Teije Middelkoop
Group leader – Developmental Mechanobiology
Institute of Molecular Genetics of the CAS
BIOCEV research center
Prumyslova 595, 25250 Vestec
Czech Republic

Prague, 27-5-2024

MSc thesis review
Author: Michaela Zdimalova
Title: 'The role of CKAP5 in mediating crosstalk between actin and microtubule cytoskeleton'

In this MSc thesis the candidate has studied the microtubule binding protein CKAP5 in vitro. CKAP5 has been implicated in numerous biological contexts, including mitotic spindle behavior and neuronal growth cone migration. However, at the molecular level the function of CKAP5 remained elusive. In this thesis, the candidate first provided a great introduction regarding neuronal growth cones and how cytoskeletal processes control their migration and navigation. She thereafter gave a detailed overview of the experimental procedures and subsequently presented her very interesting results in which she showed that CKAP5 can cross link microtubules and actin filaments. Moreover, she convincingly showed that CKAP5-mediated recruitment of actin filaments to microtubules ensures that microtubules polymerize along the actin filaments once they start growing after catastrophes. This finding is of high importance to the field as it demonstrates how CKAP5 introduces a molecular memory for growing microtubules, such that growth remains persistent. Importantly, this provides a direct molecular framework for understanding crosstalk between microtubules and actin, and provides a possible explanation for how cytoskeletal dynamics govern growth cone migration. Finally, in the discussion, her findings were put in a broader perspective.

The thesis is clearly written and both the intro and the discussion demonstrate that the candidate can conduct a critical evaluation of the relevant literature and can put experimental results in a larger perspective. Also, the candidate has proven with this thesis that she is able to perform complicated in vitro reconstitution experiments combined with high-end microscopy and quantitative data analysis. In my opinion the candidate delivered an excellent master thesis and I highly recommend her for defending it. After reading the thesis I do have several questions that I think the candidate should elaborate on during the defense:

1) In fig 4.1D: the CKAP5 density per actin filament was reported. How did you determine the actin filament density? And related to this, do the actin filaments have a characteristic average length in the experiment?

2) In fig 4.4C: The density of CKAP5 was determined two minutes after adding it. Why was 2 minutes chosen and would the timing make a difference?

3) In fig 4.6: CKAP5 binds to preformed actin filament bundles, but not to single filaments. Is It because it only binds bundles, or is it necessary to bind at least 2 actin filaments simultaneously, which is simply more likely when you have bundles? Could this be tested experimentally?

4) The affinity of CKAP5 for MT's is higher than that for actin filament bundles. Could it be that the affinity of CKAP5 for actin filaments is higher in the presence of MT's?

5) In fig 4.10B: CKAP5 localizes to the growing and shrinking +tip of MT's while a +tip preference was not observed with stabilized MT's alone (as for example in fig 4.8). Why is there this difference?

6) In the discussion it is mentioned that CKAP5 has been implicated in regulating the mitotic spindle. Do you think that the MT-actin crosslinking activity of CKAP5 is also relevant for mitotic spindle dynamics?

Kind regards,

Teije Middelkoop