Emergence of bimodal cell population responses from the interplay between analog single-cell signaling and protein expression noise

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Presented by Sanjana Gupta

Background

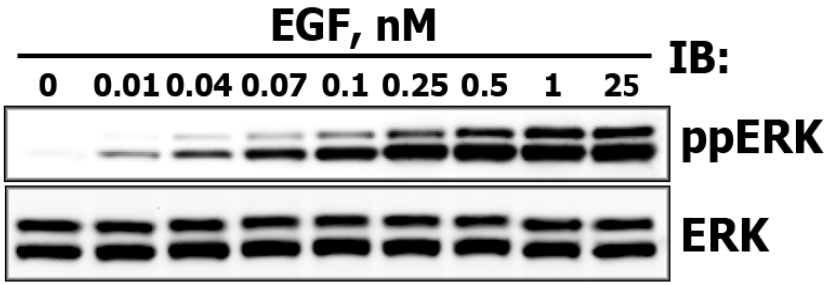
- Individual cells need to be able to convert noisy, analog signals into clear, yes-or-no cell fate decisions.

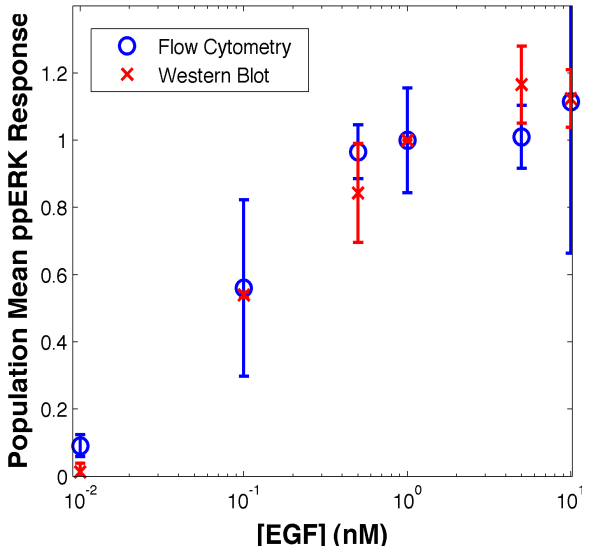
- Because of heterogeneity in protein abundance, population average measurements are not sufficient for investigating “all or nothing” responses.

- Single cell measurement techniques capable of capturing the dynamics of digital signal transduction are needed.

Analyses of ERK responses to EGF in individual cells and populations

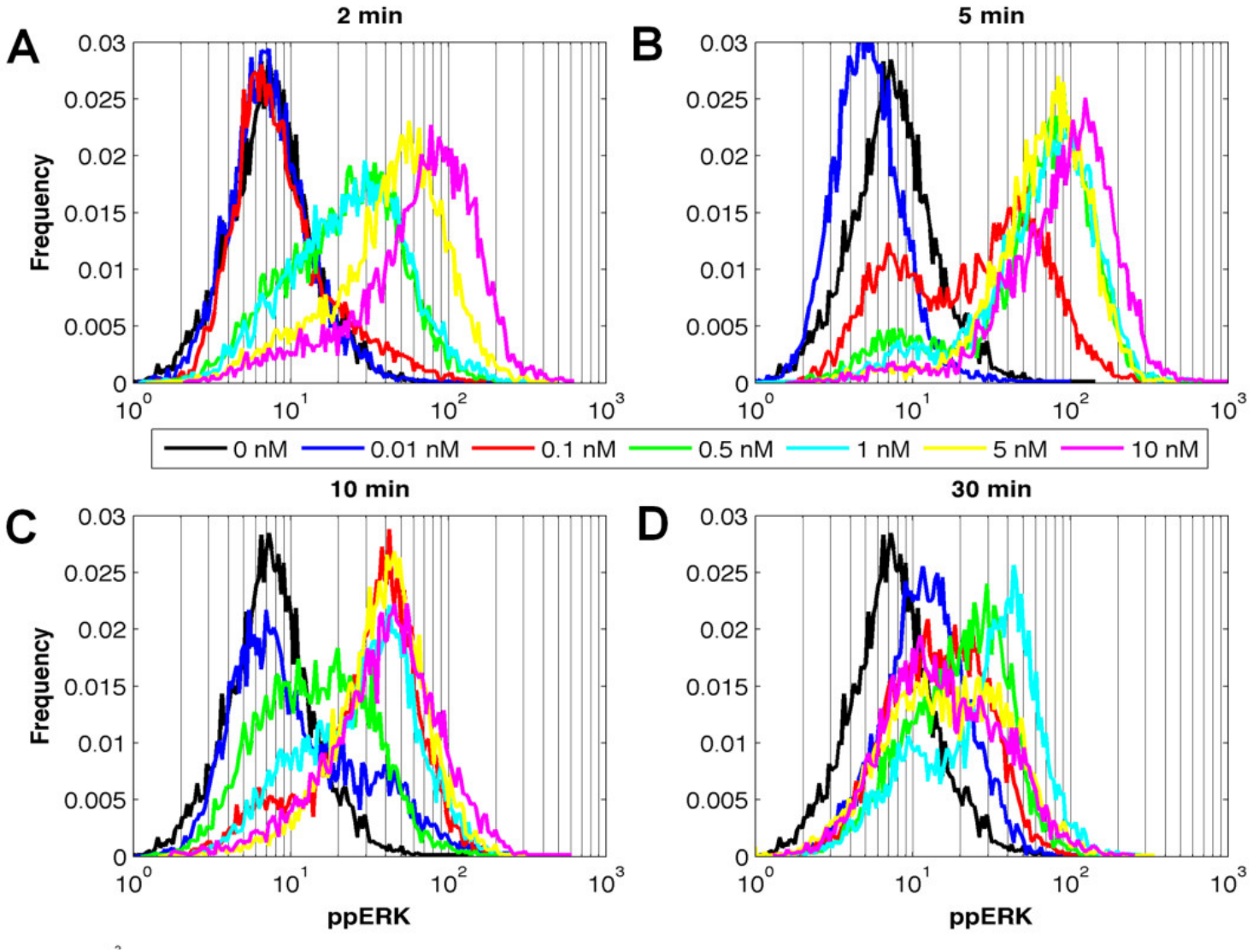
- Flow cytometry-based phosphorylation assay to determine the kinetics and dose response of ERK activation by EGF in HEK293 cells.





How do individual cells contribute to this collective population response?

Figure 1, Birtwistle et al.



