Hierarchical Model of the Ras-MAPK signalling pathway in mouse Y1 adrenocortical tumor cells

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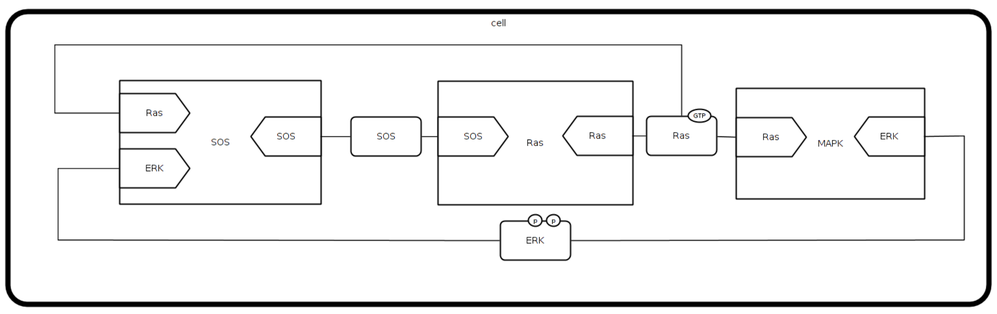
Objective

The K-Ras-driven mouse adrenocortical tumor cell line Y1 displays a surprising association of phenotypic traits, i.e., high basal levels of activated K-Ras in starved cells and induction of cell cycle arrest upon stimulation by FGF2. In addition, ectopic expression of the dominant negative mutant Ras-N17 reduced activated K-Ras basal levels and eliminated cell cycle arrest by FGF2. We are working to uncovered the molecular basis of this unexpected phenomenon by modeling the kinetics of the Ras-MAPK signalling pathway in Y1.

Our model is developed using SigNetSim, a web tool for designing quantitative biological models. It uses standard SBML representation, and is also compatible with the SBML comp package. This allows to build our model as a hierarchical model, which makes it easier to scale to large systems.

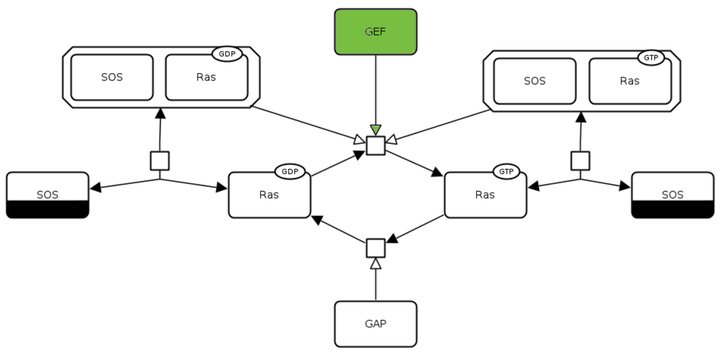
Hierarchical model

Our model is build as an assembly of three different submodels, using the SBML comp package.



Ras module

Our core Ras module is based on the model published by Das et al.[1]. It was however unable to reproduce high steady state of activated Ras. We thus introduced in the model an hypothetic additional GEF, which enable the model to reproduce this characteristic.



qRT-PCR experiment showed an over-expression of Ras-GRP4, a member of the GEF family. We are currently using CRISPR/Cas9 to deplete RasGRP4, which would corroborate that this GEF is a necessary component to achieve high RasGTP basal levels.

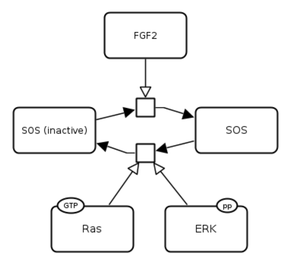
References

1. Das J. et al. (2009). Digital signaling and hysteresis characterize ras activation in lymphoid cells. *Cell*, 136(2), 337-351.
2. Huang, C. Y., Ferrell, J. E. (1996). Ultrasensitivity in the mitogen-activated protein kinase cascade. *Proceedings of the National Academy of Sciences*, 93(19), 10078-10083.

