Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

The mitogen-activated protein kinase network, wired to dynamically function at multiple scales

Paolo Armando Gagliardi and Olivier Pertz

Abstract

The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling network is a key transducer of signals from various receptors, including receptor tyrosine kinases (RTKs). It controls cell-cycle entry, survival, motility, differentiation, as well as other fates. After four decades of studying this pathway with biochemical methods, the use of fluorescent biosensors has revealed dynamic behaviors such as ERK pulsing, oscillations, and amplitude-modulated activity. Different RTKs equip the MAPK network with specific feedback mechanisms to encode these different ERK dynamics, which are then subsequently decoded into cytoskeletal events and transcriptional programs, actuating cellular fates. Recently, collective ERK wave behaviors have been observed in multiple systems to coordinate cytoskeletal dynamics with fate decisions within cell collectives. This emphasizes that a correct understanding of this pathway requires studying it at multiple scales.

Addresses

Institute of Cell Biology, University of Bern, Baltzerstrasse 4, 3012 Bern, Switzerland

Corresponding author: Pertz, Olivier (olivier.pertz@unibe.ch)

Current Opinion in Cell Biology 2024, **88**:102368

This review comes from a themed issue on **Cell Architecture 2024**

Edited by **Carsten Janke** and **Ohad Medalia**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.ceb.2024.102368

