Induction and dynamics of the RAS/MAPK system

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1 Context

Data from Armelin group

Fibroblast growth factor 2 (FGF2) is considered a bona fide oncogenic factor. Results from the group of Armelin call this into question. In fibroblast RAS dependent cell lines FGF2 triggers a senescence-like process. Attempts to select FGF2 resistant cells yielded only rare clones that either i) have lost the over-expressed ras oncogene, or ii) were dependent on FGF2 for proliferation or iii) were poorly tumorigenic [2].

Studies searching for mechanistic explanation of these results focused on the interaction between two switches: RaS-GTP/Ras-GDP and MAPK. The dynamics of this system was probed in various conditions, serum, continuous and pulsed FGF2 treatment, small and high RAS expression level.

It seems that FGF2 induces a delay of the G1/S transition and of the DNA damage response.

Furthermore, the effect of FGF2 is restricted to a small window of 2 h. After 2h, application of FGF2 has no effect on delaying cell cycle. Notice that this is shorter than the cell cycle restriction point (time after which the transition to quiescence is irreversible), which is 3.5 h.

Flow cytometry studies show that the S phase population increases continuously up to 30 min - 2 h, then has a plateau and increases again between 6 and 8 h.

Dynamics of ERK induction under FGF2 treatment.

for Y1:

A first peak, 2-5 min, robust. A second peak between 30 min and 1h (Figure 1). These peaks are also present in the level of Ras-GTP (Costa thesis)

Other reports and data

Nakayama et al Current Biology 2008 [8].

In NIH 3T3 cell line (fibroblasts) stimulated with bFGF (25 ng/ml) RAS-GTP and ERK-p have complex oscillations. A first, large amplitude, sharp peak at 1 min for RAS-GTP, between 1-10 min for ERK-p. Two more peaks for RAS-GTP 140 min, 280 min (suggesting a periodicity of about 2h), 2 small, slightly retarded peaks for ERK-p. In Sos mutants (Erk phosphorylation sites mutated), no RAS-GTP oscillations. Similar results on C3H 10T1/2 cell line. RAS and ERK oscillations are accompanied by HES1 oscillations, slightly delayed with respect to ERK, but with the same period (no first sharp peak for HES1).

Hirata et al Science 2002 [4]

Hes1 oscillates with 2h periodicity under serum treatment in a variety of cell cultures, myoblasts (C2C12), fibroblasts (C3H10T1/2), neuroblastoma (PC12), teratocarcinoma (F9). Continues for 6 to 12h. Protein is delayed about 15 min after mRNA. Hes1 protein is a inhibitor of hes1 mRNA, need protein degradation in order to have oscillations.

Shankaran et al MSB 2009 [10]

ERK-GFP fusions show periodic translocation of ERK into the nucleus under EGF treatment with a periodicity of 15 min. 1841A1 HMEC epithelial cells; period is robust with respect to EGF concentration,

cell density, cellular ERK level. Amplitude is slightly decreasing with cell density increasing with EGF concentration.

Published models

Shankaran et al MSB 2009 [10]. Discuss periodic translocation of ERK into the nucleus. Have coupled the Huang-Ferrell 1996 model for the ERK activation cascade with the nuclear-cytoplasmic shuttling model of Fujioka et al 2006. No gene transcription considered, the origin of oscillations is purely biochemical (post-translational). Model reproduces the 15 min shuttling period.

Shankaran and Wiley, Current Biology 2010 [11]. Discusses several possible origins of ERK oscillations such as biochemical negative feed-back, transcription negative feed-back, sequestration of kinases and phosphatases. indicating the correct time scales. 5-15 min for the SOS feedback on RAS, 40-100 min for the transcriptional feedback of DUSP on ERK. They notice that the 2h period reported by Nakayama et al can not result from the SOS feedback on RAS, could eventually result from the DUSP feedback on ERK. However, they do not propose a mathematical model to test this idea.

Schoebert et al Nature Biotechnology 2002 [9]. Propose a model of EGF induced MAPK activation including expression of target c-fos gene.

Nakakuki et al Cell 2010 [7]. Propose the same thing as Schoebert al, use data and model to show binary (all or none) recognition of input signal shape by early response genes. The model contains coherent phosphorylation feed-forward, that generically generate all or none response.

Bernard et al Phil Trans R Soc 2006 [1]. Discusses models for transcriptional feed-back for HES1 leading to oscillation. The models are phenomenological, based on delayed ODE, no biochemical mechanism proposed.

Liu et al Biophysical Journal 2011 [6]. Propose a model based on sequestration of kinases and phosphatases. Probably the idea is not original, see also Chikarmame et al J Theor Biol 2007, Markevich et al J Cell Biol 2004. They propose a mathematical analysis of the model based on quasi-state approximation leading to a 3 variables reduced model, application of AUTO algorithms to study bifurcations.

