H. Korswagen

Thesis Martina Hajduskova

The nematode C. elegans has a large family of nuclear hormone receptors of which the function is still poorly understood. In this study, Martina Hajduskova has focused on one of the best-conserved nuclear hormone receptors, nhr-25, an ortholog of Drosophila FTZ-F1 and mammalian SF-1.

Previously, the laboratory of her supervisors Masako Asahina and Marek Jindra has shown that nhr-25 plays a crucial role in C. elegans development. Loss of nhr-25 disrupts embryonic development and interference with nhr-25 function during larval development leads to defects in the division of the seam cells. During each larval stage, the seam cells divide and then reestablish contact by elongating along the antero-posterior axis. Intriguingly, in the absence of nhr-25, the seam cells fail to elongate and form clusters of cells. Another effect of nhr-25 knock down is that the homeobox gene mab-5 is ectopically expressed. In a recent study, her supervisors have investigated the function of nhr-25 during the asymmetric division of the somatic gonad precursor cells (SGPs) that gives rise to the so-called distal tip cells. They found that nhr-25 counteracts the Wnt/beta-catenin asymmetry pathway (that controls the asymmetry of this division) by inhibiting SYS-1/beta-catenin and POP-1/Tcf mediated transcription, most likely through a direct interaction with SYS-1. The Wnt/beta-catenin asymmetry pathway plays a central role in many other asymmetric cell divisions and in her thesis research, Martina Hajduskova has investigated the role of nhr-25 in the asymmetric division of the T cell.

The T cell gives rise to hypodermal and neural lineages in the hermaphrodite and produces copulatory structures called rays in males. Martina Hajduskova found that NHR-25 is required for the correct asymmetric fate specification of the T cell lineage. Intriguingly, she found that nhr-25 cooperates with sys-1/beta-catenin and pop-1/Tcf during the T cell division, which is in contrast to the inhibitory role of nhr-25 during the SGP division. These results indicate that the role of NHR-25 in the Wnt/beta-catenin asymmetry pathway is more complex than was previously anticipated, with a positive or negative regulatory role depending on cellular context.

I very much enjoyed reading Martina Hajduskova's thesis. The introduction provides a comprehensive overview of what is currently known about the mechanism of asymmetric cell division and the function of the Wnt/beta-catenin asymmetry pathway in this process. Her work on the role of nhr-25 in the T cell lineage is of the highest standard and was recently published in the Journal of Cell Science, one of the top journals in the field of cell biology. There is also a section with as yet unpublished observations that provides important information for future experiments. All in all, I find the thesis of high quality and interest and I enthusiastically recommend that Martina Hajduskova will be granted a PhD degree.

Of course I have a number of questions, some of which are as described below.

-One of the most important conclusions of this work is that (depending on the cellular context) nhr-25 can act positively or negatively in the Wnt/beta-catenin asymmetry pathway. One of the best-studied examples of Wnt/beta-catenin asymmetry signaling is the EMS division in the early embryo. Does nhr-25 knock down positively or negatively influence E fate induction?

-An important property of nhr-25 knock down is that seam cells fail to establish contacts with each other after division. To what degree is this also the case in scm::nhr-25(RNAi) and can this provide an explanation for the ectopic mab-5 expression and (some of) the male tail defects?

-The ectopic mab-5 expression and male tail defects of nhr-25 knock down could also result from derepression of bar-1/beta-catenin mediated canonical Wnt/beta-catenin signaling. Is there any relationship between nhr-25 and the canonical Wnt/beta-catenin pathway?

-What is the explanation for the low penetrance seam cell phenotype of Pwrt-1::nhr-25(RNAi)?

-At the International C. elegans meeting two weeks ago, some abstracts suggested that nhr-25 is a heterochronic gene. Can a defect in developmental timing explain some of the nhr-25 seam and male tail phenotypes?

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Evaluation of Ph.D. thesis:

"Functional analysis of the nuclear receptor NHR-25 during *C. elegans* development"
by Martina Hajdušková

Dear Madame/Sir,

I am delighted to write this letter of evaluation of the Ph.D. thesis entitled "Functional analysis of the nuclear receptor NHR-25 during *C. elegans* development" by Martina Hajdušková. In this thesis, the author described the function of the nuclear hormone receptor NHR-25 during postembryonic development of the nematode *Caenorhabditis elegans*, in particular, its regulatory function in asymmetrical cell division.

NHR-25 is shown to function in various developmental processes in *C. elegans*, i.e. embryogenesis, molting, epidermal and vulval cell fusion and elongation, and somatic gonadal development. The author focused on the regulatory function of NHR-25 in cell division and differentiation of stem-like seam epidermal cells. Hypodermal cell-specific *nhr-25(RNAi)* induced the extra cell division of the seam cell, resulting in ectopic ray neuron formation and disruption of the tail morphology in males. More specifically, the author demonstrated that the loss of *nhr-25* activity in *C. elegans* caused failure of asymmetric cell division of the T cells (Tail seam cells). In normal development, the anterior daughters of the T cells (T.a) produce primarily hypodermal cells, while the

posterior daughters (T.p) generate neural cells. However, in the *nhr-25(RNAi)* animal, the T cells underwent symmetrical cell division; both T.a and T.p produce hypodermal cells, indicating that *nhr-25* is required for the polarity of the T cell division. In *C. elegans*, the polarity of the T cell division is known to be regulated by the Wnt signalling genes. The authors examined the genetic interaction between *nhr-25* and the Wnt pathway (and its related) genes, such as *pop-1*, *rnt-1* and *tlp-1*, and elucidated that *nhr-25* functions in parallel to the Wnt signalling pathway. The author demonstrated that NHR-25 is a positive regulator of the Wnt signal in the fate decisions of the T cells.

