Signalling dynamics, cell decisions, and homeostatic control in health and disease

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Abstract

Cell signalling engenders cells with the capability to receive and process information from the intracellular and extracellular environments, trigger and execute biological responses, and communicate with each other. Ultimately, cell signalling is responsible for maintaining homeostasis at the cellular, tissue and systemic level. For this reason, cell signalling is a topic of intense research efforts aimed to elucidate how cells coordinate transitions between states in developing and adult organisms in physiological and pathological conditions. Here, we review current knowledge of how cell signalling operates at multiple spatial and temporal scales, focusing on how single-cell analytical techniques reveal mechanisms underpinning cell-to-cell variability, signalling plasticity, and collective cellular responses.

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Introduction

In 1854, physiologist Claud Bernard postulated that the self-regulation of their internal environment is a precondition for the existence of complex systems such as the human body. Sixty years later, Walter B. Cannon defines this fundamental feature of living organisms as *homeostasis* [1]. Despite its etymology (*i.e.*, keeping similar), homeostasis is a highly dynamic process during which only a few internal physicochemical properties are maintained within a narrow range, that is, they are homeostatically controlled. In complex organisms, homeostatic control is critical at the system, tissue, and cellular levels. For example, the brain, lungs, kidneys, and red blood cells contribute to pH regulation in plasma; adult stem cells regenerate tissues to maintain their integrity; cells maintain the basal concentrations of Ca^{2+}, Na^+, and K^+, within narrow ranges.

Homeostatic control relies on biochemical networks we refer to as *cell signalling*, which engender cells the capability to sense physicochemical cues, process information and execute the most appropriate biological responses (Figure 1a). Here, we highlight recent conceptual and methodological advancements that are helping us better understand molecular mechanisms underpinning homeostatic control. Moreover, we discuss how disease often stems from the corruption of cell signalling and the consequent deregulation of homeostasis.

Signal reception

Cells sense many physicochemical cues with a diversified complement of signalling machinery. Figure 1a—b depicts ERBB receptor tyrosine kinases (RTK) as an instructive example for receptor-mediated signalling [2,3]. The ERBB receptor family comprises four transmembrane proteins ERBB1-4 that bind more than ten ligands with varying specificities (Figure 1b) [4]. Binding favours ligand-dependent homo- and heterodimerisation of the receptors, autophosphorylation of their cytoplasmic domains and engagement of effector proteins. Arguably, receptors do not just convert a molecular event (binding) to a biochemical signal that will be further relayed by signal transduction pathways. Different ERBB dimers exhibit both varying affinities for downstream effectors and different regulatory sites. Therefore, ERBB dimers establish distinct positive and negative feedbacks with signal transduction networks [5—7]. These interactions eventually result both in the spatial propagation of the signal [8,9] and into characteristic temporal dynamics that encode the identity, amplitude, and duration of the originating stimulus.

Cell signalling is also initiated intra-cellularly and without receptors, for example, in the DNA damage response [10]. There are several types of DNA lesions, each requiring specific repair mechanisms. For instance, double-strand breaks are detected by two protein complexes, namely, Ku (XRCC5 and XRCC6) and the MRN