1.1 Model and questioning

All the previous approaches favored one among the multiple potential mechanisms generating oscillations, without critical discussion of the choice. Furthermore, the RAS switch have never been included in a mathematical model of oscillations of ERK.

We propose to build a model coupling the RAS and MAPK switches. In a first step we combine the Huang and Ferrell 1996 model of MAPK [5] and the model of RAS activation by Das et al (Cell 2009) [3]. We add to these the biochemical feedback via dephosphorylation of SOS. Preliminary tests show the capacity of this system to oscillate, however the period is short (5-15 min).

What we expect from the model?

– to explain the observed, two time scale dynamics; a first rapid sharp peak, followed by slower period oscillations (damped, a second peak only in Armelin data, not damped, several peaks in Nakakuki et al).

– to reconcile several datasets. including the results by Shankaran.

– to reveal the possibilities of multiple time-scale feed-back. a possible interpretation is the following. ERK is a multipotent transcription factor. We need pulses rather than sustained activity of ERK (a similar example is NFkB). A rapid initial sharp peak is needed for triggering processes in early G1. Slower succession of peaks is needed for a second wave of processes. Which ones? Why?

Mathematical analysis of the model

Use Auto or Mathcont to generate bifurcation diagrams. This will say something about the robustness of the oscillations. Investigate how the oscillations depend on the hyper-sensitivy of each of the two switches. Investigate dependence on total RAS and total MAPK content.

Refinements of the model

1)Introduce nuclear-cytoplasm shuttling like in Shankaran et al. Investigate how the behavior is affected. Use a compartimentalized model. Discuss compartimentalization of RAS (some ideas in Lorentzen

et al Science Signalling 2010).

2)Introduce transcriptional feedback, first via DUSP on ERK. Investigate whether data by Nakayama et al can be reproduced (figure 2).

3)Introduce transcriptional feedback via an hypothetical phosphatase that may act on RAS, instead of ERK. Repeat discussion.

5) Apply models to Armelin data (figure 1).