0955-0674/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

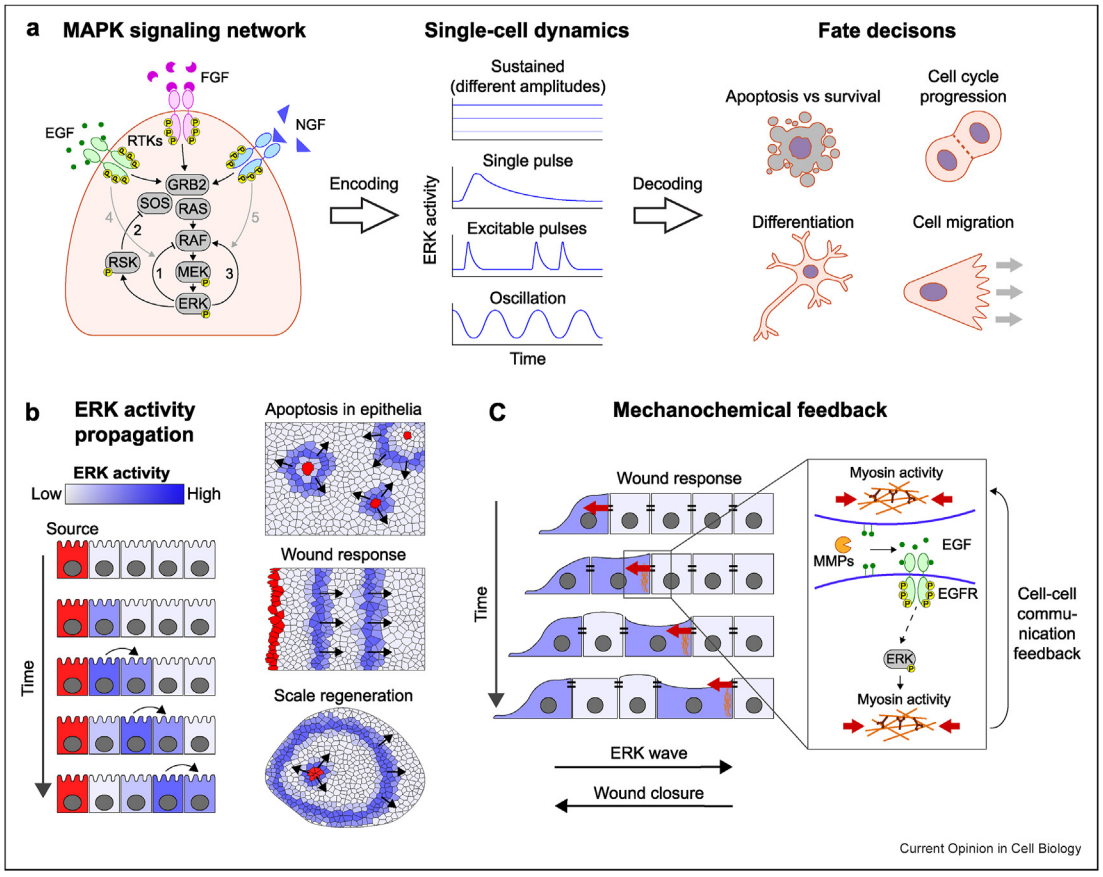
Introduction

The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway serves as a major transducer of signals from receptor tyrosine kinases (RTKs) and other plasma-membrane receptors, including G-protein-coupled receptors and integrins. RTKs' activation turns on the small GTPase Ras, which subsequently initiates a signaling cascade involving rapidly accelerated fibrosarcoma (RAF), mitogen-activated protein kinase kinase (MEK), and ERK kinases (Figure 1a). Upon activation, ERK phosphorylates multiple cytosolic substrates and a portion of ERK translocates to the nucleus, where it phosphorylates nuclear substrates, thereby inducing broad transcriptional programs. The MAPK network has been the subject of intense biochemical research for four decades. Western blots with phospho-ERK antibodies have typically measured steady-state cell population—averaged outputs of ERK activity, providing the intuition that ERK is either in an ON or OFF state. Early studies however have suggested that ERK activity dynamics [1,2] (from now on referred to as ERK dynamics), rather than steady states control fate decisions, providing an explanation on how one signaling pathway controls a large number of fates. The advent of fluorescent biosensors to measure single-cell ERK activity in the past decade, reviewed in Ref. [3], clearly shows that ERK dynamics are crucial for cell-fate decision-making. Importantly, the heterogeneity of single-cell ERK dynamics means that population-average measurements can be highly misleading. Here, we review recent developments into our understanding of how the MAPK network can produce a wide variety of single-cell ERK dynamics. Furthermore, we report on the recent findings that, thanks to mechanochemical feedback loops, multicellular ERK wave patterns can emerge and allow for coordination of cytoskeletal dynamics and fate decisions. This emphasizes that a correct understanding of the MAPK network requires its measurement and manipulation at adequate length and time scales.

MAPK network wiring with distinct topologies encode different ERK dynamics

Live imaging of fluorescent biosensors has revealed a rich repertoire of transient [4], sustained [4,5], pulsatile [6], oscillatory ERK dynamics [7,8] that fluctuate on minute timescales (Figure 1a). In the classic PC12 model system, epidermal growth factor (EGF) and nerve growth factor (NGF), respectively, trigger transient and sustained ERK activity [2,4]. Fibroblast growth factor (FGF) induces another behavior consisting of sustained ERK dynamics of different amplitudes, depending on the FGF concentration [5]. Thus, three growth factors (GFs) evoke different ERK dynamics in PC12 cells in a GF concentration—dependent manner. In epithelial systems, EGF triggers population heterogeneous nonperiodic 20- to 30-min-long ERK pulses

Figure 1



The MAPK pathway produces single-cell and collective dynamics.
(**a**) The MAPK pathway is equipped with multiple positive- and negative-feedback loops: (1) ERK-RAF negative-feedback loop [2,4]; (2) ERK-RSK-SOS negative-feedback loop [46]; (3) ERK-RAF positive-feedback loop [2]; (4) modulation of the ERK-RAF negative-feedback loop by EGF stimulation [4]; (5) modulation of the ERK-RAF positive-feedback loop by NGF stimulation [4]. The different wiring of each receptor tyrosine kinase (RTK) allows the MAPK pathway to differentiate among multiple inputs by producing different temporal dynamics. Cells then are capable to decode these different ERK dynamics into specific cellular responses and cell-fate decisions. (**b**) The ability of ERK dynamics to be propagated from cell to cell produces signaling waves of different shapes and sizes. These ERK activity waves coordinate different self-organization processes at the tissue level. Examples are epithelial homeostasis, wound repair, and fish-scale regeneration. (**c**) A mechanochemical feedback loop allows the ERK wave to propagate during wound repair. ERK activity leads to activation of myosin contraction that, in turn, leads to stretching of the adjacent cells, causing ERK activation.
Abbreviations: EGF = epidermal growth factor; ERK = extracellular signal-regulated kinase; RAF = rapidly accelerated fibrosarcoma; NGF = nerve growth factor; MAPK = mitogen-activated protein kinase.

[6,9]. Similar behavior is observed in other in vitro and *in vivo* systems [10–12]. Here, the system is excitable, and the EGF concentration encodes the ERK pulse frequency, which in turn controls the efficiency of cell-cycle entry [6]. In epithelial cells, the repertoire of pulsatile ERK dynamics is even greater, when cells are stimulated with GFs that bind to different ErbB receptors [13]. Here, the heterogeneity of ERK dynamics complicates the identification of the temporal patterns associated with each specific stimulation. However, a data-driven machine-learning approach can extract prototypical patterns in the single-cell ERK dynamics timeseries to provide better intuition about GFs specificity [13]. Another type of ERK dynamics consists in periodic oscillations [7,8,14–16]. A comprehensive review that describes how single-cell ERK dynamics patterns emerge can be found here [17].

This rich set of ERK dynamics patterns emerge through the wiring of the MAPK network with feedback structures (Figure 1a). The RAF-MEK-ERK cascade structure converts graded GF input concentrations into switch-like, all-or-nothing ERK responses [18]. Furthermore, negative- and positive-feedback loops from ERK to RAF lead to oscillatory behavior [19], and EGF-dependent transient or NGF-dependent sustained ERK dynamics in PC12 cells [2,4]. Competition of FGF binding to its main receptor (FGFR) and coreceptor (heparan sulfate proteoglycan), when coupled to an MAPK network with weak negative feedback, converts

different FGF concentrations into sustained ERK activity of different amplitudes in PC12 cells. Thus, EGF, NGF, and FGF can each lead to distinct ERK dynamics by wiring the MAPK network in different ways. A powerful approach to dissect these different circuitries is to dynamically perturb the MAPK network by application of growth factor pulses using microfluidics and to record resulting ERK dynamics [4,5]. This approach probes the network at relevant timescales revealing possible feed-forward network circuitries modulating negative- and positive-feedback loops in the PC12 cell system [4]. This was also instrumental to understand how receptor interactions in the FGFR system can produce amplitude-modulated sustained ERK activity in response to different FGF concentrations [5]. Importantly, dynamic application of GF inputs can lead to synthetic ERK dynamics that reprogram fate independently of GF identity [4,5].