Figure 1
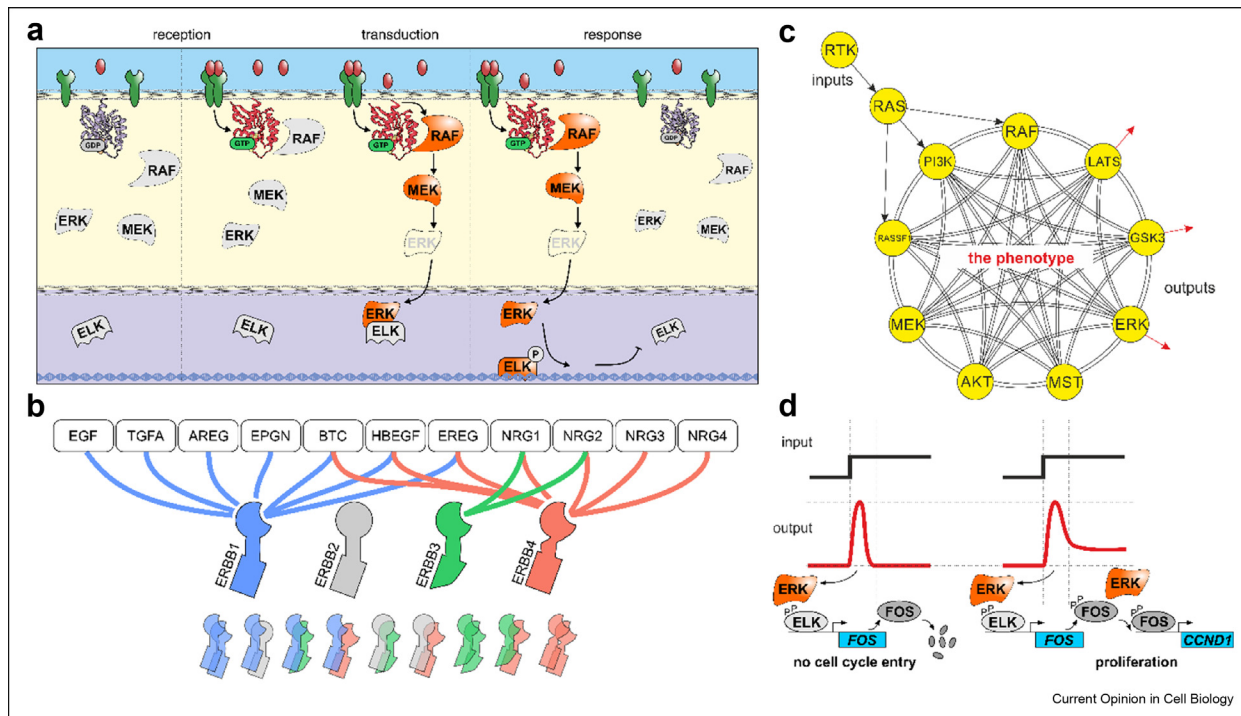
Cell *signalling. a)* Diagrammatic representation of signal reception, transduction, and response, exemplified by ERBB receptor tyrosine kinases and MAPK signalling. Ligand binding triggers receptor dimerisation at the plasma membrane, leading to their cytoplasmic domain phosphorylation and the recruitment of signalling complexes (not shown). ERBB activation then facilitates the exchange of GDP for GTP in small GTPases (e.g. RAS proteins), inducing the recruitment of effector kinases. Here, we depict one of the several pathways that amplify and relay the initiating signal to the nucleus, the MAPK pathway (RAF→MEK→ERK). In the nucleus, ERK phosphorylates ELK leading to transcription of target genes, biological response, and termination of the originating signal. *b)* Diagrammatic representation of ERBB ligand reception. ERBB1 (also known as EGFR) binds the epidermal growth factor (EGF), transforming growth factor alpha (TGFA), amphiregulin (AREG), heparin-binding EGF-like growth factor (HBEGF), betacellulin (BTC), epigen (EPGN) and epiregulin (EREG). ERBB2 has no ligand but can heterodimerise with other ERBB receptors. ERBB3 has very low kinase activity on its own but forms active dimers upon binding to neuregulins (NRG1/2). ERBB4 binds to several ligands of ERBB1/3, and NRG3/4. *c)* Once a molecular event (ligand binding) is transduced to a biochemical signal (RTK phosphorylation), a network of networks (here we depict MAPK, PI3K, and hippo pathways) further process the biochemical signal in space and time to trigger the most appropriate biological response. *d)* Signalling dynamics favour the activation of specific transcriptional programs depending on the stability of target transcripts and proteins. For example, ELK induce the transcription of FOS that is rapidly degraded. However, if ERK activation is sustained, ERK phosphorylate also FOS, leading to proliferation through Cyclin D1 (CCDN1) expression [14].