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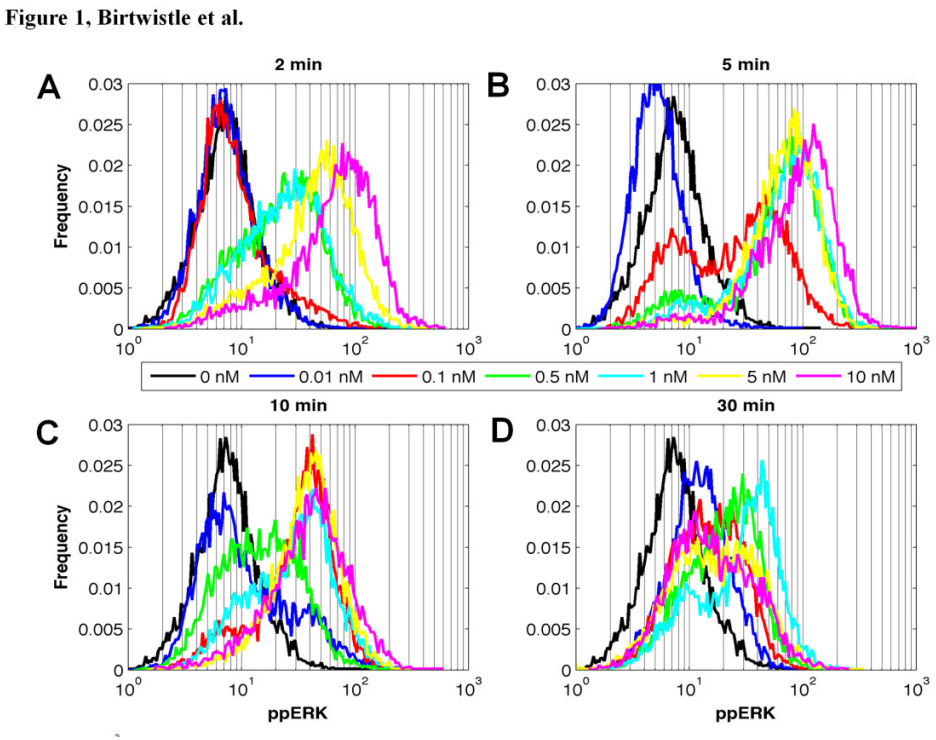
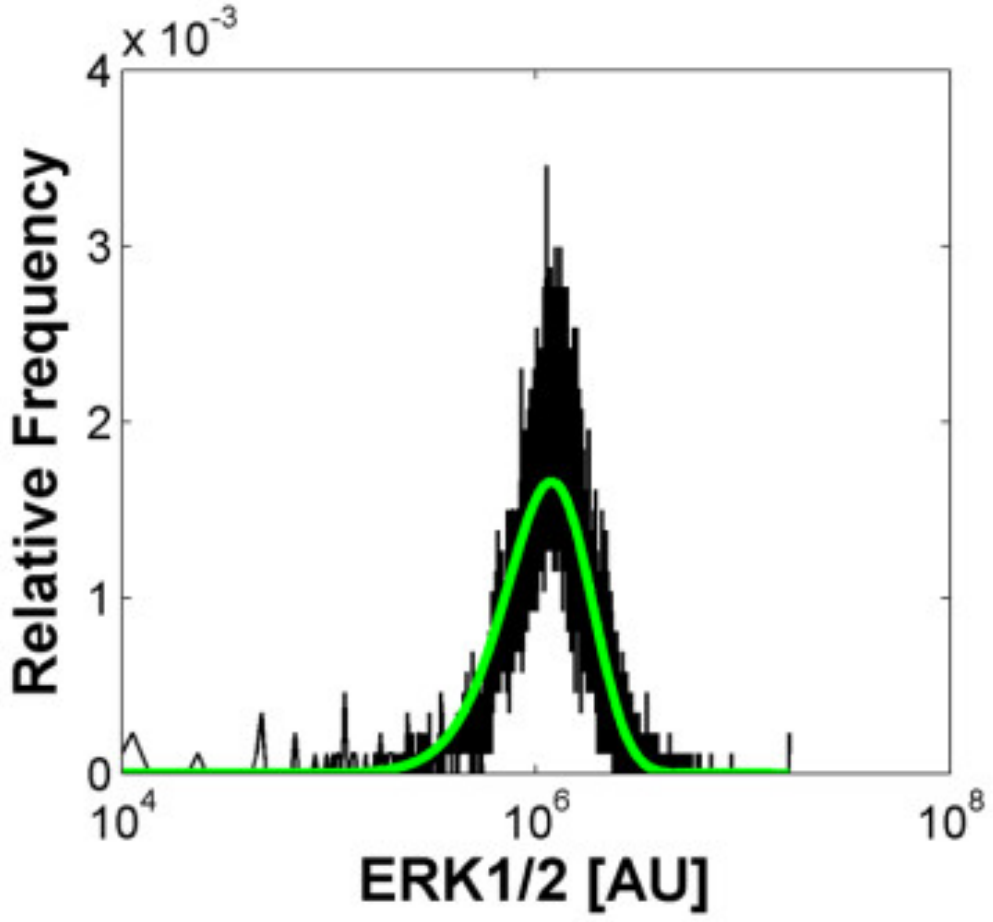


- Increase in mean values of ppERK was dose-dependent: **Analog signaling**
- A fraction of cells contain ppERK levels similar to those of the basal state.
- The height of this shoulder decreases with increasing EGF dose, but the position remains unchanged.
- **Digital on/off behaviour**

