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**Review of Master Thesis:**
The regulation of transcription by the nuclear receptor NHR-25 in *Caenorhabditis elegans*, by Linda Merglová, 2009

Presented Master Thesis by Linda Merglová is describing an investigation of the transcription regulation by worm nuclear receptors NHR-25 and NHR-23. The author prepared four transgenic constructs with two different minimal promoters and two different binding sites for nuclear receptors mentioned above. After successful transformation of all four constructs into worms and integration of two of them, Linda analyzed the expression from these constructs at the tissue level. One of the integrated construct showed strong expression which was further analyzed by RNAi. Results with RNAi led the author to analyze further the morphological defects caused by RNAi against NHR-23 which also brings new knowledge about the role of this nuclear factor.

The thesis has all the formal requirements, with one exception mentioned further, without too many mistakes, written by overall good English – only rarely the sentence composition makes it harder too understand. The graphical design is also good making the orientation in the thesis easy. The only exception is a missing part defining the aims of the work. The aims are not clear even from the introduction or results, so I would consider this a significant weak point of the thesis.

The introduction has all the parts important for the thesis, it is readable and helpful for someone who is not an expert in the subject. However the introduction is too general, more specific information related to the particular project is too short lacking the important message for the thesis. This is especially true for the parts 1.3.1 and 1.3.2 describing NHR-25/23 themselves. This, in combination with undefined aims, makes the thesis rather a description of the performed experiments.

The results and discussion sections are well written, describing all the important findings, supported by images of very good quality. It is hard to make any strong conclusions from the present state of investigation. Thus Linda appropriately discusses more the technical problems she encountered although she also touches in her discussion the significance of her findings.

QUESTIONS:

1. Since I am not so familiar with studies of transcription factors I would imagine that the tissue specific expression is regulated in a complex way requiring much more than the minimal promoter (especially from a different gene) and a sequence of one binding site. The author also mentions this in her discussion, particularly saying that "it is possible that more elements are required to see the authentic enhancer activity in the epidermis". Obviously the system Linda used for her work is often used but the information for which questions it is used is not really described in the introduction and the particular questions Linda wanted to solve by her work are not defined in missing aims. Is it an analysis of tissue specific expression or is it an investigation of sequences in the binding sites or some other type of questions? Thus I would like to ask Linda to discuss these things more during her defense since it is quite important for her work.

2. The previous question is related to the next one – in the first part of Results, the author claims without any reference or additional information that "the sequence of NHR-23 binding site is TCTAGGTCA" which is quite important for the rest of the thesis. So what is actually known about this binding site and its particular sequence? This should be in the introduction. Was the aim of the thesis to investigate the specificity of this particular sequence? etc.

3. On page 35, Linda is discussing the expression pattern of 94TCT construct and she is using a significant difference between expressions of this construct and 94TGA as one of the arguments against the expression being simple artifact. However 94TCT was integrated into the genome while 94TGA was not. What does the author think about the possibility of integration influencing the expression of the construct by positional effect of the integration site – can this influence the expression compared to the extrachromosomal construct? The RNAi against nhr-23 would nicely support the binding to 94TCT. However, besides the effect of RNAi against nhr-25 complicating the specificity of this sequence, there could be a problem of the results with RNAi against nhr-23: as author says, this RNAi affects the gut development where she was observing the expression. Is it possible that the GFP expression was lowered due to inappropriate gut development instead of an effect of lacking the transcriptional activator?

RÉSUMÉ: I would like to stress that Linda's work might be very important in obtaining new knowledge about the role of certain nuclear hormone receptors and that it is rather difficult to conduct these studies using animal development and experiments in vivo. I am sure that if the problems she encountered are worked out, the presented system might be very helpful in this goal.

I believe that Linda mastered research skills appropriate for the Master degree and if she compensates the biggest weakness of her thesis – undefined aims – in her defense presentation, I would recommend her work for successful Master degree defense.