Acknowledgements

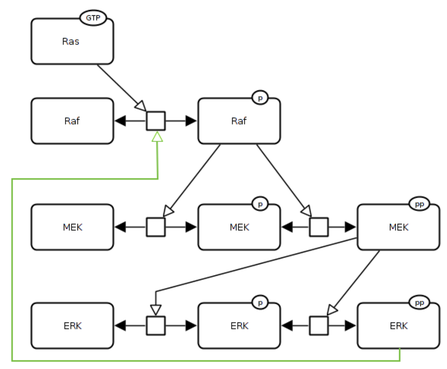
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SOS module



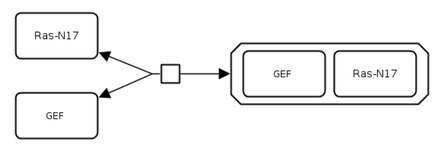
The SOS module is still a very simple one, where FGF2 activates SOS. The inactivation of SOS occurs thanks to two negative feedbacks, from activated Ras and activated ERK. We are planning to improve it, to incorpore more mechanistic details, and more growth factors.

MAPK module



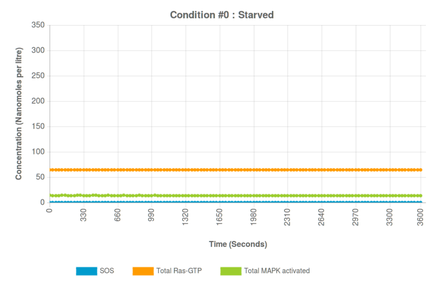
Our MAPK module is a classical MAPK model published by Huang and Ferrell[2]. Howether, we couldn't reproduce the fast inactivation of MAPK, which led us to add another negative feedback to our model. We are planning to validate this hypothesis by removing this feedback.

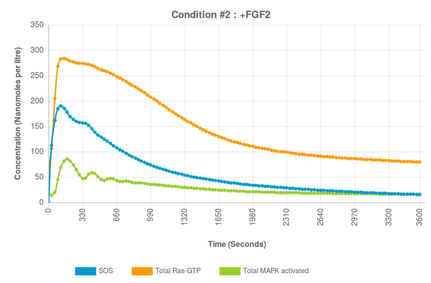
Ras-N17 module



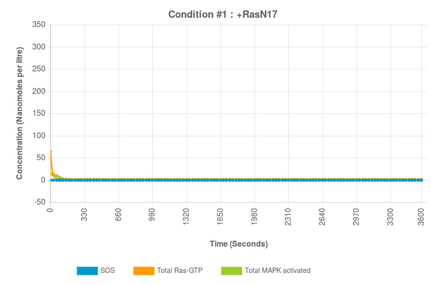
This module just contains one reaction, where GEF binds with Ras-N17, preventing it from activating Ras.

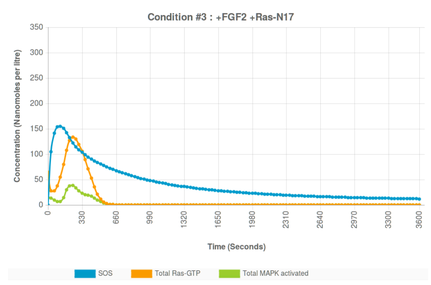
Simulations





Our model is able to reproduce the high basal level of activated Ras and activated MAPK on starved cells. It can also reproduce the Ras and MAPK activation upon FGF2 stimulation.





Additionaly, our model can reproduce the effect of Ras-N17 on both starved and FGF2-stimulated cells.

Future work

- We are currently working to validate our hypothesis regarding GRP4 using CRISPR.
- We also add to plan other modules to investigate the cell cycle arrest, including ERK nuclear translocation and its targets activations, and cell cycle control.

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