This approach was pushed further by building a genetic circuit comprised of an optogenetic FGFR coupled to a spectrally compatible ERK biosensor [8]. This circuit can probe how light-evoked dynamic RTK inputs are interpreted into ERK dynamics at scale, allowing the authors to perform a RNAi screen against 50 nodes of the MAPK network. Surprisingly, knockdown of most of the nodes does not lead to altered ERK dynamics, suggesting that the MAPK network is robust against perturbations. This robustness emerges at least in part from two simultaneously functioning negative-feedback loops: the classic ERK-RAF feedback loop, and a feedback loop from the ERK substrate p90RSK to SOS (Figure 1a). Inhibition of the latter breaks network robustness and sensitizes the MAPK network to additional drugs. This exemplifies how studying signaling networks dynamics can provide nonintuitive insights about their properties and provide opportunities for directly targeting their robustness. Together, these works illustrate how the tripartite MAPK network, when coupled with different RTK-dependent feedback structures, can elicit a rich set of ERK dynamics. The latter are then subsequently decoded into transcriptional programs that actuate the fate decisions. We refer to a recent review that describes this process [20].

ERK activity waves as a dynamic signaling motif in cell collectives

Recently, a new dimension in MAPK signaling has emerged by the observation of waves of ERK pulses in epithelial cells. This collective behavior was first noticed by *in vivo* imaging of the mouse-ear epithelium, in which ERK waves occurred spontaneously or in response to wounds [21]. The dynamic ERK-wave signaling motif was then observed in a wide variety of in vitro cellular systems (Figure 1b). In the wound healing of epithelial Mardin-Darby canine kidney cells, collective ERK waves originate from the wound edge and propagate toward the interior. These waves spatiotemporally control myosin activity pulses that coordinate collective motility [22–24]. ERK waves can also be triggered by apoptotic cells in a variety of epithelial cells [25] or in the fly pupal notum [26] (Figure 1b). Here, ERK waves locally induce a transient survival fate in the cells neighboring the apoptotic lesion, ensuring that these cells remain alive until the lesion has been repaired. This mechanism scales to the intensity of the environmental insults that induce apoptosis and ultimately contributes to epithelial homeostasis by maintaining epithelial barrier function. Apoptosis-triggered waves also regulate enterocyte differentiation during tissue patterning in human colon monolayers [27]. Similar ERK waves can also provide the forces for extrusion of oncogenic cells from an epithelium [28]. ERK waves spatially control lumen formation in developing mammary acini [29]. Here, ERK waves dynamically position two spatial domains of high and low ERK pulse frequencies that respectively control survival (high ERK pulse frequency at the acinus periphery) and apoptosis leading to lumen formation (low ERK pulse frequency in the inner part of the acinus). In mature acini, apoptosis-triggered ERK waves then mediate homeostasis.

ERK waves are also prevalent in *in vivo* systems. During fish-scale regeneration, ERK waves can last multiple days and involve hundreds of cells [30] (Figure 1b). In Drosophila, ERK waves control invagination of the tracheal placode [31]. In the mouse, ERK waves regulate collective motility-controlling cochlear duct development [32]. In Planaria, a biochemical approach suggests the existence of ultrafast ERK waves that propagate along longitudinal muscles in response to wounding [33].

The reports mentioned earlier, which have emerged in the last 8 years, suggest that ERK waves are a ubiquitous dynamic signaling motif prevalent throughout animal life. Surprisingly, ERK waves can function at a wide variety of time and length scales. ERK waves in the fly pupal notum only extend one row from the apoptotic lesion [26]. In marked contrast, ERK waves propagate for about 2 days during fish-scale regeneration [30]. Propagation speed also shows a wide range of values, from 10 μm/h in the regenerating scale in zebrafish [30] to 1 mm/h ultrafast ERK waves that propagate in longitudinal muscles during wound response in Planaria [33]. These differences reflect different mechanisms of propagation that will be discussed in the following.

The processes potentially controlled by ERK waves can considerably vary in their time scales as well. ERK waves can control cytoskeletal dynamics that feed in the regulation of collective motility on timescale of minutes [23]. In contrast, fate decisions regulated by ERK dynamics range from timescales of few tens of minutes, such as during Drosophila development [34], to multiple hours, such as in the regulation of survival and cell-

cycle progression in adult mammalian cells [6,25]. We propose that ERK waves allow coordination of collective motility with proliferation and survival fates at different temporal scales, which might be advantageous during, for example, wound healing. Such coordination of multiple functions has been observed in developing mammary acini, in which the transition from high to low ERK pulse frequencies allows a shift from rapid motility and proliferation to slower motility and quiescence [29].

