Spatiotemporal Regulation of ERK by Dual-specificity Phosphatases
University of Bristol IN Cell 1000

WT Equipment Grant – “Use of a high content image analysis platform for biomedical research”

Multi-user equipment

Craig McArdle – Functional profiling of GnRHRs

Stafford Lightman – Transcriptional regulation by glucocorticoids

Christos Paraskeva – proliferation, death and differentiation of colonic epithelial cells

Andrew Newby – migration, proliferation and survival of vascular smooth muscle cells

Guy Rutter – roles of AMP-activated protein kinase in hypothalamic neurone nutrient signalling
Cell signalling - Mechanisms of Hormone Action
reproductive endocrinology – GnRH, gonadal steroids

IN Cell 1000 Projects

Context-dependence of steroid receptor signaling
- translocation and transcription in breast and bone cells
(ER/GFP, GR/GFP, ER/GR/GFP)

Benit Maru
Cell Signalling - Mechanisms of Hormone Action
reproductive endocrinology – GnRH, gonadal steroids

IN Cell 1000 Projects

GnRH receptor trafficking
Receptor internalisation and trafficking from ER to PM
(HA-GnRHRs)

Ann Finch & Kris Sedgley
IN Cell 1000 Projects

ERK signalling: compartmentalisation and kinetics

Jim Caunt
&
Steve Armstrong
Stimulus

Growth Factors, GPCRs

MAPKKK

- B-Raf
- A-Raf
- c-Raf

MAPKK

- MEK1/2

MAPK

- ERK1/2

Response

Growth, Differentiation, Development

Stress, Cytokines, Growth Factors, GPCRs

MAPKKK

- MLK3
- ASK1
- MEKK1/4

MAPKK

- MKK4/7

MAPK

- JNK1/2/3
- p38α/β/γ

Stimulus

Growth Factors, GPCRs

MAPKKK

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Response

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MAPKK

- MKK4/7

MAPK

- JNK1/2/3
- p38α/β/γ

Response

Inflammation, Apoptosis, Growth, Differentiation
In resting cells, ERK is chiefly cytoplasmic and bound to its upstream regulator, MEK, either in a free heterodimer or in larger multiprotein complexes.

Cytoplasmic targets: cytoskeletal regulators, kinases, cytoplasmic phosphatases etc.

Nuclear targets: Ets family transcription factors, IEGs, nuclear phosphatases etc.
Phosphorylation of ERK by MEK causes dissociation and nuclear translocation.

Dephosphorylation of ERK by nuclear phosphatases facilitates reassociation with MEK and nuclear export.
### Schematic Representation of Regulatory Mechanisms for ERK Activation and Cellular Responses

<table>
<thead>
<tr>
<th>Regulators</th>
<th>Differences in ERK activity</th>
<th>Cellular responses</th>
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</thead>
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<tr>
<td><strong>Temporal regulators</strong></td>
<td>Sustained ERK activation</td>
<td>Proliferation</td>
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<td>Differentiation</td>
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<td>Filamentous growth</td>
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<td>PKC, Rap1, Sprouty, ...</td>
<td>Time</td>
<td>Fibroblasts</td>
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<td>PC12 cells</td>
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<td>Yeast</td>
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<td><strong>Strength-controlling regulators</strong></td>
<td>Weak ERK activation</td>
<td>Proliferation</td>
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<td>Apoptosis</td>
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<tr>
<td>β-arrestin, IMP, KSR, MEK1</td>
<td>Time</td>
<td>Fibroblasts</td>
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<tr>
<td>MP1, ...</td>
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<td>Carcinoma cells</td>
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<tr>
<td><strong>Spatial regulators</strong></td>
<td>Strong ERK activation</td>
<td>Cell-cycle arrest</td>
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<td>Differentiation</td>
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<td></td>
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<td>Survival</td>
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<tr>
<td>β-arrestin, calponin, LSP1</td>
<td>Time</td>
<td>Proliferation</td>
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<tr>
<td>p14, paxillin, PEA-15, Sef</td>
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<td>Proliferation</td>
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<tr>
<td></td>
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<td>Proliferation</td>
</tr>
</tbody>
</table>

Caunt & McArdle; Trends Endocrinol. Metab., 2006
Dual-specificity Phosphatases (DUSPs) Regulate MAPKs by Removing Both Phosphate Groups Required for Activation

Dephosphorylation and anchoring

Signalling

Dephosphorylation and release

Required for Activation

MAPKs

ERK

MEK

ERK

DUSP

DUSP

ERK
Nuclear integrity and definition: DAPI stain (blue)

Total ERK2 localisation: ERK2-GFP (green)

Localisation of phosphorylated (active) ERK2: anti phospho-ERK2 Ab (red)

ppERK2  ERK2-GFP  DAPI
Graded Activation and Trafficking of ERK2-GFP Revealed by Frequency Distribution of Single Cell Data
This model of Tyr kinase receptor versus PKC-mediated activation of ERK allows exploration of the factors affecting transient or sustained ERK responses, which in turn influence cell fate.
Targeted siRNA Screening of Dual-specificity Phosphatases (DUSPs)

DUSPs comprise a diverse array of enzymes with a range of substrates, including non-protein targets.

10 genes in this group form a structurally similar subgroup of MAPK phosphatases (MKPs) which specifically dephosphorylate MAPKs and are characterised by MAPK docking motifs.

Other DUSPs do not contain these motifs and are termed “Atypical”, but can also directly dephosphorylate MAPKs (e.g. DUSP3 acts on ERK: Kang et al. Nat. Cell Biol. 2006).
siRNA Screening Parameters

- EGF
- PDBu

ppERK2 Intensity

ERK2-GFP N:C Ratio

Time (mins): 0, 60, 120, 180, 240

ppERK2 Intensity: 0, 200, 300, 400

ERK2-GFP N:C Ratio: 1.4, 2.1, 2.8

siRNA Screening Parameters
<table>
<thead>
<tr>
<th></th>
<th>EGF</th>
<th>PDBu</th>
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<tbody>
<tr>
<td>A</td>
<td>Ctrl siRNA</td>
<td>Ctrl siRNA</td>
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<tr>
<td>B</td>
<td>ERK siRNA</td>
<td>ERK siRNA</td>
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<tr>
<td>C</td>
<td>WT ERK2-GFP</td>
<td>WT ERK2-GFP</td>
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<tr>
<td>D</td>
<td>D319N ERK2-GFP</td>
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</tbody>
</table>

**Legend:**

- **Basal**
- **EGF**
- **PDBu**
- **Ctrl siRNA**
- **ERK siRNA**
- **WT ERK2-GFP**
- **D319N ERK2-GFP**

**Color Scale:**

- **Blue** (Low)
- **Red** (High)
Summary

The knock-down add-back system is a highly informative and robust method for studying ERK signalling and offers information unobtainable with other methods.

Observation of activation state and trafficking of ERK in response to different stimuli indicates varying signal termination mechanisms.