Delft, Jan 15th 2021

Dear KIND directors,

Hereby we, Martin Depken and Liedewij Laan, both associate professor at BN are applying for Kavli seed money with our proposal:

"Collaboration or competition in Cdc42 regulation?"

In this proposal we describe how we will integrate the modeling expertise of Depken with an experimental project aimed at reconstituting and understanding regulation of the key polarity protein Cdc42, so far executed in the Laan lab. We will use the seed money to obtain the last necessary data and to interpret this data in microscopic terms by fitting molecular models. Our goal is to submit a paper by the end of the project, which will allow us to apply for further external funds to deepen our collaboration in the future.

Yours sincerely,



Liedewij Laan



Martin Depken

1. Applicant

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2. Title of the project

Collaboration or competition in Cdc42 regulation?

4. Abstract

Polarity establishment/symmetry breaking has long fascinated biologists and physicists alike. The redundancy evolved by living systems adds an extra layer of complexity, and precise mechanisms have often been hard to untangle. Building on early findings from the Laan lab, we here suggests a short-term intense effort to complete a study of a reconstituted system--where redundancy is avoided but key components retained. Through modelling and fitting of existing data, followed by cycles of further experiments and analysis, we will be able to determine essential parts of the structure of the network surrounding Cdc42 regulation in polarity establishment in living systems.

5. Benefit to the synergy of KIND

We propose to start a new collaboration with the help of KIND, combining the biological knowledge and experimental data from the Laan group, with the molecular-modelling expertise of the Depken group. Our collaboration will allow us to quantitatively characterize the recently reconstituted biomolecular reaction network, governed by the notoriously-difficult-to-work-with but very important regulatory protein Cdc42. As both groups bring in complementary expertise on this important problem, the collaboration initiated here has the potential to be the start of a long term collaboration.

Competition or Collaboration in the regulation of Cdc42?

How cells work, and moreover, how they work so well given they need to function in an ever fluctuating and changing environment, is one of the big questions is biology. The subfield of nanobiology asks how the interactions between biological molecules in space and time create robust and efficient biological function. We address this problem using polarity establishment in budding yeast as a model system. During polarity establishment, a pattern of Cdc42 (the key polarity-establishing protein) forms on the cell membrane from an initial cytoplasmic state (**Figure 1**). The formation of the polarity site is crucial, since it marks where the daughter will grow and cells that fail to polarize die.

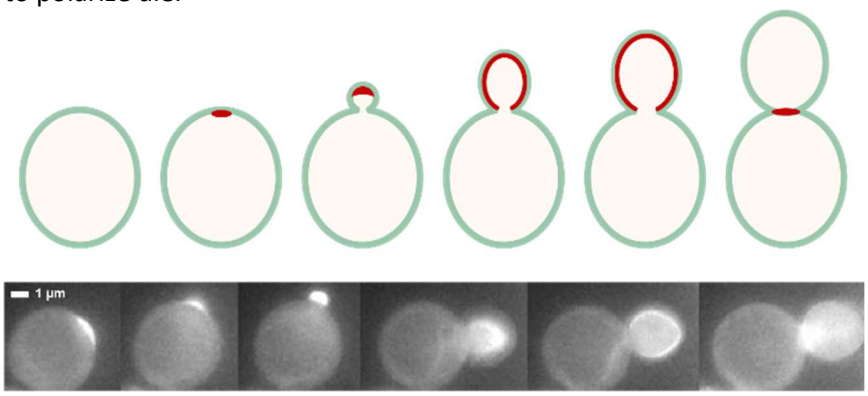


***Figure 1**: Polarity establishment and its role in the cell cycle in budding yeast. The cartoon depicts Cdc42 localisation in red. From the first to the second cartoon polarity is established. The bottom part shows live cell microscopy pictures where Cdc42 is labelled.*

Polarity establishment has been a popular topic for decades, and all the key proteins (approximately 35) have been identified, and their localisation and dynamics within the cell have been quantified (Daalman et al 2020). Based on these observations, several reaction-diffusion models have been proposed (Goryachev an Leda, 2017). Though these models are similar in spirit and predictions, they disagree about which specific molecular interactions are crucial for proper polarity establishment. Due to the high level of connectivity and redundancy present in the interaction networks of polarity establishing proteins, the molecular interactions within the living cell are very hard to dissect. This has led several groups, including ourselves, to argue that a minimal *in vitro* system needs to be developed, as is presently being done in the Laan lab.

Reconstitution of the polarity network

Cdc42 is an enzyme, which cycles between an active GTP-bound and an inactive GDP-bound state through hydrolysis of GTP to GDP followed by the exchange of Cdc42-bound GDP for GTP. The localisation of Cdc42 on the membrane is controlled through at least three interconnected feedback loops. For the reconstitution of a minimal system, we first focus on the feedback loop that controls Cdc42 cycling through GEF and GAP proteins. These two protein types respectively enhance *hydrolysis of GTP to GDP* and *exchange of Cdc42-bound GDP for GTP* (**Figure 2a**).