complex (MRE11, RAD50, and NBS1 also known as NBN); single-strand breaks are sensed by RPA proteins, TOPBP1 and ATRIP. Ku, MRN, and RPA/ATRIP recruit to the site of damage the three kinases DNA-PKcs (PRKDC), ATM and ATR, respectively. Active DNA-PKcs, ATM, and ATR then coordinate specific repair processes (*e.g.*, non-homologous end-joining, homologous recombination and Fanconi anaemia repair pathways, respectively). At the same time, DNA-PKcs, ATM, and ATR amplify the signal initiated by an individual DNA lesion both at the site of damage and towards other cell compartments leading to cell cycle arrest, or cell death in the presence of unresolved damage.

ERBB and DNA damage signalling are two very different examples of signal reception amongst a large variety of diverse signalling mechanisms. Both examples, however, illustrate how initiating events usually trigger the formation or activation of multi-molecular signalling platforms. These structures, nowadays more frequently but not universally referred to as signalosomes [11−13], initiate signal transduction from sensors to downstream effector pathways, from sites of reception to different cellular locations, and integrate other modulatory inputs and feedbacks from downstream networks. Signal reception already embeds signal processing characteristics that are often poorly understood but critical in shaping downstream cellular responses.

Signalling pathways and signalling dynamics

The realisation that spatiotemporal dynamics plays a fundamental role in cell signalling can be tracked back to the early discovery of the action potential in 1843 by

Emil du Bois-Reymond [15]. Nerve conduction is indeed a specialised form of cell signalling. Neurons integrate numerous signals and encode information both in the amplitude of an action potential travelling along the axon and the frequency of a neuron's firing, that is, in the temporal dynamics. With the advent of the modern biochemistry of the 20th century, the importance of spatiotemporal dynamics in all tissues and at all scales became increasingly apparent, for example to explain morphogens [16] and cell cycle control [17]. ERBB signalling also exhibits distinctive spatiotemporal dynamics.

Differences in negative feedbacks in ERBB1—ERBB1 and ERBB3—ERBB4 heterodimers, for example, trigger transient or sustained activation of the mitogen-activated protein kinase (MAPK) pathway in response to EGF and NRG1, respectively (Figure 1a, c) [18]. Furthermore, cross-talk between signalling pathways permits cells to integrate different signals. For example, when stimulated with EGF, pheochromocytoma rat cells (PC-12) exhibit the characteristic transient response in extracellular regulated kinases (ERK1/2, also known as MAPK1/3) activity [19]. Instead, the nerve growth factor (NGF) binds the neurotrophic receptor tyrosine kinase 1 (NTRK1, also known as TrkA) and triggers sustained ERK activity. Differences in signalling dynamics also depend on the promiscuity of receptors with several signalling pathways and their cross-talk [20—24]. Santos et al. [21], for example, have reconstructed topological maps of MAPK signalling (see Figures 1c and 3a), demonstrating that either through direct or indirect interactions, different stimuli reshape network topologies encoding for distinct signalling dynamics, eventually resulting in different responses such as proliferation or differentiation of PC-12 cells in response to EGF and NGF, respectively.