Mechanisms of ERK wave formation

Most of the observed ERK waves are trigger waves that emerge through mechanochemical feedback, rather than sensing a gradient of GFs. On the one hand, the MAPK network exhibits high sensitivity to mechanical stimuli, such as stretch, shear stress, substrate stiffness, or protrusive activity [35,36]. Conversely, ERK also controls cell mechanics by regulating myosin contraction through phosphorylation of myosin light chain [37]. ERK waves in epithelial wound healing [23], apoptosis-mediated homeostasis [25], acinar morphogenesis [29], and extrusion of oncogenic cells [28] all seem to involve a conserved mechanochemical feedback loop. Here, ERK-mediated activation of myosin contractility in one cell activates matrix metalloproteinases (MMPs) that leads to cleavage of pro-EGF ligands, then activation of epidermal growth factor receptor (EGFR) and production of a new ERK pulse in the adjacent cell. ERK-mediated activation of myosin contractility in this cell will then stretch the next cell, leading to a repetitive relay system, producing the ERK wave [24] (Figure 1c). Additional mechanosensing mechanisms feeding into the ERK wave are reviewed here [38]. Different epithelial cell systems display waves of different magnitudes, most likely reflecting different strengths of the mechanical linkage between cells [25]. This phenomenon can be captured in a mathematical model in which different biochemical and mechanical parameter spaces explain how ERK waves of different sizes can emerge [39]. Knockout of individual EGFR ligands only leads to subtle ERK wave defects, suggesting that ERK waves are propagated through an EGFR ligand mixture [40]. Further adding to the complexity of this system, the hepatocyte growth factor (HGF)—receptor MET—wires the MAPK network to produce sustained ERK activity and lamellipodial extension in wound-edge leader cells [41]. Note that crosstalk of mechanochemical feedback loops with the MAPK network does not only necessarily produce ERK waves but can also link curvature sensing to control mechanical forces leading to repetitive patterning during lung branching morphogenesis [42].

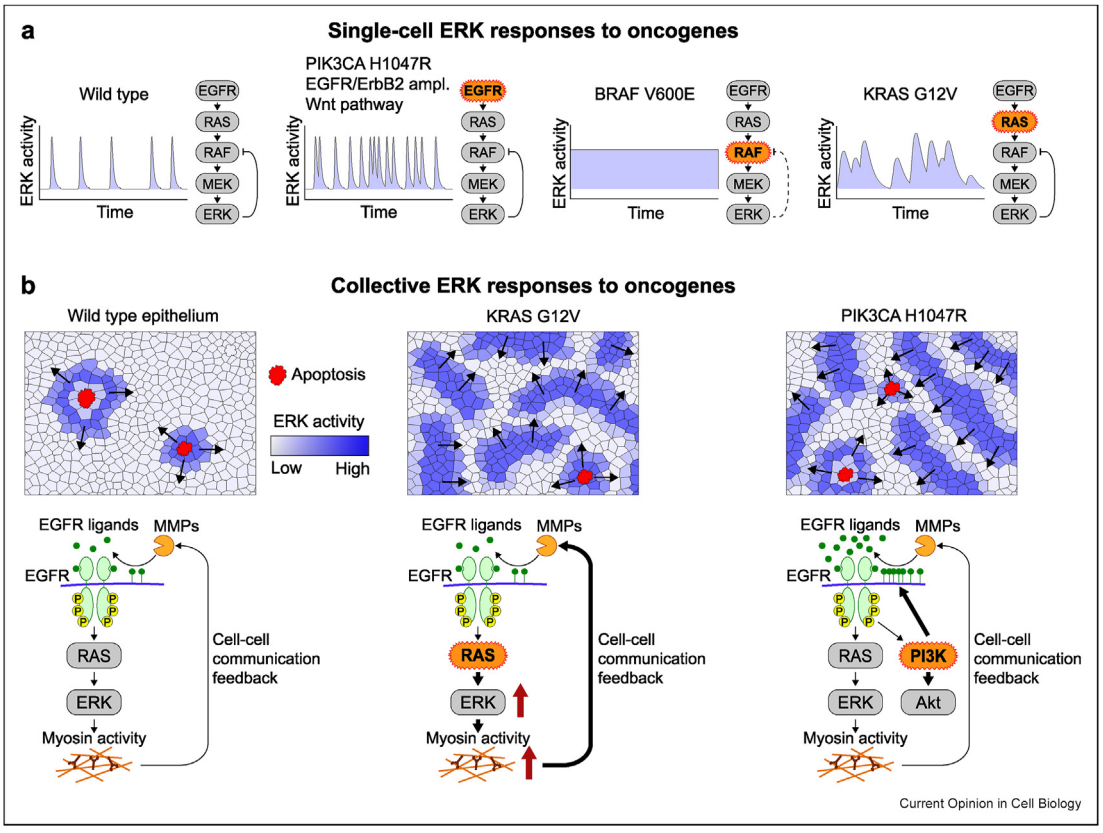
The long-lasting ERK waves involved in fish-scale regeneration take advantage of a different excitable system in which the negative feedback is provided by ERK-dependent expression of negative regulators such as dual specificity phosphatases (DUSPs), explaining their slower kinetics [30]. Key new insights from these findings are that ERK waves can operate at a variety of time and length scales, which results both from different MAPK-network feedback structures, and emergent properties of collective behavior. In epithelial ERK waves, the finding of reciprocal coupling of the MMPs/pro-EGF/EGFR/MAPK system with ERK-dependent mechanical feedback blurs the classic idea of causal hierarchy in which the EGFR is the master regulator of the system, often depicted as feed-forward signaling network. The findings that different EGFR ligands and HGF fine tune different spatial processes in the epithelial cell collectives provide new insight about the function of these GFs that was not available using classic biochemical methods.