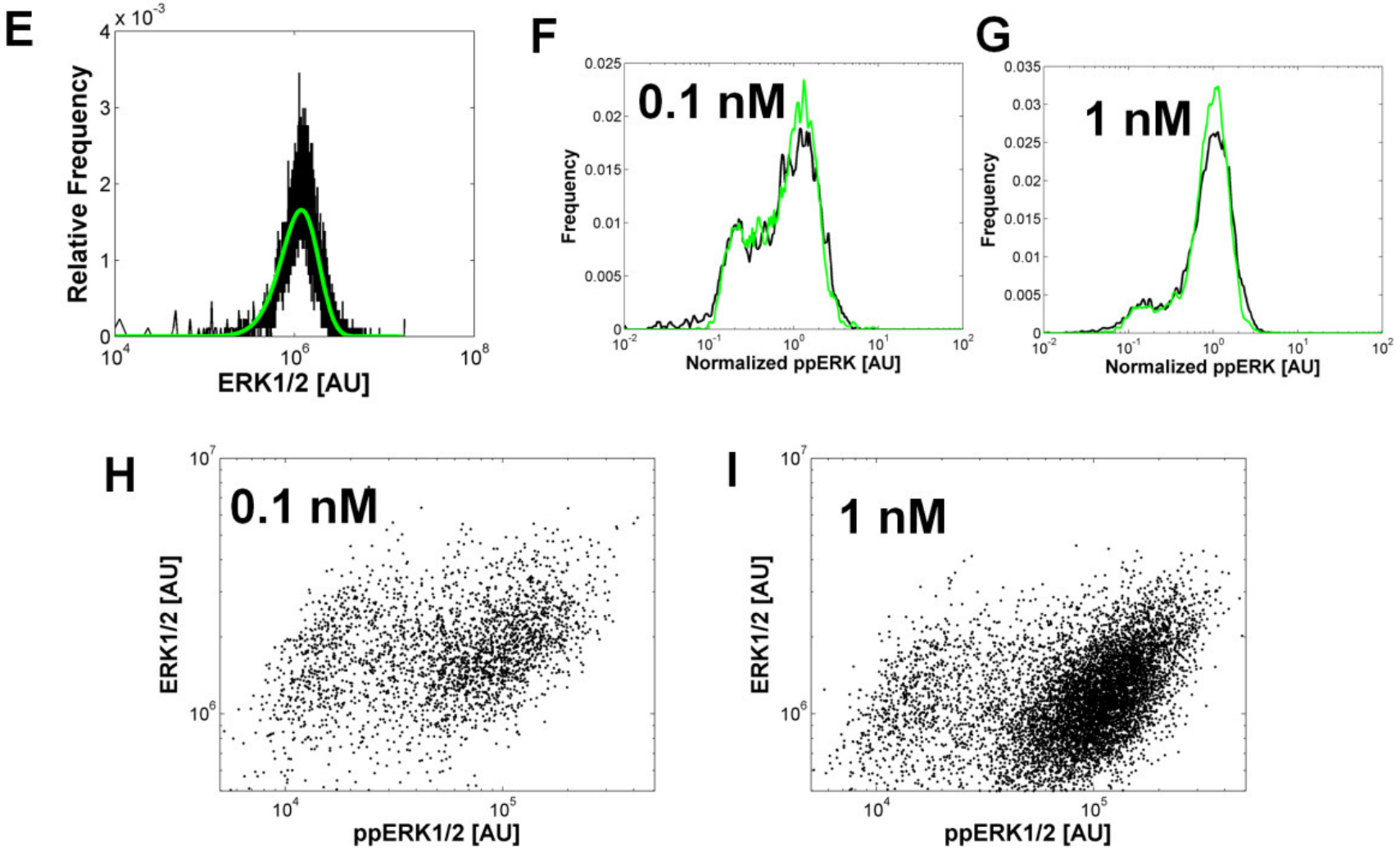
How does cell to cell variability in total Erk abundance affect the ppERK response?

E



- Substantial cell to cell variability in total ERK levels.
- Data is well approximated by a gamma distribution.

How does cell to cell variability in total Erk abundance affect the ppERK response?



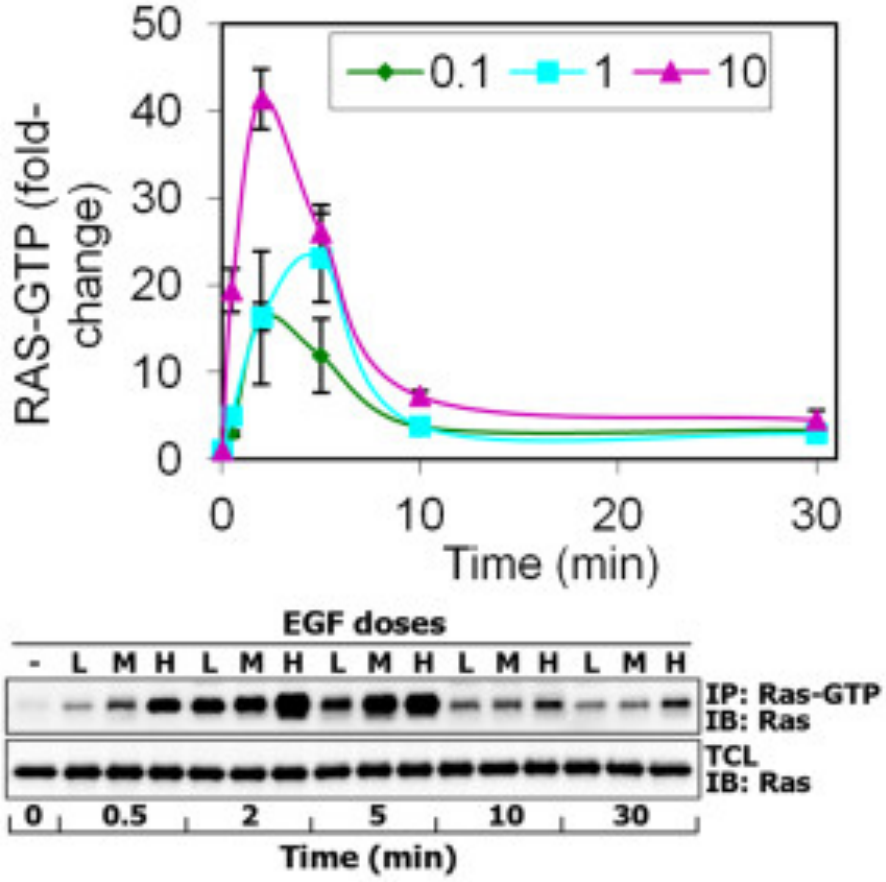
“Although cell to cell variability in ERK abundance contributes to ppERK response variability, it does not control bimodality, raising the question of what other factors contribute to the observed bimodality”