Figure 2

Current Opinion in Cell Biology
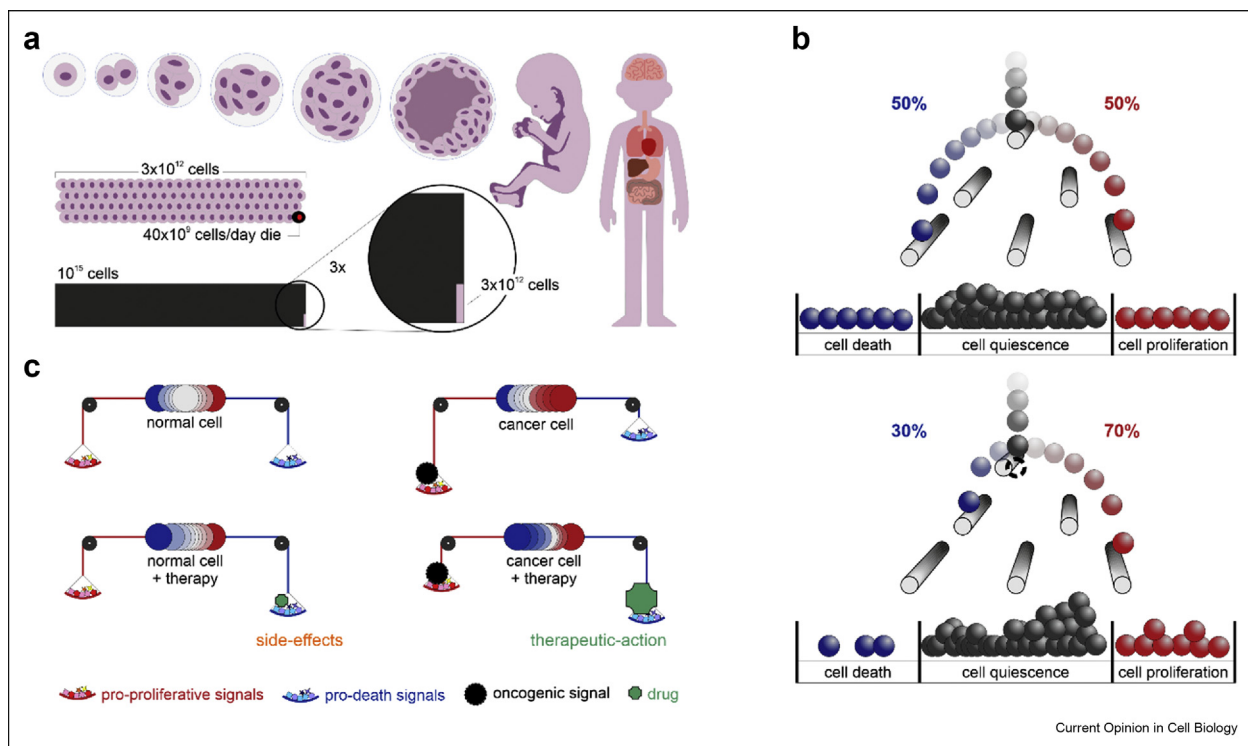
Cell decisions and homeostasis. a) *Cellular decisions are essential during development and in adult tissues. Even excluding the hematopoietic system with its very high turnover, of the 3 trillion cells making up an adult human body, up to 40 billion die each day* [40]. *Turnover of cells varies significantly across tissues (being lowest in the brain and the highest in the gut and hematopoietic system); however, by the end of our lifespan, a human body might have replenished 1000 times more cells than the number of cells it has at any given time.* **b)** *Cell fates exhibit stochastic characteristics, here described by a Galton board. Cells are depicted by marbles falling onto pins (biochemical events). Each step is deterministic, but marbles eventually fall into a 'cell fate' box randomly following distributions determined by the geometrical configuration of the pins. Most cells are quiescent within a tissue, and an equal number of cells die and are born, on average, to maintain tissue homeostasis. Molecular cues favour one or the other cell fate, inducing tissue regeneration or leading to aberrant homeostasis.* **c)** *Genetically identical cells of the same type can thus exhibit vast cell-to-cell variability of non-genetic origin. Biochemical signals are then shifting the balance in the cellular population (in the analogy of the Galton board, the pins are moved), favouring homeostasis in healthy tissues or causing cell fate imbalances in disease (e.g. excess proliferation in cancer). Therapies aim to restore homeostasis in target tissue avoiding homeostatic disruption in others.*