Tomáš Doležal , České Budějovice, 25.5.2009

**Review of Master Diploma Thesis submitted by Bc. Linda Merglová under the title „The regulation of transcription by the nuclear receptor NHR-25 in *Caenorhabditis elegans*.**

The submitted thesis is focused on study of nuclear receptors in *C. elegans* using recombinant DNA technology, transgenic organisms and RNA interference. It took me some time to think out how to briefly characterize the text. Perhaps the best way would be to say that it is written economically. Maybe even a little bit more than it is necessary.

The whole text spans 43 pages including a list of references and contains some nicely written parts, well supported by the references to the published scientific literature, as well as poor pieces which look like written by somebody else. However this is quite common and understandable, as diploma theses are usually the first larger and more important written works of the university students and of course are often written under the time-pressure. More specifically: The beginning of the introductory part doesn't contain any references, but here and there some funny sentences like "Transcription factors are proteins necessary for the transcription", and resembles thus more a textbook chapter than a scientific introduction. However the second part of the Introduction starting with the paragraph #1.3.meets most of the requirements expected from the M.Sc. thesis. Similarly the Materials and Methods chapter is quite well written, but contains shoddy description of electron microscopy methods used. Some solutions (toluidine blue, water with detergent, 0.2 M buffer) are not sufficiently described. Did author really use vinegar acid for specimens washing? Results are well written however some figures or tables are insufficiently described or contain even no legend (see Table 6) which makes them difficult to understand for ignorant in the field, like I am. Also some marks announced in figure legends got finally lost on their ways to photographs, like an arrow showing the tail neuron in Fig. 3.5 or the arrows showing molting defects in Fig. 3.6 C, D. Regarding the Discussion chapter, I would expect that it will contain more reflections from the scientific literature already published. I would also move the small paragraph announcing the objectives of the work from the Discussion chapter to the very beginning of the thesis.

From the more scientific point of view, I am happy that I can say that Linda Merglová got a great opportunity to be involved in an exciting research as well as to learn many new techniques. To summarize her main achievements, she used a DNA recombinant technology to prepare new DNA vectors containing a gene encoding green fluorescence protein under the control of one of two different minimal promoters and either NHR-25 or NHR-23 binding sites. She prepared new transgenic worm strains and succeeded to obtain two different worm strains bearing some of these DNA vectors integrated in their genomes. She tried to characterize localized expression of green fluorescence reporters in newly constructed strains as well as to investigate the nature of GFP expression by RNAi targeted against the *nhr-25* and *nhr-23* mRNAs. Linda obtained some preliminary results which have a great potential to be further developed. I would like to raise few questions and comments:

1. The statement that "The sequencing revealed that all new constructs have the correct inserts" does not correspond well with data in Table 2.
2. Why did author use TGAAGGTCA motif as NHR-25 binding site and not a TCAAGGTCA motif used in (Asahina 2006, Developmental Cell 11, 203–211). What would be the expected difference, if any?
3. Author hypothesizes that more than two NR binding sites could provide stronger activation of the reporter gene and suggests using 7 binding elements. Why 7?
4. Could author explain in more detail the rationale of her—yet disproved—hypothesis about the possibility that NHR-25 and NHR-23 could function as repressors in your system? (page 29). Please include the real positions of the regulatory elements in your vectors to

your speculation. Plasmid maps described in Fig 3.1 do not allow getting an exact impression about the regulatory elements sizes and spacing.

5. Could author comment a marked decrease of number of GFP expressing 94TCT worms in L4 stage of their *nhr-25* knock-downs as depicted in Fig. 3.7 Could she provide any hypothesis about switch in rate of GFP expression reduction during progression from L3 to L4 stages when comparing 94TCT *nhr-25* and *nhr-23* knock-downs?
6. Could author speculate about the reasons for expansion of nuclear receptor family in *C. elegans* in comparison to other multicellular eukaryotic models?

It is a pity that I cannot attend personally the Linda's thesis defence. She took part in a nice research project. She learnt a lot of new techniques, prepared new vectors and worm strains as well as obtained some interesting results which could be further developed. She also learnt that science including writing about the science is a hard work with sometimes unpredictable outputs. If I compare the thesis submitted by Linda Merglova with those I used to see every year at our department, I would suggest to be evaluated as "velmi dobrá".

May 28, Prague

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