of their significance in many countries. This has advantages in that often the papers are discussed in an indepth way, which may be are less palatable to a broader audience. Nonetheless, I feel that Martin should be able to gain some papers in higher impact factor journals and should perhaps try to present his work in such a manner. His more recent study in Scientific Reports, describing the sensitivity of replication forks to freeze thawing is a step in this direction.

Finally, I will make some comments of relevance to the goal of this appraisal. As I understand the goal is to assess Martin's ability to carry out high quality work, and his potential ability to supervise PhD students and undertake lecturing to undergraduate and PhD students. As stated above, Martin has clearly carried out some good quality work both intellectually and technically. He has displayed an extensive understanding of the field and an ability to explain and convey the important questions in his writing. Finally, for me at least he has raised my interest and enthusiasm to think about the questions considered. I am certain therefore that he will be a motivating lecturer and will guide PhD students in a motivating manner to achieve good technical abilities coupled with the ability to reason intellectually..

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

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Q1. Page 34 line 5. You say that Ku binding stabilises chromatin. I like this idea but what is the actual evidence. Is it via end-tethering or by stopping end-fraying or end-resection. Does it require eg XLF, XRCC4? What experiments show that it stabilises chromatin?

Q2. Page 100 and Flak 2008. Could you use this method to assess whether repair occurs with distinct kinetics in open/closed regions– ie at later times do DSBs in more compacted regions persist. If not what other approaches could be used?

Q3. Figure 32 /page 100. 53BP1 is beautifully shown not to be in the exclusion zone (where HP1b exists). Is this because it cannot penetrate (as proposed) or because DSBs cause relaxation of the DNA in HC and the DSBs then migrate to the periphery of the HP1b dense zone. Could you distinguish these two possibilities – eg was a control for NBS or gH2AX done – what would that tell you?

Conclusion

The habilitation thesis entitled "DNA Damage and Repair upon Cell Exposure to Different Types of Ionizing Radiation ? the Importance of Chromatin Context and New Perspectives of Cancer Radiotherapy" by RNDr. Petr Falk, Ph.D. ***fulfils the*** requirements expected of a habilitation thesis in the field of Genomics and proteomics.

17. 3. 2020

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Summary of the report.

This habilitation thesis discusses a range of studies examining the responses of normal or cancer cells to ionizing radiation (IR) with a focus on how chromatin structure influences the response. The work covers an appropriate mix of basic, mechanistic research on IR-induced DNA damage induction and repair coupled with more translational questions, such as procedures to radiosensitise cancer cells (or protect normal cells), and the impact of freeze-thawing. The thesis commences with an in-depth introduction,that is extremely well written, informative and provides the background for the research questions addressed. This is followed by a discussion of the more significant papers, which again is of high quality. The figures are outstanding and convey key thoughts/models extremely well. The publications encompass important questions in the field. State of the art technology has been exploited for many years, which is a strength of the work. Collectively, I find this a strong habilitation thesis, which was a joy to read and includes work of significance to the damage response field. Although there are many highlights, the work showing that heterochromatin protects against the oxidative damage caused by low LET radiation is a particular strength.