Dynamical model of the Ras-mediated AP-1 activation in mouse Y1 adrenocortical tumor cells.

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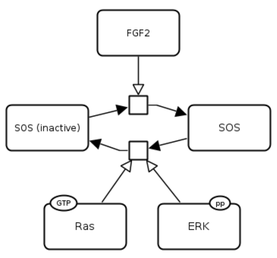
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Objective

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. We are working to uncovered the molecular basis of this unexpected phenomenon by modeling the kinetics of the Ras-MAPK signalling pathway in Y1, and its subsequent activation of the AP-1 complex.

Our model is developed using SigNetSim, a web tool for designing quantitative biological models. It allows user to write a model as a collection of submodels, which makes it easier to design large systems. This model describes starved cells activated by growth hormones, which will activate SOS. SOS will then activate the Ras signalling pathway, which will subsequently activate the MAPK pathway. Once activated, ERK will be able to translocate to the nucleus, where it will activate the transcrition of the two main components of the AP-1 complex, C-Fos and C-Jun.

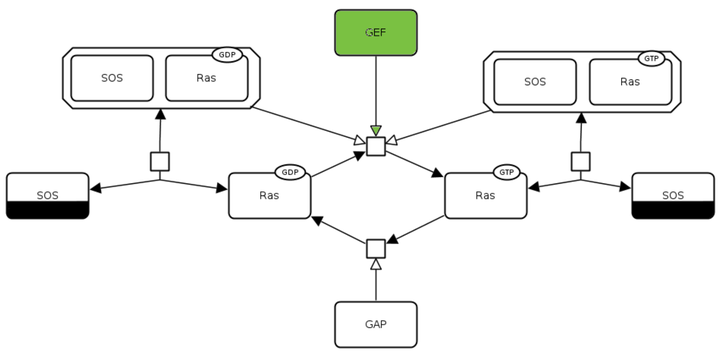
SOS module



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

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.

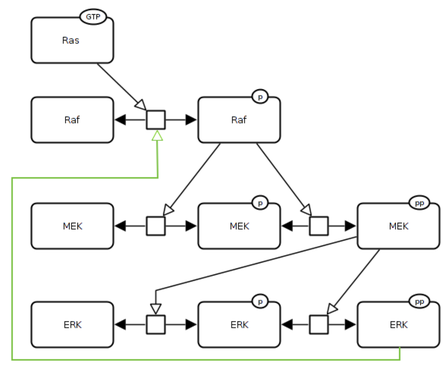
References

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

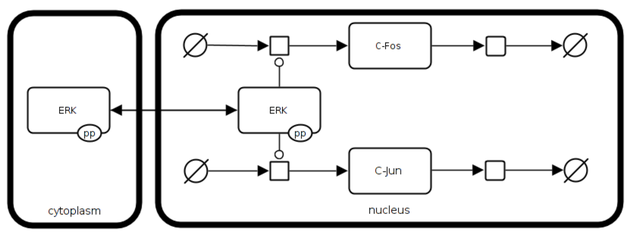
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MAPK module



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

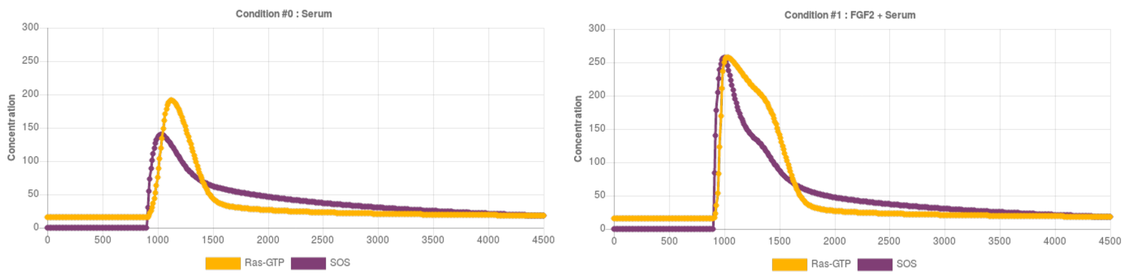
AP-1 activation module



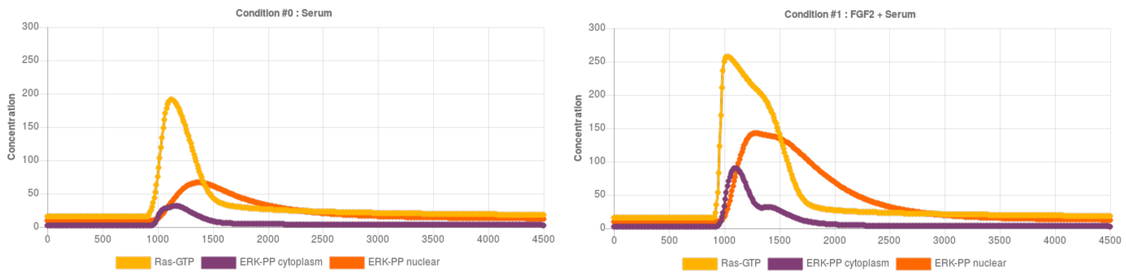
Once activated, ERK will move to the nucleus, where it will bind to the DNA to start the transcription of various proteins, including C-Fos and C-Jun.

Simulations

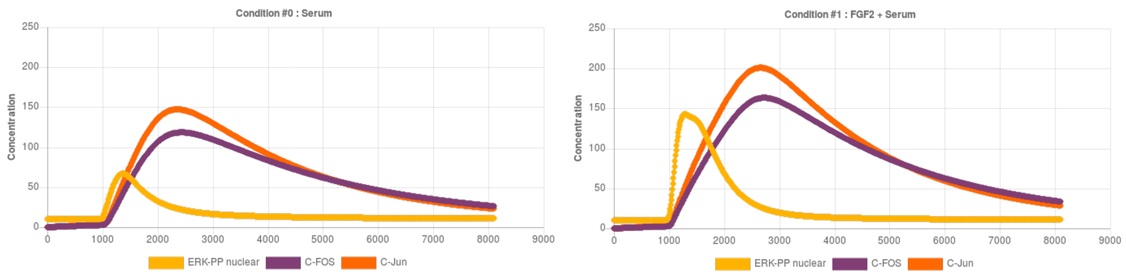
Our cells are first starved for 48h, then treated with either 10% FBS (Fetal Bovine Serum), or with 10nM FGF2 + 10% FBS. In these simulations, the treatment is given after 15m.



We can observe here that despite the absence of SOS in the first 15 minutes, the basal level of active Ras is not zero. The treatment will then activate SOS, which will itself activate Ras.



Active Ras will then activate ERK, enabling it to enter the nucleus.



Finally, ERK in the nucleus will bind to the DNA and start the transcription of C-Fos and C-Jun.

Future work

- Our lab isolated Y1 sublines which do not show the same phenotype, and we plan to improve our model to reproduce their dynamical behavior.
- We are studying transcriptomics data to elaborate further our model of transcription and cell cycle initiation.

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