As a general impression of this thesis, I see the author studied this field very well and conducted her research theoretically. Judging from the thesis, the author seems to show remarkable determination and originality in her approach to research. The "Introduction" section guides the readers to understand the background of this field. I could follow well in this thesis, because results and discussion in "Research article" are presented in a logical order. The figures (Nomarski and fluorescent photographs) are in excellent quality. I would like to emphasise this paper is excellent as a Ph.D. thesis because the materials and methods section was well documented in detail, facilitating future students to easily continue this work. I would also like to point out the author showed a sincere attitude as a scientist toward problem solving. The author continued the research to answer the questions that were raised during the study for "Research article", and (partly) succeeded in presenting the answers in the "unpublished preliminary data" section. Their findings would provide invaluable information to many scientists in this field (including me, working on *rnt-1* gene) to understand the mechanism of asymmetric cell division. Lastly, I conclude that this is an excellent thesis and I have no criticism regarding methodology, results or discussion. Thus, Martina Hajdušková absolutely deserves her Ph.D., and I evaluate the thesis with the highest score: excellent (summa cum laude).

Sincerely yours

Kagoshima

Hiroshi KAGOSHIMA

PhD Student: Martina Hajdůšková

Oponent: Vítězslav Bryja, PhD. (Inst. of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic)

The work of Martina Hajduskova describes detailed analysis of the role of nuclear receptor NHR-25 during development of the worm *Caenorhabditis elegans*. The study is specifically focused on the function of NHR-25 in the biology of seam cells, which represent epidermal stem cells in the worm. Most of the results are achieved by knockdown of nhr-25 targeted to seam cells and their precursors, followed by detailed analysis of mutants using morphological methods, lineage tracing and genetic analysis. The presented thesis represents a huge amount of work with different methodologies.

According to my opinion the most important finding, which requires detailed discussion (and further experiments), is dual role of WNT x NHR-25 interaction in assymetric cell division depending on the cell type. NHR-25 antagonizes Wnt asymmetry pathway in the gonad but synergizes with the same pathway in the T cell. What could be the reason for this difference? Since I am not in the C. elegans field I can speculate wildly and think of several possibilities:

(i) There is a cell type specific co-factor, which decides whether the outcome of the interaction will be positive or negative.

(ii) In one cell type (eg. gonad) NHR-25 acts as the master of the asymmetry, and is blocked by WRM-1/β-catenin (as shown by Asahina et al. 2006), whereas in other cell type (eg. T cell) TCF/β-catenin-dependent transcription is crucial and enhanced by NHR-25.

(iii) In the somatic gonad precursor cells and in the T-cell asymmetric division two β-catenins play a role, and these β-catenins (SYS-1 and WRM-1) have opposing function on NHR-25-driven transcription. Cannot this provide a clue?

(iv) POP-1 (analogically to TCFs in vertebrate cells) acts as a repressor in one cell type and as the activator in the other. NHR-25 always synergizes with POP-1 but with different (opposing) consequences.

(v) The molecular mechanisms of asymmetric cell division in the SGPs and T cells are fundamentally different.

Can the author discuss, confirm or reject individual hypothesis, and suggest a possible approaches to test them?

More questions to "Unpublished preliminary data" section:

Ad "Does NHR-25/SF-1 physically interact with TLP-1/Sp1?"

- In the Fig. 28 crucial controls are missing – mainly IP GFP/WB GFP blot to assess the efficiency of the bait pulldown. To get conclusive results, one should also add known binding partners of TLP-1 to the experiment as a positive control.

Ad "Can misexpression of nhr-25 revert the cell fate of the somatic gonad precursor cells?"

- HSP promoter-driven overexpression of NHR-25 gave both "all distal" and "all proximal" Sys phenotypes. What can this implicate about mechanism of action of NHR-25?

Ad "Can dominant negative form of NHR-25 be designed?"

- Has anyone directly tested if NHR-25 can dimerize (eg. using immunoprecipitation of NHR-25 tagged with two different tags)?
- Is here any information about (putative) ligands of NHR-25?
- Is the expression of NHR-25alpha and NHR-25beta different in the SGPs and T-cells? How would you determine it?
- Fig. 34 – This seems to be a viable approach to test whether any form of NHR-25 can act as dominant negative. Haven´t you manage to perform similar experiments with NHR-25beta in the meantime?

In summary, the proposed thesis clearly demonstrates the ability of Martina to perform independent experimental design and laboratory work. According to my opinion this thesis would be ranked among the best 25% in the Faculty of Science, Masaryk University. As such, the thesis of Martina Hajdůšková fulfills the criteria for obtaining a PhD degree and I fully support granting a PhD degree to her.

In Brno July 2, 2009

Vítězslav Bryja, PhD.