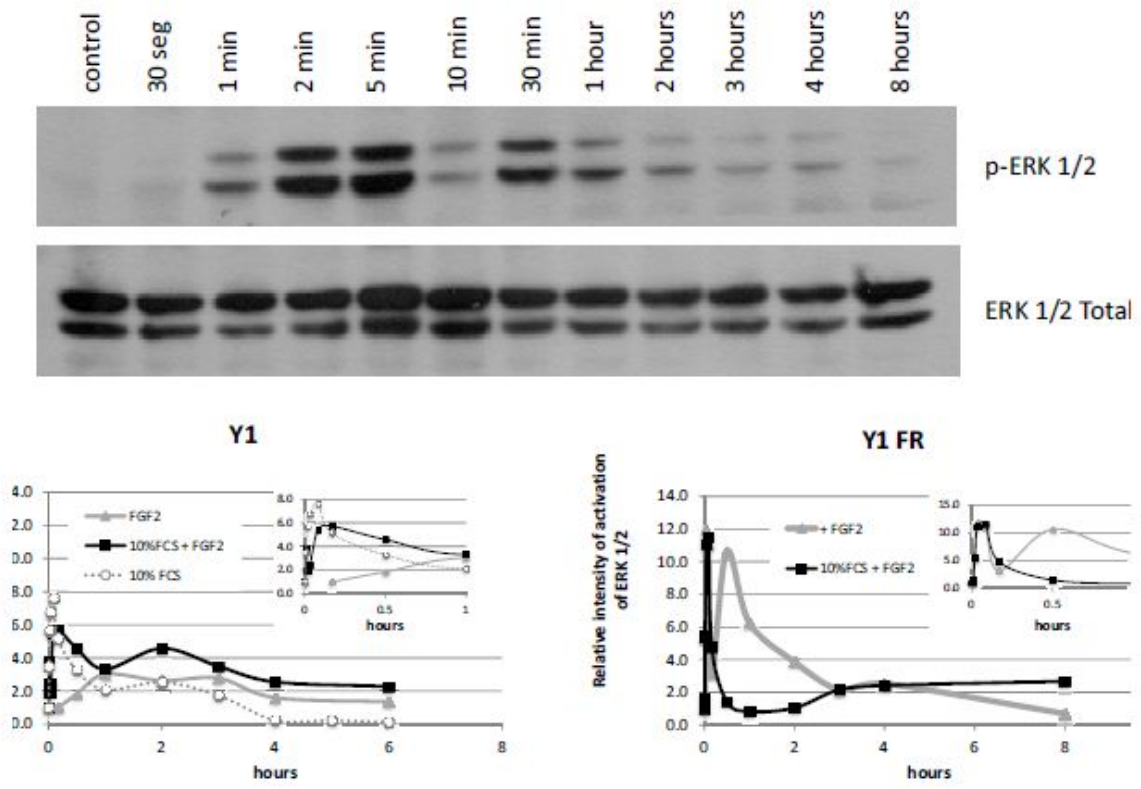

Figure 1: Activation kinetics of ERK in Y1 line and in a FGF2 resistant sub-line (Y1 FR) Up, Western-blot, showing the concentration of ERK-p (active) in Y1, under continuous FGF2 treatement (10 ng/ml). Down, same thing for Y1 and Y1 FR, after relative quantification.

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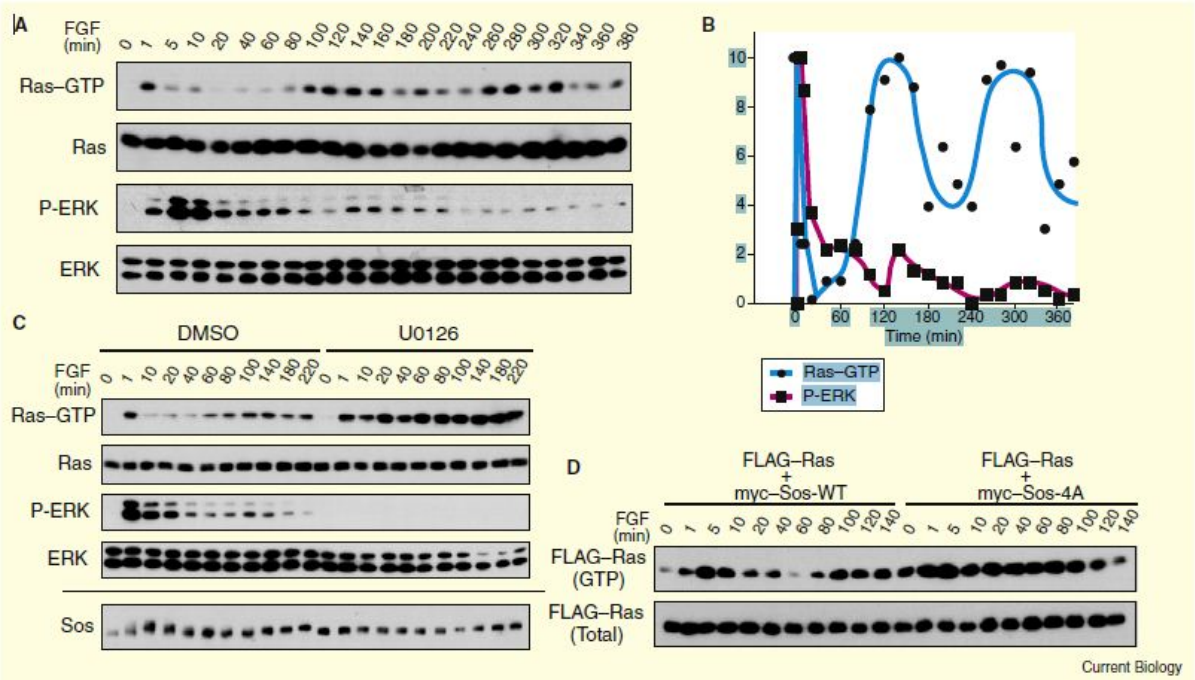

Figure 2: FGF-induced oscillatory activation of Ras and ERK. (A) The levels of RasGTP, Ras (total), phosphorylated ERK (P-ERK) and ERK (total) were determined in NIH 3T3 cells stimulated with bFGF (25 ng/ml). (B) Quantification of the signals of RasGTP and P-ERK. (C) ERK-dependent phosphorylation of Sos leads to Ras/ERK inactivation in NIH 3T3 cells. The phosphorylation levels of Sos, in addition to Ras-GTP and P-ERK levels, were determined during FGF stimulation in the presence or absence of U0126 (20 M). DMSO or U0126 was added 30 min before bFGF stimulation. Essentially the same results were obtained in two independent experiments. The upper four rows were from the same series and the bottom row from the other series. (D) Expression of mycSos-4A, but not mycSos-WT (wild-type), abolished FGF-induced Ras activity oscillations. NIH 3T3 cells were cotransfected with FLAGRas (250 ng) and mycSos-WT or mycSos-4A (750 ng). Cells were stimulated with bFGF (25 ng/ml) at time 0 and incubated for the indicated times. Three independent experiments gave similar results.

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