Consequence of oncogenic mutations on single-cell and collective ERK dynamics

The prevalence of oncogenic mutations or aberrant expression of components of the MAPK network or pathways that crosstalk with it begs the question on how dysregulation impacts on single-cell or collective ERK dynamics. With respect to single-cell responses, overexpression of EGFR [28] or ErbB2 [8] receptors augments ERK pulse frequency in MCF10A cells (Figure 2a). This results from an increased RTK input on the MAPK network with intact feedback loops. The same effect is observed in response to activation of pathways that crosstalk with the MAPK pathway, such as aberrant Wnt activation [43], or a PIK3CA H1047R mutation that activates PI3K/Akt signaling [29] (Figure 2a). In both cases, this involves EGFR activation, explaining the increase in ERK pulse frequency. In marked contrast, mutations within the core of the MAPK network lead distinct ERK dynamics by rewiring feedback loops. BRAF V600E leads to sustained ERK dynamics due to insensitivity of mutated BRAF to the ERK-RAF negative-feedback loop [28] (Figure 2a). In contrast, KRAS G12V [44] leads to wider, noisy ERK activity pulses most likely because mutated KRAS strongly activates the RAF—MEK—ERK tripartite structure with an intact negative-feedback loop from ERK to RAF (Figure 2a).

Aberrant oncogenic signaling can also feed into emergent properties regulating collective ERK dynamics. Recently, Gagliardi et al. developed ARCOS, a computational tool for automatic recognition of collective signaling events, allowing for quantification of ERK waves in response to KRAS G12V and PIK3CA H1047R mutations. Beyond the cell autonomous effects described earlier, both mutations increased the size and frequency of ERK waves in MCF10A cells [44] and do not necessarily require initiation by apoptotic cells (Figure 2b). In the case of the KRAS G12V mutation, longer-lasting ERK pulses might lead to increased

Figure 2



Oncogenic mutations alter single-cell ERK dynamics and collective ERK waves.
(**a**) Different oncogenic alterations affect the pulsatile ERK dynamics of mammary epithelial cells. Mutations at the receptor level (EGFR or ErbB2 amplification) or those that feed to EGFR input (PIK3CA H1047R or Wnt) result in increased ERK pulse frequency. BRAF V600E bypasses the ERK-RAF negative-feedback loop, causing sustained ERK dynamics. KRAS G12V corrupts the dynamics of the pathway but keeps a pulsatile behavior, thanks to the intact ERK-RAF negative-feedback loop. (**b**) Oncogenes can also alter emergence of collective ERK activity patterns. While in WT mammary epithelium ERK waves are typically triggered by apoptosis, we observed the emergence of apoptosis-independent waves in the presence of KRAS G12V and PIK3CA H1047R mutations. We speculate that KRAS G12V induces more ERK waves via reinforced mechanochemical feedback loop. On the contrary, the PIK3CA H1047R mutation determines increased release of EGFR ligands, which makes the system more prone to form ERK waves. Abbreviations: ERK = extracellular signal-regulated kinase; EGFR = epidermal growth factor receptor; WT = wild type.

myosin contractility, augmenting the MMPs/EGF-ligands/EGFR/ERK mechanochemical feedback loop. In the case of PIK3CA H1047R cells, increased expression of the EGFR-ligand amphiregulin [45] potentiates the excitability of the EGFR receptor. Thus, at least, part of the aberrant ERK output results from an emergent property that depends on cell interactions in the epithelial collective (Figure 2b).

Having access to a system-level view of the different scales at which MAPK signaling functions informs about potential nontrivial "weak" nodes that can be pharmacologically targeted to best switch-off oncogenic signaling for each respective mutation. This is not accessible using the classical population-average biochemical experimental paradigm. In the case of an ErbB2 driven system, coinhibition of an ERK-RSK2-SOS feedback that leads to loss of network robustness drastically reduces residual single-cell signaling than when RAF, MEK, or ERK nodes are targeted individually [8]. In the case of the PIK3CA H1047R mutation that "hacks" EGFR signaling to increase the size of the collective ERK waves, inhibition of EGFR or MMPs might be synergistic with PI3K inhibition. Targeting MAPK network properties or emergent properties feeding into collective behavior might therefore realize the potential of personalized cancer medicine using combinatorial targeted therapy.

Conclusions and future perspectives

Studying ERK dynamics with single-cell resolution has clearly augmented our understanding of how the MAPK network is wired to produce different ERK outputs that control a wide variety of fates, as well as how emergent properties allow single cells to coordinate different fates that occur at different timescales in a tissue (e.g.

motility versus proliferation and survival). We anticipate that evolving technologies to measure and manipulate the MAPK network at relevant time and length scales in cells, organoids, and tissues will allow us to further characterize the rich set of dynamic behaviors we have observed so far. Having access to this knowledge will allow us to target new nontrivial properties of the MAPK network to realize the potential of personalized medicine in cancer and other pathologies such as Rasopathies.

Funding

This work was supported by Swiss National Science Foundation grants 310030_185376 and 310030_215581 to O. Pertz.

CRediT authorship contribution statement

Gagliardi PA: Writing—Original Draft, Writing—Review and Editing, Visualization; Pertz O: Writing—Original Draft, Writing—Review and Editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgement

We thank Dr. Maciej Dobrzyński for critically reading the manuscript. We apologize to all the colleagues that we could not cite and that made significant contribution in understanding single-cell extracellular signal-regulated kinase signaling dynamics.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Marshall CJ: **Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation**. *Cell* 1995, **80**:179–185.