Figure 3
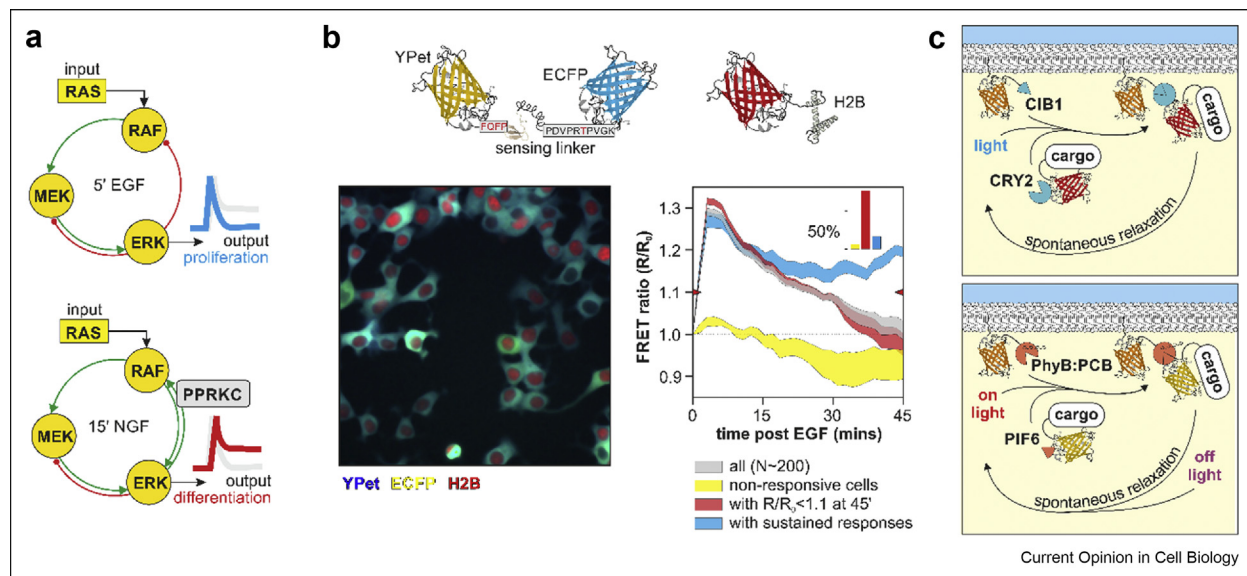
Studying signalling dynamics *in space and time.* **a)** *Diagrammatic representation of MAPK network topology as determined by Santos et al. [21]. In response to EGF and NGF, ERK exhibit transient or sustained activation. Different dynamics are determined by distinct feedback mechanisms enacted by growth factor receptors, and result in different cell decisions (proliferation versus differentiation). However, each cell might respond with a different dynamic to the same stimulus. Panel **b)** shows MCF7 cells stably expressing the EKAREV ERK FRET-based sensor and a nuclear marker treated with 100 ng/ml EGF. The large majority of cells respond with the transient activation of ERK (red), similar to the average response (grey). However, ~10% of cells either do not respond (yellow) or respond with sustained ERK signalling (blue). The non-responders and the sustained responders were classified as those cells that either do not cross the 1.1 FRET ratio threshold (red marks on the ordinate), or cells that raise above the marker initially but do not return below 1.1. Methods can be found in De et al. [27]* **c)** *Diagrammatic representation of optogenetic tools utilised to control cell signalling.*

The temporal evolution of a biochemical signal can take many different shapes, including periodic oscillations. For example, upon induction of double-strand breaks by γ-irradiation, several cell types exhibit periodic pulses of TP53 and DNA damage checkpoint kinase activities (*e.g.*, ATM and CHK1/2); however, single-strand breaks resulting from the repair of damage caused by UV radiation trigger a sustained response [10,25,26]. Pulsatile or oscillatory dynamics are triggered by the opposing effects of positive and negative feedbacks. For instance, the E3 ubiquitin ligase MDM2 is a transcriptional target of TP53 that induces its rapid degradation through the ubiquitin—proteasome system. Upon DNA damage triggering recruitment and activation of ATM, ATM phosphorylates and activates CHK kinases. ATM and CHK kinases then phosphorylate and stabilise TP53, which induces its negative regulator MDM2. Depending on the balance between stimuli and negative feedback, TP53 exhibits oscillatory dynamics that are backpropagated to ATM via the activity of WIP1 phosphatase. We have recently shown that these oscillations can also propagate to the MAPK pathway; additionally, MAPK signalling attenuates TP53 pulses when stimulated by NRG1 but not EGF because of the different ERK dynamics they trigger [27].

