pThesis Nagagireesh Bojanala.

The development of *C. elegans* has been studied to unravel molecular pathways that govern important biological processes like cell differentiation, migration and apoptosis. Formation of the vulva by a limited group of epidermal cells is required for egg-laying in hermaphrodites. Investigating mutants with defective vulval development exemplifies how instrumental genetic studies can be for delineating complete signal transduction cascades. Importantly, identical or very similar signaling cascades also function in humans to control development and homeostasis. Thus, studying *C.* elegans can have a major impact on understanding the molecular basis of human disease.

One of the proteins whose expression affects vulval development is the nuclear hormone receptor NHR-25. Dr. Asahina and her coworkers have greatly contributed to understanding the function and mode of regulation of this protein. The work described in this thesis forms an interesting extension of that work. Especially the demonstration that post-translational modification of NHR-25 affects its transcriptional activity is noteworthy. It nicely illustrates that well designed studies can unravel the mechanism by which transcription factors orchestrate robust cell behavior during development.

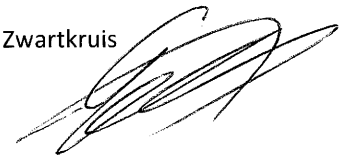
In his thesis, Nagagireesh Bojanala starts out with a comprehensive review of vulval development in *C. elegans*. It includes a description of the architecture of the vulva during larval stages that covers aspects like asymmetric cell division and polarity. In addition, it pictures the major signaling pathways like those of LIN-3/EGF, LIN-12/Notch and the MuV genes. This is done in an orderly fashion with a good balance between general concepts and more detailed information. As such, this chapter is an excellent starting point for all students, who are new to the field. I would not be surprised if the profound theoretical knowledge of Nagagireesh Bojanala in part stems from the comparative studies in *C. briggsae* to which he contributed, as can be seen in the final chapters.

The most intriguing parts of the thesis in my view are the experimental chapters 3 and 4. In a collaborative effort with the Yamamoto lab, both physical and genetic interactions between NHR-25 and the sumo-protein SMO-1 are demonstrated. The precise sumoylation sites within NHR-25 are mapped by *in vitro* assays. By means of reporter assays, sumoylation of NHR-25 is shown to decrease transcriptional activity in HEK293 cells as well as in *C. elegans*. I have great respect for the careful analysis of altered vulva morphogenesis in RNAi and genetic experiments, as these are crucial for the final conclusions. The cell lineage work deserves a special compliment. Publication of this work in PloS Genetics underscores that also other colleagues in this field highly appreciate the quality of this work.

Chapter 4 provides a further description of the precise role that NHR-25 plays in vulva morphogenesis. Altered gene expression patterns and abnormal cell migration are accurately documented. Interestingly, genetic interactions with putative null alleles of the semaphorin *smp-1* and its ligand *plx-1* are identified. Finally, a role for *lin-3* pathway in inducing *nhr-25* expression is shown.

In summary, the thesis of Nagagireesh Bojanala very nicely covers both the theoretical background as well as experimental work focused around the role of NHR-25 in vulval development. I consider the quality of his work as high. Therefore, I enthusiastically recommend that Nagagireesh Bojanala will be granted a PhD degree.

Dr. ir. G.J.T. Zwartkruis



As you realize, interesting papers always leave space for a number of questions. And I listed some of these below.

In the result section of chapter 3, where *in vitro* sumoylation assays are done, you suggest that the SMO-1 is added in a sequential order. The reason for this is that, over time, you observe the appearance of a single, a double and a triple band. I would argue that exactly the same pattern is seen if SMO-1 was added in a random order. Do you agree and could you think of an experiment to discriminate between these options?

Sumoylation of NHR-25 clearly affects its transcriptional activity. For example, the increase in expression of the 8xNR5RE(WT) in fig. 5B is very convincing. The underlying mechanism, however, remains rather elusive. One clear claim in the text is that sumoylation affects DNA binding, but I am actually not so convinced by the data. For example, in figure 7A, I barely see a decrease in the EMSA, while the protein is heavily sumoylated.

A major conceptual problem in experiments that deal with posttranscriptional modifications on a given protein is that when one tries to interfere in these modifications, many other proteins (so also proteins NOT under investigation) are affected simultaneously. Working around this bottleneck is far from simple. With respect to your studies, one could for example argue that the evidence that the effects seen following *smo-1* RNAi or overexpression are not mediated via NHR-25, but rather indirectly via numerous other proteins that bind SMO-1 or are sumoylated. Would you be so kind to share with me the arguments of why you think the effects do occur via NHR-25?