2. Santos SDM, Verveer PJ, Bastiaens PIH: **Growth factor-induced MAPK network topology shapes Erk response determining PC-12 cell fate**. *Nat Cell Biol* 2007, **9**:324–330.

3. Nakamura A, Goto Y, Kondo Y, Aoki K: **Shedding light on developmental ERK signaling with genetically encoded biosensors**. *Dev Camb Engl* 2021, **148**, dev199767.

4. Ryu H, Chung M, Dobrzyński M, Fey D, Blum Y, Lee SS, Peter M, Kholodenko BN, Jeon NL, Pertz O: **Frequency modulation of ERK activation dynamics rewires cell fate**. *Mol Syst Biol* 2015, **11**:838.

5. Blum Y, Mikelson J, Dobrzyński M, Ryu H, Jacques M, Jeon NL, Khammash M, Pertz O: **Temporal perturbation of ERK dynamics reveals network architecture of FGF2/MAPK signaling**. *Mol Syst Biol* 2019, **15**, e8947.

6. Albeck JG, Mills GB, Brugge JS: **Frequency-modulated pulses of ERK activity transmit quantitative proliferation signals**. *Mol Cell* 2013, **49**:249–261.

7. ** Raina D, Fabris F, Morelli LG, Schröter C: **Intermittent ERK oscillations downstream of FGF in mouse embryonic stem cells**. *Development* 2022, **149**, dev199710.
Here, the authors identify single-cell intermittent ERK activity oscillations in mouse embryonic stem cells. This suggests that in this cell type, ERK dynamics is at the interface between pulsatile and oscillatory behavior.

8. ** Dessauges C, Mikelson J, Dobrzyński M, Jacques M, Frismantiene A, Gagliardi PA, Khammash M, Pertz O: **Optogenetic actuator – ERK biosensor circuits identify MAPK network nodes that shape ERK dynamics**. *Mol Syst Biol* 2022, **18**.
This paper combines optoGFR, an optogenetic actuator for MAPK pathway, with an ERK biosensor to explore the nodes in the MAPK network that are important for single-cell ERK signaling dynamics. It shows that the ERK substrate p90RSK2 constitutes a crucial feedback loop that is necessary for ERK activity oscillations. p90RSK2 silencing or inhibition reduces the robustness of the MAPK pathways, and synergizes with MAPK pathway inhibition.

9. Aoki K, Kumagai Y, Sakurai A, Komatsu N, Fujita Y, Shionyu C, Matsuda M: **Stochastic ERK activation induced by noise and cell-to-cell propagation regulates cell density-dependent proliferation**. *Mol Cell* 2013, **52**:529–540.

10. Regot S, Hughey JJ, Bajar BT, Carrasco S, Covert MW: **High-sensitivity measurements of multiple kinase activities in live single cells**. *Cell* 2014, **157**:1724–1734.

11. De La Cova C, Townley R, Regot S, Greenwald I: **A real-time biosensor for ERK activity reveals signaling dynamics during C. elegans cell fate specification**. *Dev Cell* 2017, **42**:542–553.e4.

12. Hiratsuka T, Bordeu I, Pruessner G, Watt FM: **Regulation of ERK basal and pulsatile activity control proliferation and exit from the stem cell compartment in mammalian epidermis**. *Proc Natl Acad Sci USA* 2020, **117**:17796–17807.

13. Jacques M, Dobrzyński M, Gagliardi PA, Sznitman R, Pertz O: **CODEX, a neural network approach to explore signaling dynamics landscapes**. *Mol Syst Biol* 2021, **17**, e10026.

14. * Simsek MF, Chandel AS, Saparov D, Zinani OQH, Clason N, Özbudak EM: **Periodic inhibition of Erk activity drives sequential somite segmentation**. *Nature* 2023, **613**:153–159.
This article shows that during somitogenesis the Her1–Her7 oscillator periodically lowers ERK activity. The ERK oscillations then drive somite segmentation. This article demonstrates that oscillations of ERK dynamics can drive repetitive pattern formation, such as somitogenesis.

15. Shankaran H, Ippolito DL, Chrisler WB, Resat H, Bollinger N, Opresko LK, Wiley HS: **Rapid and sustained nuclear–cytoplasmic ERK oscillations induced by epidermal growth factor**. *Mol Syst Biol* 2009, **5**:332.

16. * Wilcockson SG, Guglielmi L, Araguas Rodriguez P, Amoyel M, Hill CS: **An improved Erk biosensor detects oscillatory Erk dynamics driven by mitotic erasure during early development**. *Dev Cell* 2023, **58**:2802–2818.e5.
In this article, the authors report the development of an improved ERK-KTR biosensor to be more specific for ERK kinase activity, and to be insensitive to Cdk1 kinase activity. They tested it in zebrafish and Drosophila embryos. They identify a period before mitosis when ERK activity shuts down.

17. Ram A, Murphy D, DeCuzzi N, Patankar M, Hu J, Pargett M, Albeck JG: **A guide to ERK dynamics, part 1: mechanisms and models**. *Biochem J* 2023, **480**:1887–1907.