Stochastic dynamic modeling explanation of the data

- EGF activates RAS, which activates ERK

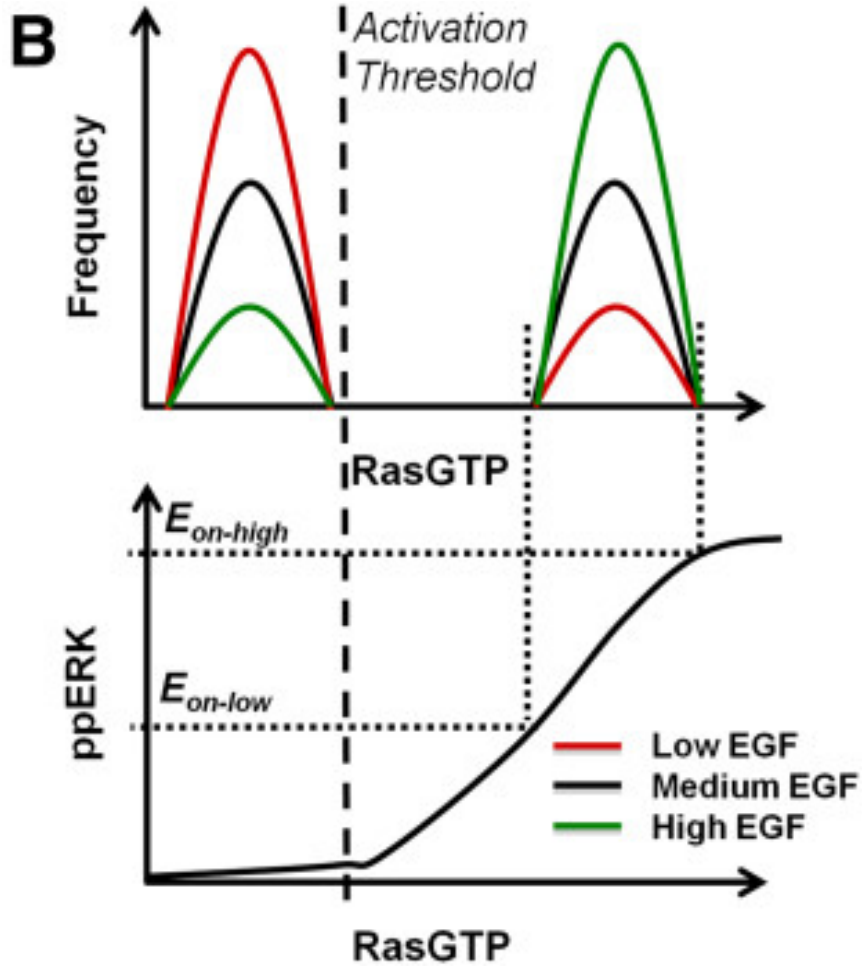
- The population average dose and dynamic responses of GTP-bound active RAS were assayed via pull-down and Western blotting and then quantified.

A



"A recent study suggested that in T lymphocytes, a positive feedback between RasGTP and its activator guanine exchange factor Son of Sevenless(SOS) leads to bistability and hysteresis in Ras activation"

Can bistability in RasGTP explain the bimodal ppERK populations?



Stimulation by EGF would affect the relative fraction of cells in the two populations, but not their means

A defined high mean RasGTP population would induce a defined high mean ppERK population.

Can bistability in RasGTP explain the bimodal ppERK populations?

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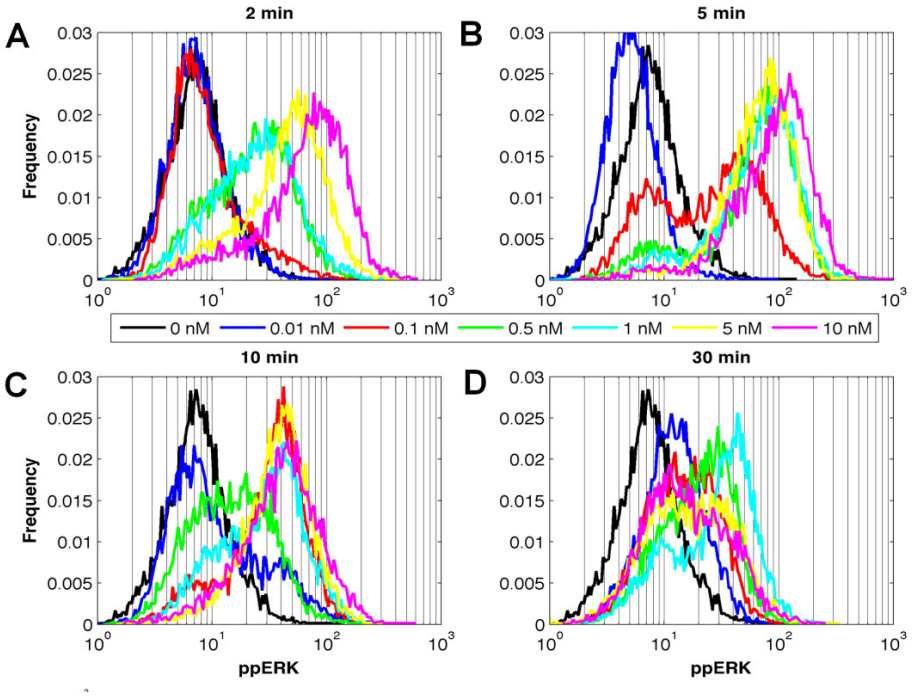

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Examining different configurations of the MAPK/ERK cascade

Distributions of active ERK display bimodal/ shouldering behavior with increasing EGF dose

The “ERK-on” population mean increases with increasing EGF dose at early time points, but decreases with time at constant EGF dose.

