

# RAF1 Coordinates Proliferation and Motility

Varga A<sup>1,2</sup>, Ehrenreiter K<sup>2</sup>, Aschenbrenner B<sup>2</sup>, Kocieniewski P<sup>3</sup>, Kochanczyk M<sup>3</sup>, Lipniacki T<sup>3</sup>, and Baccharini M<sup>2</sup>

**Short Abstract** — RAF1 and BRAF are two main RAF kinase isoforms in the ERK pathway. We have constructed detailed computational model of the ERK pathway, accounting for the regulatory sites of both RAF1 and BRAF, and their effects on assembly and coordination of RAF1-BRAF and RAF1-ROK $\alpha$  complexes, promoting respectively survival/proliferation and motility.

**Keywords** — RAF1, BRAF, isoforms, ROK $\alpha$ , cell fate, proliferation, migration, MAPK, MEK, ERK, rule-based modeling

## I. BACKGROUND

THE RAF/ERK cascade plays a principal role in the signal transduction of growth factors and initiating proliferation. The RAF kinase possess three isoforms: RAF1, BRAF, and ARAF that share similar homologous regulatory phosphorylation sites and activation mechanism but differ in activity and protein-protein interactions (PPIs) [1]. Specifically, RAF1 interacts with BRAF promoting ERK activation and proliferation but also with ROK $\alpha$ , which promotes cytoskeleton rearrangement and cell motility. These interactions are mutually exclusive and depend on the phosphorylation status of RAF1-Ser621. In this study we explored the coordination of the two responses [2].

## II. RESULTS

### A. Computational Model

We have constructed a model of the cascade that accounts for the regulation of RAF1 and BRAF by phosphorylation and protein-protein interactions. The model accounts for the phosphorylation state of the N- and C-terminal 14-3-3 binding sites, the NtA domain, and dimerization interface (by a negative feedback from ERK). Of particular importance is the N-terminal 14-3-3 binding site of RAF1 – Ser621; when phosphorylated 14-3-3 stabilizes RAF1-BRAF complex via crosslinking, or crosslinks RAF1 in an inactive form, when unphosphorylated RAF1 binds ROK $\alpha$ . The model features a detailed mechanism of RAF1 and

BRAF activation by RAS-GTP recruitment, homo- and heterodimerization, and stabilization by 14-3-3. The model comprises a system of 277 ODEs generated from the system of 85 rules specified in BNGL. The model was manually fit to reproduce the observed time profiles of (1) the phosphorylation of RAF1 sites, (2) ERK activation, (3) RAF1-BRAF and RAF-ROK $\alpha$  complex formation and effects of single and multiple mutations of the regulatory sites of RAF1 and BRAF on the ERK activity and protein-proteins interactions.

### B. Model Analysis

The analysis of the model demonstrated the mechanisms of coordinating protein-protein interactions in response to growth factor stimulation. The model indicated that the recruitment of RAF1 to RAS-GTP dimer is critical for Ser621 phosphorylation and heterodimerization with BRAF. This favors early ERK activation with maximal response at 5 min after the stimulation, which coincides with the maximal level of RAF1-BRAF heterodimer and promotes cell proliferation in the initial phase of signaling. The subsequent dimer disassembly and dephosphorylation at RAF1-Ser621 promotes RAF1-ROK $\alpha$  complex assembly with the maximal level at 15 min post-stimulation, inducing cytoskeletal rearrangements and promoting cell motility.

Sensitivity analysis confirmed the importance of the RAF1-BRAF stability and regulation thereof in determining the time profile of RAF1-ROK $\alpha$  complexes. The model predicts that the negative feedback from ERK to RAF isoforms terminates ERK activity and actively promotes RAF1-ROK $\alpha$  assembly by prior disruption of RAF1-BRAF heterodimers, thus establishing a temporal sequence of cellular responses.

## III. CONCLUSIONS

We have formulated and analyzed the first comprehensive model of the RAF/ERK cascade, accounting for the combinatorial complexity of the RAF1 and BRAF activation and interactions, and explaining coordination of proliferation and motility due to growth factor signaling.

## REFERENCES

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<sup>1</sup> Department of Biophysics of the Semmelweis University, Budapest, Hungary, E-mail: [matkovicsne.andrea@med.semmelweis-univ.hu](mailto:matkovicsne.andrea@med.semmelweis-univ.hu)

<sup>2</sup> Department of Microbiology, Immunology and Genetics, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria. E-mail: [manuela.baccharini@univie.ac.at](mailto:manuela.baccharini@univie.ac.at)

<sup>3</sup> Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland. E-mail: [tlipnia@ippt.pan.pl](mailto:tlipnia@ippt.pan.pl)