Signaling Networks: The Ras-Erk Pathway in the Context of Time

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Targeting the MAPK pathway: From Ras to Mek
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Biological and engineered systems share structural and functional features:

- Component multiplicity
- Rich connectivity
- Fail-safe functioning

However, the single supervisory component of engineered systems is often replaced in biological systems by multiple control loops.
Feedback Loops Carve Network’s Output

Computational tasks of feedback regulatory loops

- **Fold-change detection**

- **Signal consolidation**

- **Noise elimination**
  - Reference: Zwang et al., Molecular Cell (2011)

- **Decoding ligand specificity**
Coordinate regulation of c-FOS by miRNAs and A delayed early gene, ZFP36
microRNA155 and other Immediately Downregulated miRs (ID-miRs) Normally Suppress FOS and other IEGs

Early induced mRNAs (20-40 min) which are predicted targets of ID-miRs

Inversed correlation between ID-miRs and early induced mRNAs
ID-miRs are commonly downregulated in mammary tumors vs peri-tumors.
Chronobiology of Ras-to-Erk Signaling: An outline

Of all physical parameters of biological processes, **time** appears to be the one most elusive to the observer.

Part 1: Pulsatility of signaling (cell cycle)

Part 2: Diurnal regulation of signaling (cell migration)
Part 1: EGF-induced proliferation of mammary cells (HMEC cells)

R Point: cell decides whether to continue

Yaara Zwang
IGF1 may replace EGF in the 1<sup>st</sup> pulse, not in the 2<sup>nd</sup> pulse.
RPPA and Transcriptomic Analyses of the Two-Pulses
10 expression profiles are induced by EGF (two pulses)
The module “Down-regulated by 2nd Pulse” comprises several p53 regulated genes. The module includes well-established p53 target genes.

And p53 associates with chromatin upon the 1st pulse, remains active during the interval and dissociates on the 2nd pulse.
Knockdown of p53 enables R-crossing in the absence of a second pulse
The 2-pulse mode of commitment might filter the "noise" of growth factor bursts, which are often short and inconsistent.

In the absence of p53 (e.g., cancer cells), this filtering mechanism is defective.
Mechanisms of pulsatility: A primed state might involve a transient trough of negative regulators (263 genes)

**1st pulse**

- 0
- 20
- 40
- 60
- 60
- 60
- 60
- 60
- 60
- 60
- 1h
- 2h
- 7h
- 7h
- 7h
- 7h
- 7h
- 20
- 40
- 60
- 60
- 60
- 1h
- 2h
- 1h

**2nd pulse**

- 1st EGF (min)
- Starve interval
- 2nd EGF (min)
- Starve interval

**1st + 2nd pulses**

- Basal state
- Refractoriness
- Primed state

Phospho-Erk is enhanced in the 2\textsuperscript{nd} pulse, along with reduced induction of DUSP1
Part 2: Circadian Regulation of EGFR Signaling
Dexamethasone (DEX) Inhibits EGF-induced Cell Migration

Mattia Lauriola
Glucocorticoids (GCs): Mechanism of Action

- GCs stimulate **gluconeogenesis** (in liver) and **fat breakdown** in adipose tissue.
- GCs are used as anti-inflammatory agents (eg, allergy) and in high doses they reduce lymphocyte proliferation (eg, lymphomas).
- GCs, along with leptin and melatonin, maintain the **sleep-wake cycle** and the feeding/fasting rhythm, and assist the master biological clock (SCN) of the hypothalamus.
GR Mediates the Inhibitory Effect of DEX on Cell Migration
GR Exploits the EGFR Gene Program by Inhibiting the Feedback Activators and Activating the Feedback Inhibitors
Module A: GR Enhances the Expression of a Set of Negative Regulators of EGFR Signaling
Module B: GR Represses the Expression of a Set of Positive Regulators of EGFR Signaling (e.g., EREG and HB-EGF)
Circadian Oscillations of Serum Cortisol Levels in Human

TIME (AM/PM)

Graph showing serum cortisol levels throughout the day, with a peak at 9am and a trough at 3am, indicating a normal diurnal rhythm.
Circadian Regulation of EGFR Feedback Genes (liver and lung; WT mice)

Module A
- Dusp1
- Errf1/Mig6

Module B
- TGF-alpha
The Hypothalamus-Pituitary-Adrenal (HPA) Axis and CRFR1 Knockout Mice

corticotropin-releasing factor (CRF)
Circadian Regulation of EGFR Negative Regulators is Defective in CRFR1-KO Mice

In Red: CRFR1-depleted mice (CRFR1−/−)
GR Inhibits the EGFR Gene Program by Stimulating EGFR Negative Feedback and Suppressing Essential TFs
Take-home Message #3: Circadian Regulation of EGFR

- Glucocorticoid block EGF-induced migration of mammary cells by suppressing the activators and activating the inhibitors of EGFR.
- Our model predicts that EGFR is suppressed during daytime.
- If correct, EGFR’s contribution to tumor progression might occur at night (in human).
- Hence, inhibiting EGFR at night might be more beneficial than daytime treatments.
HER2-overexpressing Gastric Cancer Xenografts: Superiority of Resting Phase Treatment with Lapatinib
Chronobiology of Ras-to-Mek: Messages

1. Pulsatility of RTK signaling depends on wt-p53; it might be lost in tumors and lead to unregulated cellular proliferation.

2. Growth factor signaling is strongly suppressed by the glucocorticoid receptor, implying that some tumors might progress at the resting phase.

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HER2 Recycles EGFR

HER2-overexpressing Gastric Cancer Xenografts: Superiority of Resting Phase Treatment with Lapatinib

D

Lapatinib 40mg/Kg by day

Lapatinib 40mg/Kg by night

ZT23  ZT13

E

Tumor size (mm)

Days of Treatment

Lapatinib by Night

Lapatinib by Day

p<0.05

Days

F

Tumor weight (mg)

Night  Day

Night

Day
The *Persistently Induced* module is enriched for metabolic genes.
Induction of metabolic processes is essential for R-crossing.

Lipid metabolism and membrane biogenesis initiate at the 1st pulse and might be essential for R crossing.