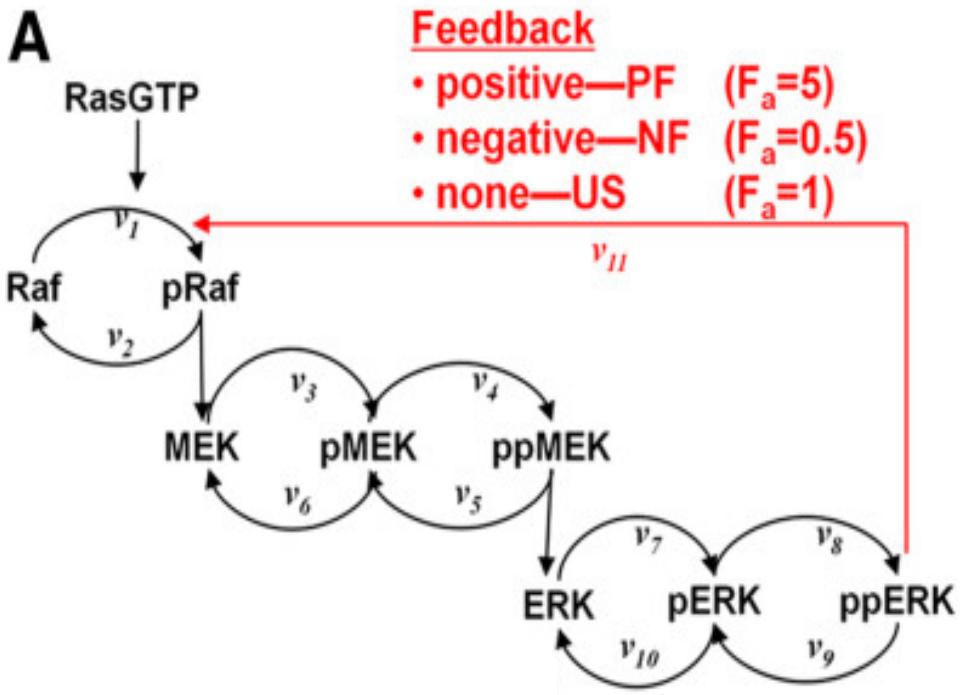
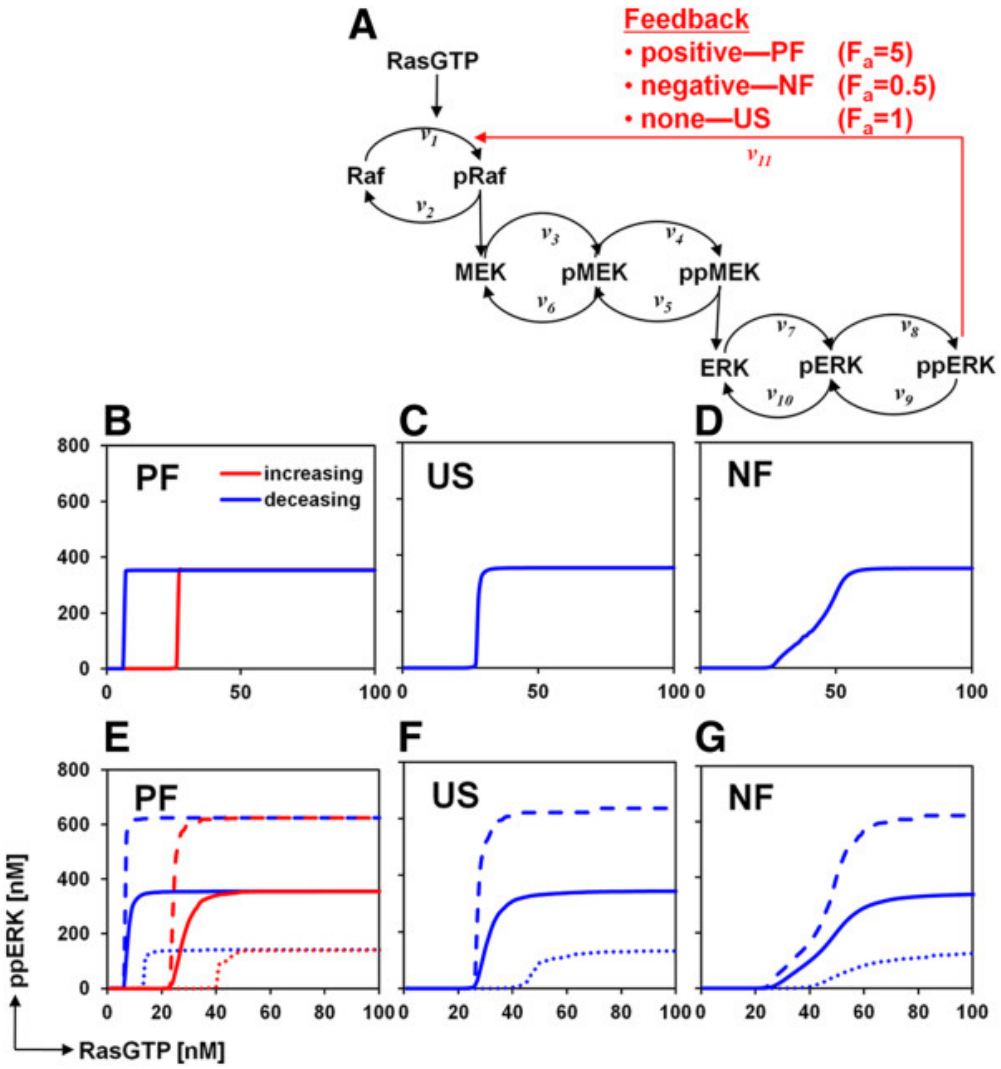


Table 1 Kinetic description of the ERK signaling cascade

N	Reaction	Rate	Kinetic constant*
1	MAP3K → pMAP3K	$v_1 = \frac{k_1^{cat}\cdot[Ras-GTP]\cdot[MAP3K]/K_{m1}}{(1+[MAP3K]/K_{m1})}\cdot g(F_a)$	$k_1^{cat} = 0.2;\ K_{m1} = 50$
2	pMAP3K → MAP3K	$v_2 = \frac{V_{max2}\cdot[pMAP3K]/K_{m2}}{(1+[pMAP3K]/K_{m2})}$	$V_{max2} = 5;\ K_{m2} = 50$
3	MAP2K → pMAP2K	$v_3 = \frac{k_3^{cat}\cdot[pMAP3K]\cdot[MAP2K]/K_{m3}}{(1+[MAP2K]/K_{m3}+[pMAP2K]/K_{m4})}$	$K_3^{cat} = 1;\ K_{m3} = 130$
4	pMAP2K → ppMAP2K	$v_4 = \frac{k_4^{cat}\cdot[pMAP3K]\cdot[pMAP2K]/K_{m4}}{(1+[MAP2K]/K_{m3}+[pMAP2K]/K_{m4})}$	$k_4^{cat} = 5;\ K_{m4} = 50$
5	ppMAP2K → pMAP2K	$v_5 = \frac{V_{max5}\cdot[ppMAP2K]/K_{m5}}{(1+[ppMAP2K]/K_{m5}+[pMAP2K]/K_{m6}+[MAP2K]/K_{i1})}$	$V_{max5} = 250;\ K_{m5} = 100$
6	pMAP2K → MAP2K	$v_6 = \frac{V_{max6}\cdot[pMAP2K]/K_{m6}}{(1+[ppMAP2K]/K_{m5}+[pMAP2K]/K_{m6}+[MAP2K]/K_{i1})}$	$V_{max6} = 250;\ K_{m6} = 100;\ K_{i1} = 80$
7	MAPK → pMAPK	$v_7 = \frac{k_7^{cat}\cdot[ppMAP2K]\cdot[MAPK]/K_{m7}}{(1+[MAPK]/K_{m7}+[pMAPK]/K_{m8})}$	$k_7^{cat} = 1;\ K_{m7} = 50$
8	pMAPK → ppMAPK	$v_8 = \frac{k_8^{cat}\cdot[ppMAP2K]\cdot[pMAPK]/K_{m8}}{(1+[MAPK]/K_{m7}+[pMAPK]/K_{m8})}$	$k_8^{cat} = 20;\ K_{m8} = 50$
9	ppMAPK → pMAPK	$v_9 = \frac{V_{max9}\cdot[ppMAPK]/K_{m9}}{(1+[ppMAPK]/K_{m9}+[pMAPK]/K_{m10}+[MAPK]/K_{i2})}$	$V_{max9} = 380;\ K_{m9} = 10$
10	pMAPK → MAPK	$v_{10} = \frac{V_{max10}\cdot[pMAPK]/K_{m10}}{(1+[ppMAPK]/K_{m9}+[pMAPK]/K_{m10}+[MAPK]/K_{i2})}$	$V_{max10} = 50;\ K_{m10} = 18\ \ K_{i2} = 100$
11	*Feedback*	$g(F_a) = \frac{\left(1+F_a\cdot([ppMAPK]/K_a)^2\right)}{\left(1+([ppMAPK]/K_a)^2\right)}$	$K_a = 100;\ F_a = 5; 1; 0.5$(PF; US; NF)

* Maximal rates, Michaelis and catalytic constants are expressed in [nM/s], [nM], and [s^{-1}], respectively. Total protein concentrations are $[MAPK3]_{total} = 200nM$, $[MAPK2]_{total} = 200nM$, and $[MAPK]_{total} = 360nM$.

