INSTYTUT
GENETYKI CZŁOWIEKA
POLSKIEJ AKADEMII NAUK

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Prof. dr hab. Jadwiga
Jaruzelska
ul. Strzeszyńska 32
60-479 Poznań

tel. +48/61/657 91 00
fax +48/61/823 32 35
e-mail:
jadwiga.jaruzelska@igcz.poznan.pl

www.igcz.poznan.pl

Review of the doctoral thesis

„The role of phosphorylation of ISGF3 components in the regulation of ISG expression and viral protection"

Ph.D Student: MSc Hanna Nowicka

This dissertation was prepared under Professor Hans Bluijssen supervision, whose research group is renowned for long-lasting research in interferon-stimulated cellular response mechanisms to viral infection. The importance of such research is unquestionable, especially at the present world-wide fight against COVID-19 pandemics and future threats.

The Author focused this dissertation on mechanisms of interferon Type-1 (INF-type 1) cellular response involving ISGF3 complex formation built of phosphorylated STAT1 and STAT2 and of IRF9 transcription factor. This complex triggers the expression of many genes with antiviral functions known as ISGs, by binding specific ISHRE motif in their promoters and providing protection against viral infection.

Although in the past research, this mechanism was considered as transient and until recently was under study only up to 24 hours after INF type-1 stimulation, it is

clear now that the expression of ISGs substantially extends over this time. Given that observation, the question was raised in this dissertation, how ISGs expression is maintained beyond 24 hours and, more precisely, is the ISGF3 complex involved in that extended expression time?

The second question addressed the role of ISGF3-related complexes built of ISGF3 components such as unphosphorylated ISGF3 (U-ISGF3) as well as phosphorylated and unphosphorylated STAT2-IRF9 complexes in both, early and later response, up to 72 hours upon INF type-1 stimulation.

Combination of experiments based on the wild-type cell lines usage 2fTGH and Huh7.5, as well as on other cell types with engineered expression of STAT1, STAT2 and IRF9 available in the lab, treatment with JAK inhibitor I of phosphorylation, viral protection assays and global gene expression analysis including RNA-seq, DGE and CHIP-seq, enabled addressing both questions.

These experiments provided a positive answer to the first question of this dissertation by showing that phosphorylation of ISGF3 components plays an essential role not only in the early but also in the extended in time transcriptional response reflected by ISGs expression upon INF type-1 stimulation in both WT cell-line models used.

As far as the second question is concerned, the experiments showed a potential role of unphosphorylated U-ISGF3 and possibly U-STAT2/IRF9 complexes in constitutive as well as INF-type 1 stimulated extended in time expression of ISGs and the resulting viral protection. However, as it is stated in this dissertation, further experiments are needed to get a more complete picture of that role.

This dissertation is of value as it represents a complex methodological approach to address an important scientific problem and I read it with interest.

I appreciated the Introduction section providing a sufficient background for the study. At some points, however, details are dense and additional pictures would have been of interest, for example, for 1.3.1. chapter describing JAKs kinases.

For Hypothesis and Objectives section, I would appreciate the short section synthesising novelty of the study that precedes a list of specific objectives.

Results section is well structured and contains chapters corresponding to specific aims listed in Hypothesis and Objective section.

I highly appreciated the Discussion section in which results got in this study are one by one confronted with previous knowledge, including papers published by the own group of the Author. I especially liked the discussion section focused on the potential significance of STAT2/IRF9 complex that in the absence of STAT1 could serve as backup system, providing protection in case of infection with virus with ability to block STAT1 function, taking the role of classical ISGF3 complex in providing viral protection.

However, considering the complexity of this study, I missed point-by-point conclusions at the end of the Discussion corresponding to specific objectives listed in Hypothesis and Objectives section.

Minor criticisms

1. Page 36, line 2 of 1.4.2.2. Fig 1.8, left and not it should show the right panel.
2. Page 62 Fig. 3.4 is very important showing the pipeline for ChIP-seq data. Unfortunately font is too small and hard to read.
3. Page 73 in Fig. 4.6 In the description A and B panels are mentioned but are not shown in the picture.
4. Page 98 Fig. 4.17 and page 102 Fig. 4.20 IRF1 transcription factor is mentioned in the description of this figure, but the Figure does not show its expression.
5. It is not explained why clone 3 was chosen for analysis.
6. Page 123 line 7 from the bottom: "Discussion about antibodies quality from CST versus Santa Cruz Company for detection of phosphorylated and total STAT1 and STAT2 proteins". Based on what it was assumed that those p-proteins were stronger than those for the total? It is not clear to me.
7. Page 123 line 9 not Fig. 4.10 should be quoted.

8. Page 132 the end of the bottom Fig. 5.1 part of description is missing.
9. Fig. 5.1, 5.2, 5.3 and 5.4 dotted lines in the bottom part of those figures are not seen.

Final conclusion

This dissertation describes experiments and results which are of value by shedding more light on the role of particular ISGF3 complexes in INF type 1-stimulated response to viral infection. It provided a more complete understanding of the role of ISGF3 and related complexes and the functional role of their phosphorylation status in the process of early and later cellular response to INF type-1 stimulation and the resulting viral protection. I read this dissertation with interest and I learned a lot. Among several aspects of this thesis, I appreciate that questions concerning future research have been raised. For example, whether prolonged ISG expression is regulated only by STAT2-IRF9 or is there any role of U-STAT2-IRF9? Another important problem that the research group plans to explore in the future is the role of 145 and 21 genes, specifically regulated by ISGF3 and STAT2-IRF9 complexes, respectively. They also plan Chip-Seq experiments to study whole–genome binding of unphosphorylated STAT1, STAT2 and IRF9 in U3C cells, stably overexpressing combinations of ISGF3 components.

Altogether, I am convinced that this dissertation with no doubt fulfills the requirements expected for a doctoral thesis. Therefore, I kindly ask the Council of the Discipline of Biological Sciences of the Adam Mickiewicz University to proceed to the next steps of this Ph.D. process.