18. Huang CY, Ferrell JE: **Ultrasensitivity in the mitogen-activated protein kinase cascade**. *Proc Natl Acad Sci USA* 1996, **93**:10078–10083.

19. Kochańczyk M, Kocieniewski P, Kozłowska E, Jaruszewicz-Błońska J, Sparta B, Pargett M, Albeck JG, Hlavacek WS, Lipniacki T: **Relaxation oscillations and hierarchy of feedbacks in MAPK signaling**. *Sci Rep* 2017, **7**, 38244.

20. Ram A, Murphy D, DeCuzzi N, Patankar M, Hu J, Pargett M, Albeck JG: **A guide to ERK dynamics, part 2: downstream decoding**. *Biochem J* 2023, **480**:1909–1928.

21. Hiratsuka T, Fujita Y, Naoki H, Aoki K, Kamioka Y, Matsuda M: **Intercellular propagation of extracellular signal-regulated kinase activation revealed by in vivo imaging of mouse skin**. *eLife* 2015, **4**, e05178.

22. Matsubayashi Y, Ebisuya M, Honjoh S, Nishida E: **ERK activation propagates in epithelial cell sheets and regulates their migration during wound healing**. *Curr Biol* 2004, **14**:731–735.

23. Aoki K, Kondo Y, Naoki H, Hiratsuka T, Itoh RE, Matsuda M: **Propagating wave of ERK activation orients collective cell migration**. *Dev Cell* 2017, **43**:305–317.e5.

24. Hino N, Rossetti L, Marín-Llauradó A, Aoki K, Trepat X, Matsuda M, Hirashima T: **ERK-mediated mechanochemical waves direct collective cell polarization**. *Dev Cell* 2020, **53**: 646–660.e8.

25. Gagliardi PA, Dobrzyński M, Jacques M-A, Dessauges C, Ender P, Blum Y, Hughes RM, Cohen AR, Pertz O: **Collective ERK/Akt activity waves orchestrate epithelial homeostasis by driving apoptosis-induced survival**. *Dev Cell* 2021, **56**: 1712–1726.e6.

26. Valon L, Davidović A, Levillayer F, Villars A, Chouly M, Cerqueira-Campos F, Levayer R: **Robustness of epithelial sealing is an emerging property of local ERK feedback driven by cell elimination**. *Dev Cell* 2021, **56**:1700–1711.e8.

27. ** Pond KW, Morris JM, Alkhimenok O, Varghese RP, Cabel CR, Ellis NA, Chakrabarti J, Zavros Y, Merchant JL, Thorne CA, *et al.*: **Live-cell imaging in human colonic monolayers reveals ERK waves limit the stem cell compartment to maintain epithelial homeostasis**. *eLife* 2022, **11**, e78837.

This article shows that waves of ERK activity are triggered by apoptotic cells in a primary culture of intestinal epithelium. These waves propagate for multiple rows of neighbors. Interestingly, the propagation of these waves corresponds to tissue patterning. Their propagation is confined to the adult enterocytes' compartment. On the contrary, the region that is not reached by the waves is the crypt with stem cells.

28. Aikin TJ, Peterson AF, Pokrass MJ, Clark HR, Regot S: **MAPK activity dynamics regulate non-cell autonomous effects of oncogene expression**. *eLife* 2020, **9**, e60541.

29. ** Ender P, Gagliardi PA, Dobrzyński M, Frismantiene A, Dessauges C, Höhener T, Jacques M-A, Cohen AR, Pertz O: **Spatiotemporal control of ERK pulse frequency coordinates fate decisions during mammary acinar morphogenesis**. *Dev Cell* 2022, **57**:2153–2167.e6.

This paper reports the observation of single-cell ERK signaling dynamics during mammary acini morphogenesis in vitro. Each stage of the morphogenetic program shows specific ERK dynamic patterns, ranging from high ERK pulse frequency in the initial proliferating stage, to the lower frequency in stage 2–4. Interesting is the observation that from stage 2, ERK activity occurs in waves. These events are important to determine the survival of outer cells and death of inner cells, to form a lumen. An oncogenic mutation in PI3K alters ERK dynamics and, consequently, the morphogenesis. This article shows how signaling waves can coordinate a complex 3D morphogenetic program.

30. De Simone A, Evanitsky MN, Hayden L, Cox BD, Wang J, Tornini VA, Ou J, Chao A, Poss KD, Di Talia S: **Control of osteoblast regeneration by a train of Erk activity waves**. *Nature* 2021, **590**:129–133.

31. Ogura Y, Wen F-L, Sami MM, Shibata T, Hayashi S: **A switch-like activation relay of EGFR-ERK signaling regulates a wave of cellular contractility for epithelial invagination**. *Dev Cell* 2018, **46**:162–172.e5.

32. Ishii M, Tateya T, Matsuda M, Hirashima T: **Retrograde ERK activation waves drive base-to-apex multicellular flow in murine cochlear duct morphogenesis**. *eLife* 2021, **10**, e61092.

33. Fan Y, Chai C, Li P, Zou X, Ferrell JE, Wang B: **Ultrafast distant wound response is essential for whole-body regeneration**. *Cell* 2023, https://doi.org/10.1016/j.cell.2023.06.019.