Steady state analysis

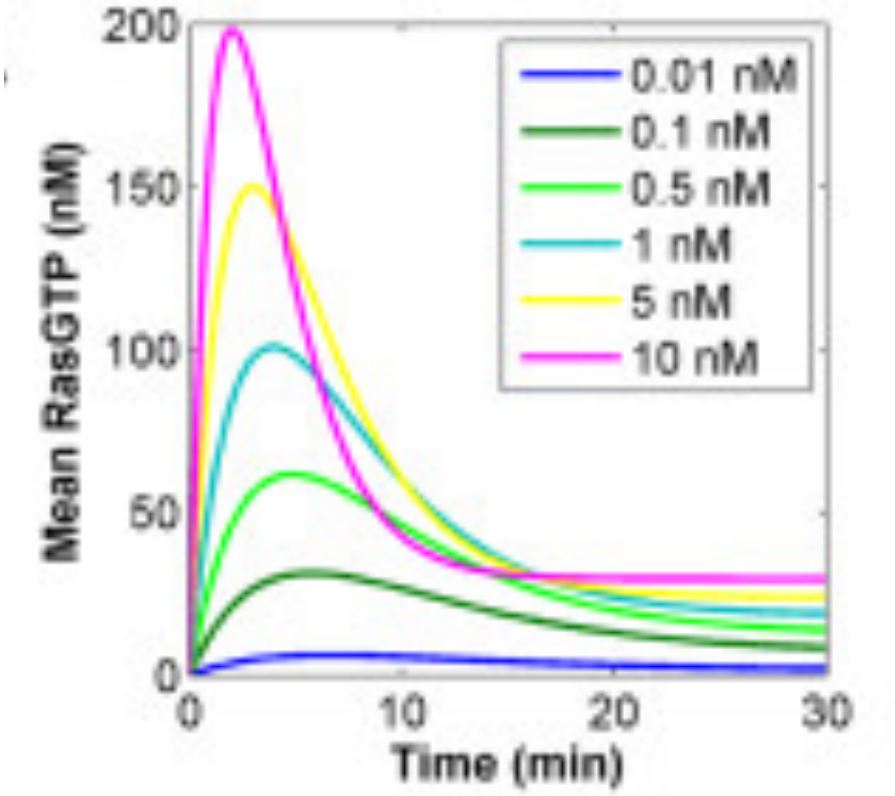


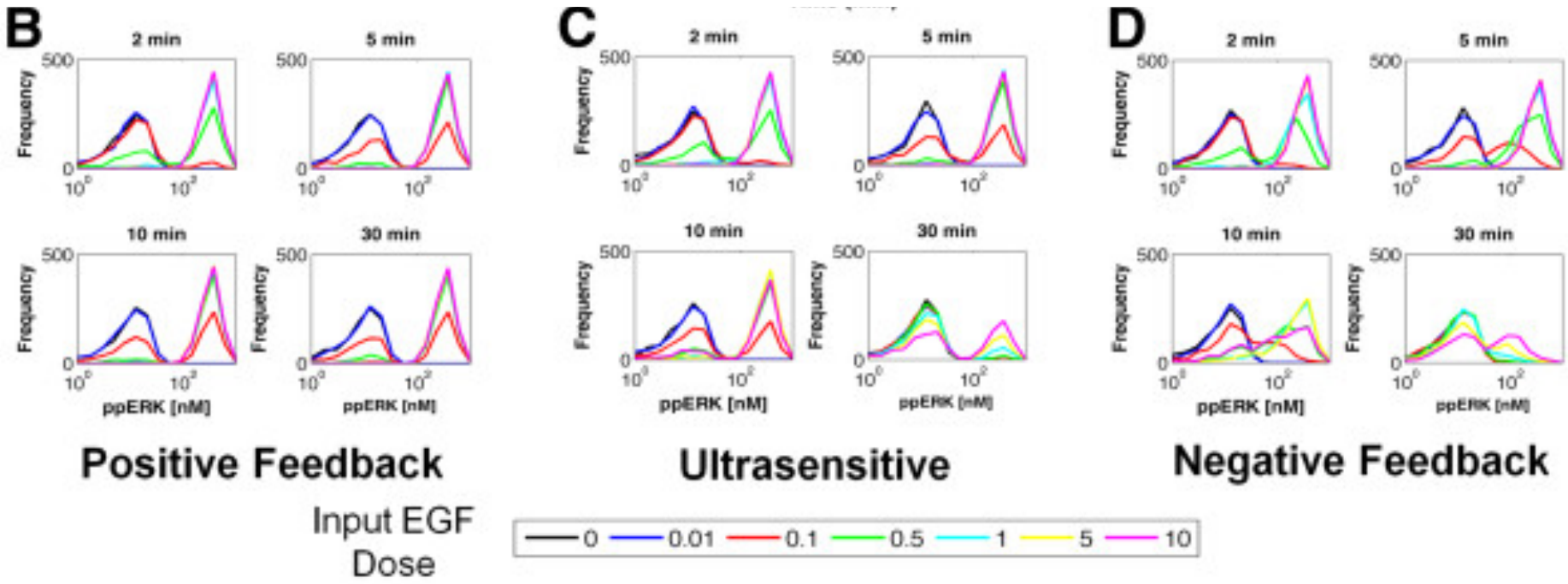
Deterministic simulations

Stochastic simulations

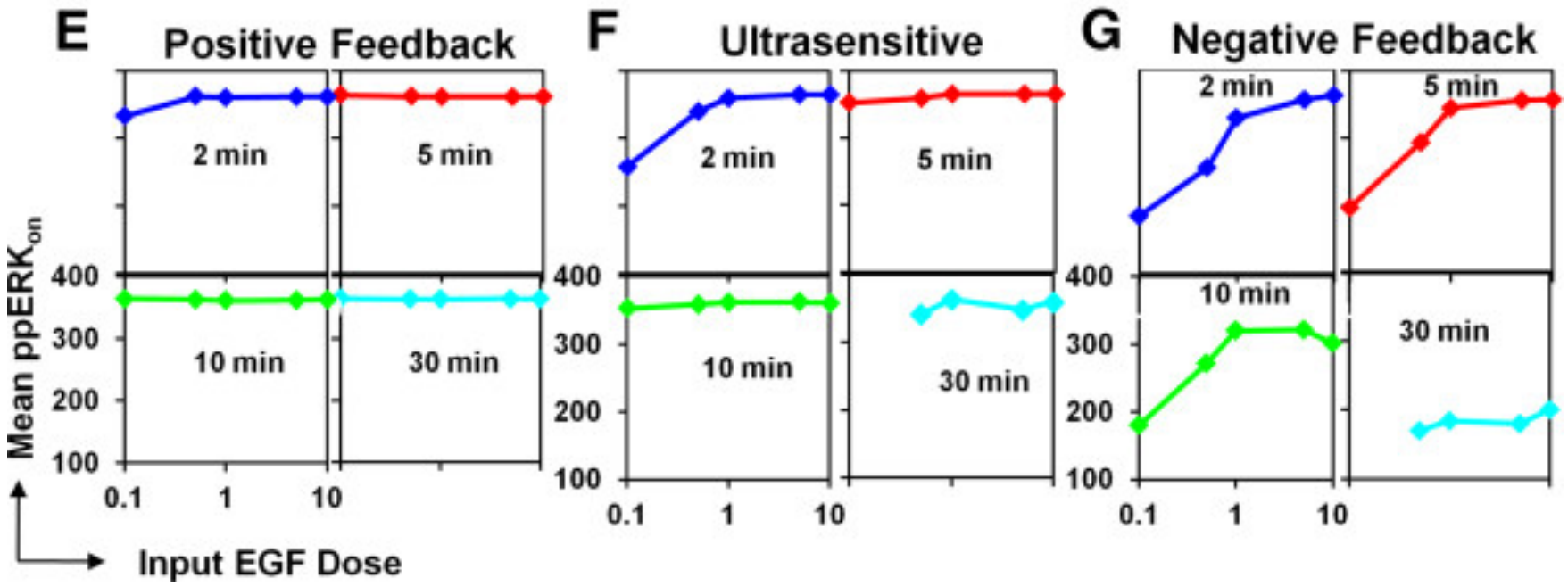
Analysis of transient responses

- Unimodal RasGTP distribution assumed
- Peak RasGTP values sampled from a gamma distribution





All three topologies exhibit time and dose-dependent bimodality or “shouldering”



Only the negative feedback model reproduce proper behavior of the ERK-on population mean:

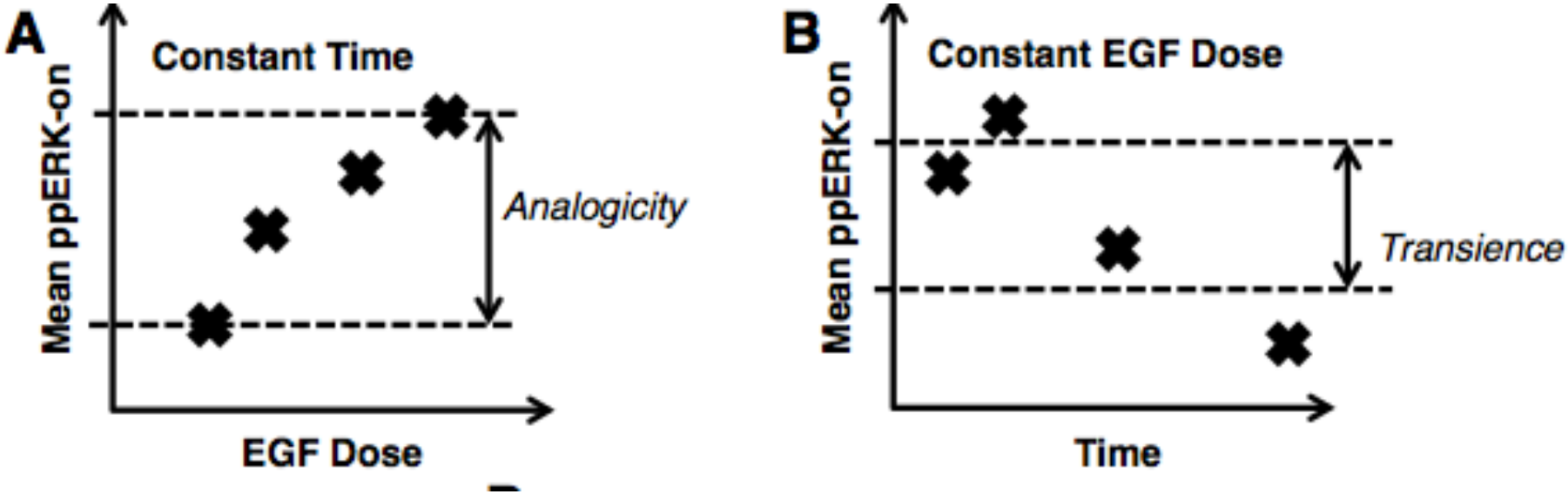
- Mean increases as a function of dose at short times
- Decreases as a function of time at a particular EGF dose

500 parameter sets, Latin hypercube sampling: Simulations were analyzed for three features

- **Analogicity:**
  Erk on population = cells with ppERK> 200nM
  Calculate the mean ppERK in the ERK-on populations if the population has > 10 cells
  Analogicity of a time point = max ERK on population mean – min ERK on population mean
  Total analogicity = analogicity(2min) + analogicity(5min)

- **Transience:** Transience of an individual EGF dose is the mean of the ERK-on population at 2 and 5 minutes minus that at 10 and 30 minutes. Sum over all doses.

