Bachelor thesis evaluation

Title: Making Transgenic *C. elegans* with Polycistronic mCherry Vector
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The presented thesis describes a preparation and analysis of transgenic *C. elegans* strains. The aim of the project was to express two different *C. elegans* proteins, NHR-25 and SMO-1, from different tissue-specific promoters. To prevent possible malfunction of the expressed proteins due to tagging, a polycistronic vector was used for transgenesis.

The first part of the thesis gives the necessary background for understanding the technique and the model system. This part is written clearly, with sufficient detail and with only few inaccuracies (e.g. page 5 – males in *C. elegans* do not occur because of *malfunction* of the X chromosome but because of *nondisjunction* of the X chromosome, page 7 – "the DNA inside of the nucleus is firstly transcribed to nuclear RNA cutting the promoter sequence off"). For a non-specialist reader it would be beneficial to elaborate more on the role of NHR-25 and SMO-1 in *C. elegans* development, as this would help to understand the observed phenotypes in the Results section. The Materials and Methods section describes the maintenance of *C. elegans* and the microinjection technique clearly and comprehensibly and gives all the necessary details. Results are well described and nicely presented in four figures, although scale bars would make the figures even better. Results together with several interesting unexpected observations are then adequately discussed.

While the scientific quality of the presented thesis is high, there are several formal shortcomings. First, out of eleven figures only four are referred to in the text. Figures 1 and 3 should include references to their source. Next, when citing literature using numbered references, the references should be numbered in the same order as they appear in the text. Last, references should be given in a uniform style, in the thesis the list of references is a display of different styles and most importantly, out of 16 journal article or book references, only 5 are given correctly, the rest lack journal names, year or issue number.

Overall, the submitted thesis is of a very good scientific quality, the candidate got well acquainted with an important model organism of developmental biology and mastered a difficult experimental technique. I fully recommend the thesis for defense. I give the thesis grade 2 due to the formal mistakes.

Questions:
1) In chapter 1.5 you mention that it is possible to generate knock-outs by microparticle bombardment. Can you briefly explain how that works?
2) You observed a suppression of the Rol phenotype when using *wrt-2* promoter and your explanation is that the overexpression of a second protein, e.g. NHR-25, effectively dilutes the concentration of mutated ROL-6 and therefore the Rol phenotype is less penetrant. According to Wormbase, *rol-6* is expressed in hyp7 while you see *wrt-2* expressed in the seam cells. How would that fit into your model?
3) The observation of Multivulva after NHR-25(3KR) overexpression is very interesting. Do you think that this phenotype is caused directly by overexpression of NHR-25 in the vulva precursor cells or that it is a consequence of the ectopic expression of NHR-25 in the hypodermis?

In Prague, June 11, 2012

Marie Macůrková