34. Lim B, Dsilva CJ, Levario TJ, Lu H, Schüpbach T, Kevrekidis IG, Shvartsman SY: **Dynamics of inductive ERK signaling in the Drosophila embryo**. *Curr Biol* 2015, **25**:1784–1790.

35. Hirata H, Gupta M, Vedula SRK, Lim CT, Ladoux B, Sokabe M: **Actomyosin bundles serve as a tension sensor and a platform for ERK activation**. *EMBO Rep* 2015, **16**:250–257.

36. Yang J-M, Bhattacharya S, West-Foyle H, Hung C-F, Wu T-C, Iglesias PA, Huang C-H: **Integrating chemical and mechanical signals through dynamic coupling between cellular protrusions and pulsed ERK activation**. *Nat Commun* 2018, **9**:4673.

37. Klemke RL, Cai S, Giannini AL, Gallagher PJ, Lanerolle PD, Cheresh DA: **Regulation of cell motility by mitogen-activated protein kinase**. *J Cell Biol* 1997, **137**:481–492.

38. Hirashima T, Hino N, Aoki K, Matsuda M: **Stretching the limits of extracellular signal-related kinase (ERK) signaling — cell mechanosensing to ERK activation**. *Curr Opin Cell Biol* 2023, **84**, 102217.

39. * Boocock D, Hirashima T, Hannezo E: **Interplay between mechanochemical patterning and glassy dynamics in cellular monolayers**. *PRX Life* 2023, **1**, 013001.

This article describes computational modeling of the emergence of mechanochemical waves of ERK. By modulating different parameters, such as the active migration forces and the mechanochemical coupling, they observe a transition from a uniform active glass to periodic spatiotemporal waves.

40. * Lin S, Hirayama D, Maryu G, Matsuda K, Hino N, Deguchi E, Aoki K, Iwamoto R, Terai K, Matsuda M: **Redundant roles of EGFR ligands in the ERK activation waves during collective cell migration**. *Life Sci Alliance* 2022, **5**, e202101206.

This article aims at solving the puzzle of which EGFR ligand mediates the propagation of ERK waves. The authors found that the propagation of ERK waves is significantly, albeit not completely, suppressed only when EGF, HBEGF, TGFα and EREG are simultaneously removed. The waves can be however restored by the re-expression of only one of them. This suggests a redundant role for EGFR ligands in ERK wave propagation.

41. ** Hino N, Matsuda K, Jikko Y, Maryu G, Sakai K, Imamura R, Tsukiji S, Aoki K, Terai K, Hirashima T, *et al.*: **A feedback loop between lamellipodial extension and HGF-ERK signaling specifies leader cells during collective cell migration**. *Dev Cell* 2022, **57**:2290–2304.e7.

This paper shows that during wound repair in kidney epithelial cells in vitro, the leader cells, at the edge of the wound, have a specific ERK signaling dynamic profile. These cells show sustained ERK activity due to HGF-MET signaling that in turn activates ERK. This is in marked contrast with the pulsatile ERK activity in the follower cells that is necessary for ERK wave propagation.

42. ** Hirashima T, Matsuda M: **ERK-mediated curvature feedback regulates branching morphogenesis in lung epithelial tissue**. *Curr Biol* 2024, https://doi.org/10.1016/j.cub.2023.12.049.

This articles shows that ERK activity plays a role during branching morphogenesis of lung epithelium. ERK activity is observed to be higher in the curved region of epithelial tissues, due to FGF1 internalization. There, ERK activity determines an increase of actin polymerization, that reduces the curvature. This generates a mechanochemical feedback loop that forms the branching points.

43. Muta Y, Fujita Y, Sumiyama K, Sakurai A, Taketo MM, Chiba T, Seno H, Aoki K, Matsuda M, Imajo M: **Composite regulation of ERK activity dynamics underlying tumour-specific traits in the intestine**. *Nat Commun* 2018, **9**:2174.

44. ** Gagliardi PA, Grädel B, Jacques M-A, Hinderling L, Ender P, Cohen AR, Kastberger G, Pertz O, Dobrzyński M: **Automatic detection of spatio-temporal signaling patterns in cell collectives**. *J Cell Biol* 2023, https://doi.org/10.1083/jcb.202207048.

This article describes a computational method to automatically identify collective signaling events of ERK activity, in multiple different contexts. This method is validated against a large variety of possible ERK waves of different shape and size, some spontaneously occurring in epithelial systems, others engineered with optogenetics, or produced via computational simulations. The article also describes how ERK waves are altered by oncogenic mutations such as KRAS G12V and PIK3CA H1047R.

45. Young CD, Zimmerman LJ, Hoshino D, Formisano L, Hanker AB, Gatza ML, Morrison MM, Moore PD, Whitwell CA, Dave B, *et al.*: **Activating PIK3CA mutations induce an epidermal growth factor receptor (EGFR)/Extracellular signal-regulated kinase (ERK) paracrine signaling Axis in basal-like breast cancer***. *Mol Cell Proteomics* 2015, **14**:1959–1976.

46. Saha M, Carriere A, Cheerathodi M, Zhang X, Lavoie G, Rush J, Roux PP, Ballif BA: **RSK phosphorylates SOS1 creating 14-3-3-docking sites and negatively regulating MAPK activation**. *Biochem J* 2012, **447**:159–166.