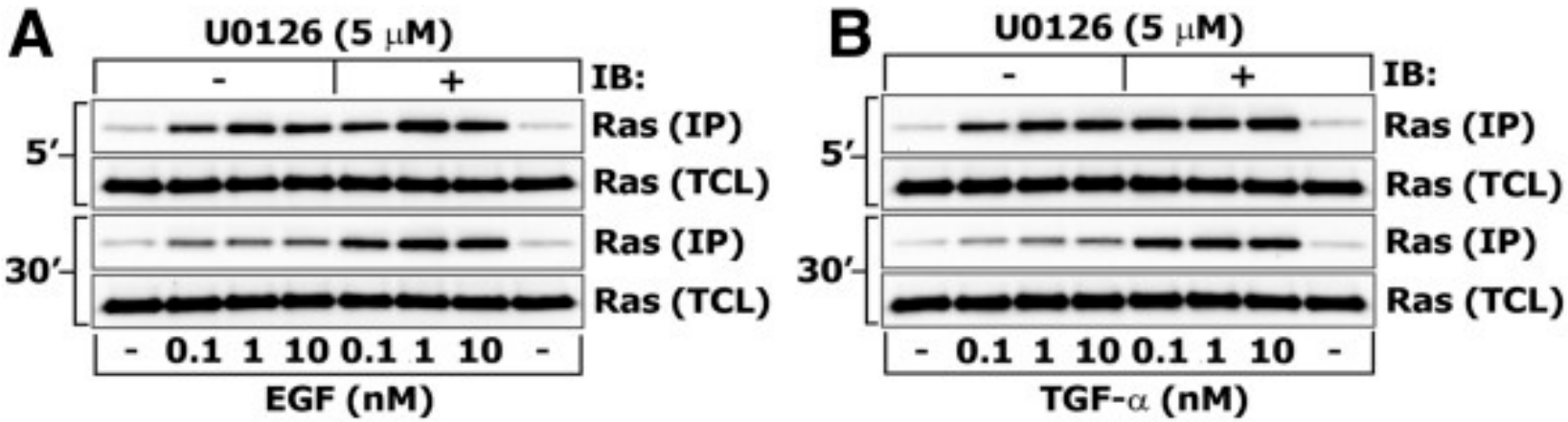
- **Bimodality:** Hartigan's Dip Test


A
Constant Time
Mean ppERK-on
Analogicity
EGF Dose
B
Constant EGF Dose
Mean ppERK-on
Transience
Time

Test of the negative feedback prediction

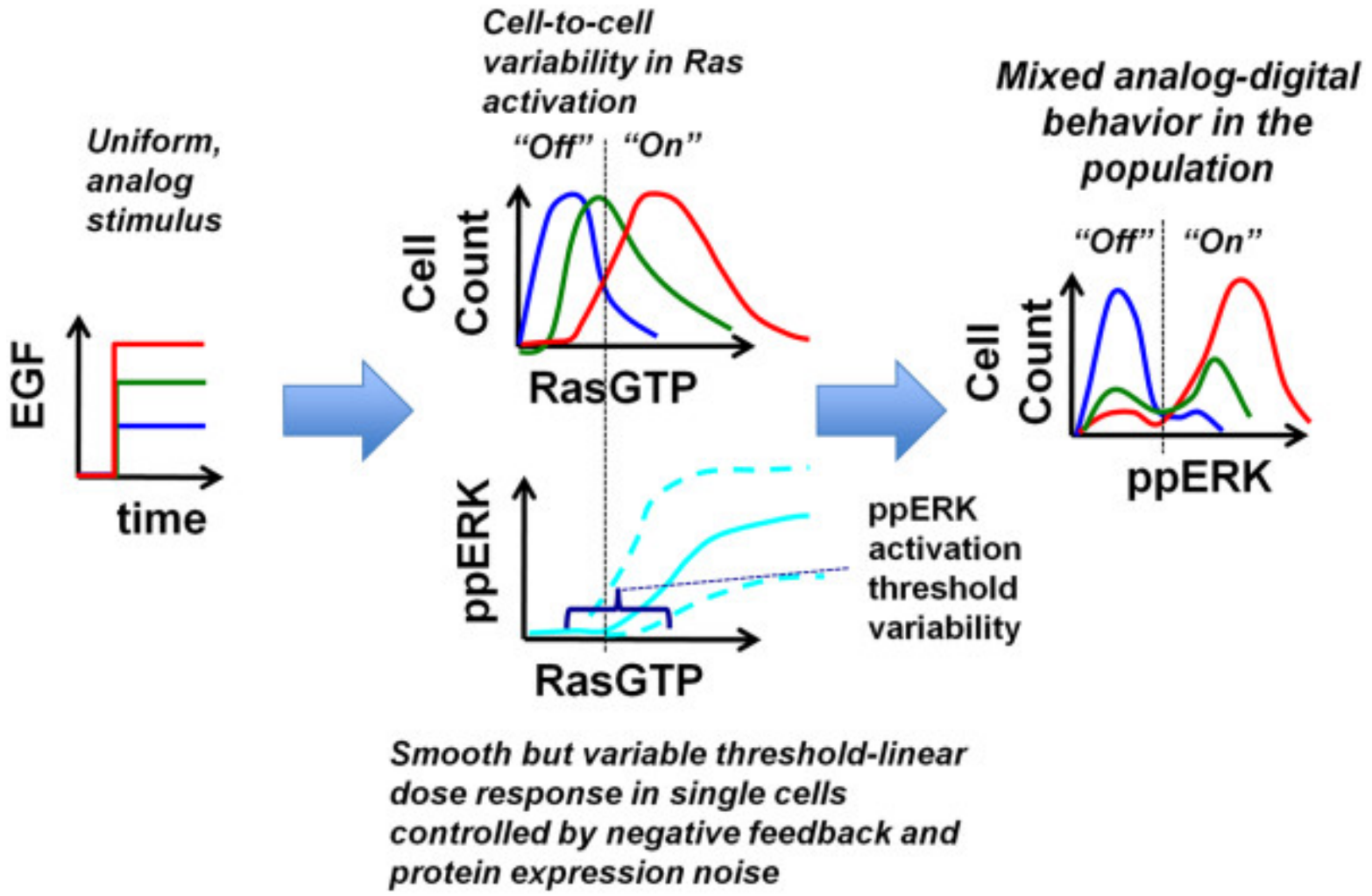
- If the feedback were negative, blocking ERK activity should increase the activation of upstream elements such as RasGTP.

- Measure the dynamic and dose response of RasGTP with and without the MEK inhibitor U0126.


A
U0126 (5 μM)
-
+
IB:
5′
Ras (IP)
Ras (TCL)
30′
Ras (IP)
Ras (TCL)
- 0.1 1 10 0.1 1 10 -
EGF (nM)
B
U0126 (5 μM)
-
+
IB:
5′
Ras (IP)
Ras (TCL)
30′
Ras (IP)
Ras (TCL)
- 0.1 1 10 0.1 1 10 -
TGF-α (nM)

Summary and conclusions

- Flow cytometry to measure EGF-induced single cell ERK activation responses in HEK293 cells
- Bimodal response distributions in cell populations
- An ERK cascade signaling model incorporating negative feedback and a graded, analog single cell dose response is shown to be consistent with observed population responses
- This analog to digital response conversion is suggested to be due to protein abundance variability
- **Thus bimodal distributions can arise from the interplay between protein expression noise and negative feedback-mediated analog single-cell responses.**



Smooth but variable threshold-linear dose response in single cells controlled by negative feedback and protein expression noise

Thank you