Therefore, a model of cell signalling where individual pathways are better understood as constituent parts of a 'network of networks' that process information and embed in their spatiotemporal dynamics the 'code' that regulates the transition between cellular states — or phenotypes (Figure 1c) is now emerging [28—30].

Cellular response
Intense research efforts spanning cell biochemistry, computational biology and biophotonics are revealing how signalling dynamics regulates transitions between cellular states. The different stability of gene transcripts and proteins is a key molecular aspect for interpreting cell signalling. For example, the mRNA of immediate early genes (IEGs), *i.e.*, genes that do not require the *de novo* expression of accessory proteins for their transcription, can accumulate seconds or minutes after a triggering signal. Stable IEG transcripts accumulate over time and can induce expression of proteins even after a transient or pulsatile signal; instead, IEGs that are transcribed in mRNAs of short half-life require a sustained transcriptional activation to induce the expression of target proteins at biologically significant concentrations [31—34].

Interestingly, under the effects of a prolonged stimulus, both short- and long-lived IEG transcripts are expressed. However, while the less stable mRNAs rapidly reach a steady-state concentration, long-lived IEGs can accumulate over time, thus encoding the duration of the

originating stimulus in the amplitude of the mRNA response, [31]. The interplay between temporal patterns of signalling molecules and transcriptional machinery provides a robust and specific mechanism to induce different cellular responses (*e.g.*, survival, arrest, cell death, and differentiation).

Protein stabilities also play a fundamental role in decoding signalling dynamics into biological responses. For example (Figure 1d), ERK can induce the expression of the immediate early gene *FOS* by phosphorylating the transcriptional activator ELK [14]. FOS is a transcription factor that is rapidly degraded once expressed. However, when phosphorylated by ERK, FOS is stabilised and initiates the transcription of target genes such as cyclin D1 (*CCND1*). This and similar mechanisms permit cells to engage in the cell cycle only in the presence of non-spurious mitogenic cues, such as constant or properly-timed pulses of growth factors. Moreover, protein stabilisation can endow cells with the memory of past events also across generations, such as the occurrence of strongly mitogenic environments [14,35,36].

These mechanisms are not restricted to ERBB and mitogenic signalling. For example, Lahav et al.[25] have shown that TP53 oscillations maintain cells in a reversible cell cycle arrest conducive to DNA damage repair, while prolonged TP53 expression results in cell death. Both the dynamics of TP53 expression and that of target genes depends on a delicate balance of mRNA and protein stabilities. For example, while TP53 is a memoryless oscillator that promptly responds to DNA damage kinases such as ATM, its transcriptional target CDKN1A integrates TP53 dynamics over longer periods, maintaining a cell cycle arrest or eventually resulting in cell death [37].

Similar mechanisms are reported for several signalling pathways and transcriptional programs [26,38,39], suggesting that time-encoding of signals and transcriptional programs is a fundamental principle underpinning biological responses.