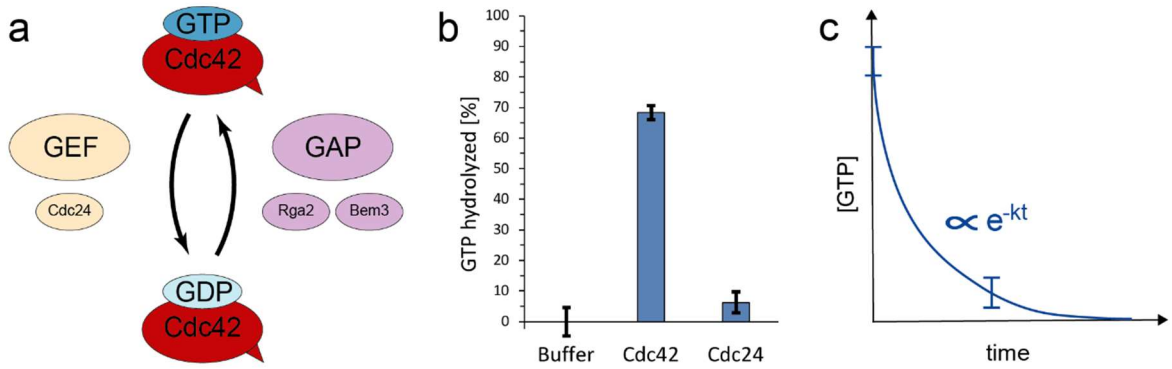


***Figure 2**: Cycling of Cdc42 a) cartoon of proteins studied and their role on Cdc42 cycling. b) Experimental data showing Cdc42 hydrolysis of GTP in our assay, in contrast to buffer or the GEF (Cdc24) alone. C) Conceptual figure shows how to obtain a cycling rate from experimental data.*

Aim of this project:
With this seed money we propose to work on two research questions summarized in two aims:

1) How much do individual GAPs or GEFs enhance the activity of Cdc42, and how does this depend on the stoichiometry or concentrations of proteins?
2) Do GEFs and GAPs interact while regulating Cdc42, and if so, (how much) do they collaborate (enhance) or compete (inhibit) the regulation of Cdc42 by the other's action?

Approach:
Aim1
Fayezeh Shamsi (PhD student in the Laan lab) has purified the following proteins: Cdc42, the GAP proteins Bem3 and Rga2, as well as the GEF protein Cdc24. Using a bulk GTPase assay, she has shown that purified Cdc42 can hydrolyze GTP, and thus that it remains active also after purification (see **Figure 2b**). She has also added Bem3, Rga2 or Cdc24 to the reaction, and observed that more GTP is hydrolysed in each case. By performing titration experiments, Fayezeh is presently measuring how the cycling rate depends on enzyme, substrate, and GAP/GEF concentrations.

In order to extract the microscopic parameters that determine the strengths of the individual interactions in the reconstituted reaction network, the system will need to be modelled, and the models need to be fitted to the data. The models will take the form of generalized Michaelis-Menten reactions, involving multiple regulatory proteins besides the traditional enzyme-substrate-product components. The microscopic modelling and data analysis will be performed by Friso Douma, a soon-to-graduate MSc student in the Depken lab.

Aim 2
After we have characterized the individual interactions of GEF and GAP proteins, we will probe their mutual interactions by combining them in different stoichiometries and concentration in our GTPase assay. This will allow us to investigate whether the regulatory proteins act independently (*neutral*), inhibit each other's binding (*compete*), or enhance each other's recruitment (*collaborat*e). We will here construct coupled versions of the models introduced under **Aim 1**, and fit out the same microscopic parameters. Based on how these parameters are effected, we will be able to determine if the interactions are neutral, competitive, or collaborative. Preliminary data suggests that the action of Cdc24 varies depending on if Rga2 or Bem3 is also present.

Our output goal is to submit a research paper before the end of the project (Sept 1st 2021).

References:
W. K.-G. Daalman, E. Sweep, L. Laan (2020)
The Path towards Predicting Evolution as Illustrated in Yeast Cell Polarity
Cells **2020**, 9(12), 2534;

A. Goryachev, M. Leda (2017)
Many roads to symmetry breaking: Molecular mechanisms and theoretical models of yeast cell polarity.
Mol. Biol. Cell **28**(3), 370 – 380

Requested budget:

Team

To achieve these aims, Fayezeh Shamsi (a PhD student in the Laan Lab, who will hand in her thesis before May 1st) will collaborate with Friso Douma (a Nanobiology MSc student in the Depken lab, who will hand in his MEP thesis before February 1st). Fayezeh is the person who has performed the experiments mentioned in this proposal, and Friso has already successfully built and analysed reaction models during his MEP.

Fayezeh will perform the experiments and Friso will build and fit models to the data. Based on modelling results, additional experiments will likely need to be performed to discriminate between models. We thus expect the collaboration to be close, which will help deepen the nascent collaboration between our groups. Douma will be hired from Feb 1st-Sept 1st as a research assistant and Shamsi will be hired as a Post-Doc from May1st-Sept 1st. Laan will pay lab materials and other lab work related costs from other resources.

Costs:

4 months post-doc	~ 20keuro
7 months research assistant	~ 30 keuro
Total:	**50 keuro**