Along the same line, one could argue that interfering in the function of SMO-1 results in a general de-repression of many genes. The control shown, i.e. a mutated version of the NR5ER construct is in that respect not very informative: it is silent en remains silent. The only other gene shown is a reporter gene of NHR-25, which is also up-regulated following *smo-1* RNAi. Did you check other genes as well to exclude a general de-repression effect?

I was intrigued by the migratory defects that you observe following *nhr-25* knock down and especially the strong adherence of the Pnp cells to the ventral cuticle caught my attention.
Could you speculate on putative target genes or do you envision a role for NHR-25 independent of its capacity to activate transcription. I wondered what is known about the adhesion molecules that tether the hypodermal cells to the cuticle and the process that results in the disassembly of cell-cuticle contacts?

In mammalian cells, Ras is known to decrease cell-ECM contacts, leading to enhanced migration or even metastasis in cancer. Given the fact that that you find NHR-25 to be a downstream target of LIN-3/LET-60, do you have any indication that human orthologues play a similar role?

In your genetic analysis you found enhancement of the *nhr-25* phenotype by *plx-1* and *smp-1*. Did also test the reverse situation, so did you try to suppress the *smp*-1 phenotype by gain of function mutations in the LIN3/LET-60 pathway.

In your review, you point out that post-embryonic RNAi for *nhr-25* does not interfere in expression of *lin-3::gfp* in the anchor cell. If we assume that *nhr-25* RNAi is indeed effective enough to abolish its activation of the enhancer element ACEL, what do you think is the most likely explanation for this observation?

PhD thesis evaluation

Title: Modulation of *C. elegans* vulva organogenesis by nuclear hormone receptor NHR-25
Author: Nagagireesh Bojanala

Scientific quality: The presented thesis maps various aspects of organ development regulated by a single transcription factor, the nuclear hormone receptor NHR-25/SF-1/LRH-1. The author uses C. elegans vulva as a model system, one of the best characterized models of organogenesis in animals. In the first part of the thesis – formed by a research article published in PLoS Genetics – the author presents findings about NHR-25 posttranslational modification by *C. elegans* SUMO protein SMO-1 and about physiological role of such modification. Sumoylation inhibits NHR-25 dependent transcription and restricts NHR-25 activity only to a subset of vulva precursor cells, thus promoting correct cell fate specification. In the second part of the thesis the author presents unpublished data describing NHR-25 role in vulva cell migration, cell fate specification and terminal differentiation, and also possible crosstalk of NHR-25 with other signalling pathways regulating vulva differentiation. All the presented data are of high quality and bring novel insights into vulva organogenesis. The author tries to interpret and discuss the unpublished data. Although I appreciate the complexity of the system and I agree with the conclusions, I miss a bit broader discussion of the results, for example, comparison between NHR-25 and SF-1 functions, as SF-1 is no longer a purely steroidogenic factor.

Formal quality: The thesis is written in a fairly standard format. The literary introduction gives the necessary background to the topic, possibly slightly more background information about sumoylation would be beneficial as NHR-25 – SMO-1 interaction forms a substantial part of the results. I would prefer to include figures in the text, not after the text, as it complicates reading (same goes for Chapter 4). Materials and Methods section is sufficiently detailed, unpublished results in Chapter 4 are adequately documented and presented. The biggest formal shortcomings however are in the references, both in text and in the list of references. The in-text citations are not presented in a uniform style (and vs &, names with initials, etc…), some names are spelled incorrectly (Shemmer vs Shemer), some references in the reference list are incomplete (missing year, some have doi, some not,…). Overall, the whole thesis would greatly benefit from more careful editing.

Questions:

1) Does sumoylation of NHR-25 play a role in any other NHR-25 dependent event in *C. elegans* development or is it specific to vulva?
2) Do you have any physiological explanation why *nhr-25* expression should temporarily disappear during Christmas tree stage of vulva development and re-appear later?
3) NHR-25 is clearly required for cell contacts and cell migration. Can you compare the migration phenotype observed in vulva cells with the phenotype caused by loss of *nhr-25* in seam cells? Is it possible that the underlying mechanism is similar in both cell types?
4) How do you think NHR-25 can regulate actin cytoskeletal re-arrangements?

Conclusion: The presented thesis definitely demonstrates that Nagagireesh Bojanala is able to independently conduct experiments, document the results, interpret them, discuss and present them to a wider scientific audience. Part of the presented data underwent a peer-review process and was published in highly impacted journal and there is no doubt about quality of the data. Nagagireesh Bojanala proved to be a competent scientist and, judging also from the supplementary articles, he is now an expert on *C. elegans* vulva development. I recommend the thesis for defense.

Prague, June 5, 2015

Marie Macůrková