Homeostasis, heterogeneity, and disease

In response to a stimulus, cell signalling thus co-ordinates molecular machinery (*e.g.*, ERBB-dependent cytoskeletal rearrangements, or DNA damage repair) with the transition between cellular states to maintain cell homeostasis and function. Similarly, by integrating cell-autonomous and non-cell-autonomous mechanisms, signalling determines cellular decisions that regulate the development of multi-cellular organisms and the homeostasis of adult tissues (Figure 2a). We can describe each step of these mechanisms deterministically; however, it is increasingly evident that the behaviour of cells within a population can be better described with stochastic models [41]. For example, the homeostasis of skin and the oesophageal epithelium is maintained by stem cells that, when proliferating, exhibit a given probability to either differentiate or self-renew. This probability is fine-tuned to ensure tissue renewal [42—44]. However, cell signalling corruption through cell-autonomous mechanisms or cell-to-cell communication can shift this balance, leading to diseases such as cancer (Figure 2b,c).

Therefore, a better understanding of these processes is critical to elucidate pathogenicity mechanisms and improve disease management. A notable example is provided by the difficulty in explaining the distribution of mutations that often occurs within signalling pathways that drive carcinogenesis in different tissues [45—47]. Even a given oncogenic driver (*e.g.,* KRAS) can exhibit distinct mutational patterns or gene amplifications that remain difficult to explain by tissue-dependent mutagenesis [48,49]. We can hypothesise that mutations result both in quantitative and qualitative alterations of cell signalling. Gene truncations, deletions, and inactivating mutations can remove essential nodes of biochemical networks; gene amplifications and activating mutations can drive excessive signalling. However, we now appreciate how these alterations might also exert more subtle effects on cell signalling, still corrupting signalling dynamics and drastically disrupting homeostatic control [23,30]. For example, similar mutations (*e.g.*, non-synonymous KRAS G12 mutations) result in similar yet quantitatively different signals [50,51]. Specific alterations might bring oncogenic signalling within a narrow range (the 'sweet spot') that confers clones with a fitness advantage in specific permissive tissues [51—53]. This sweet spot is likely to vary across tissues, resulting in tissue-dependent mutational landscapes of the same protein (*e.g.*, KRAS) or proteins related to the same pathways (*e.g.*, other RAS isoforms, ERBB signalling, and RAS-dependent pathways).

In the first instance, a deeper understanding of cell signalling could lead to improved strategies for patient stratification (*e.g.*, a specific mutation in a specific tissue might require a specific therapeutic approach). Moreover, therapeutic interventions do not always eradicate all cancer cells, resulting in disease relapsing often with resistant or more aggressive tumours. Genetic heterogeneity of cancer, particularly in advanced disease, is undoubtedly a major contributor to therapeutic resistance. However, we are increasingly recognising how the plasticity of signalling pathways and cell-to-cell heterogeneity of non-genetic origin contribute significantly to limiting the efficacy of clinical interventions [54,55]. Cell signalling can indeed induce transitions to cellular states refractory to therapy, either before or in response to treatment [32,56—58]. Moreover, current pharmacological interventions aim to alter the activity of specific nodes in biochemical networks. However, feedback mechanisms (*e.g.*, reviewed in the study by Roesch et al. [32]) within the target pathway or other related

signalling networks often compensate for the loss of signalling induced by inhibitors.

Arguably, the elucidation of mechanisms underpinning the development of an organism, tissue homeostasis, pathogenesis, and management of disease strongly depends on our capability to understand cell signalling and cell-to-cell variability.

Perspectives and conclusions

Since the advent of modern cell biochemistry, the scientific community has made immense progress in understanding cell signalling and cellular decisions. However, traditional tools for cell biochemistry offered limited spatial and temporal resolution, for example, through cell fractionation and snapshot detection of biochemical activities at different times with immunoblotting.

We have illustrated how cell signalling is a dynamic process that evolves in space and time at different scales [59], from the fast dynamics of molecular interactions, occurring in seconds and tens of minutes required by immediate response genes, and minutes to days required by biological responses; from nanometer-scale conformational changes of signalling proteins, the propagation of signals between cellular compartments to macroscopic cell-to-cell communication mediated by paracrine and endocrine mechanisms.

For these reasons, fluorescence microscopy has been an invaluable tool to deepen our understanding of cell signalling as it provides low invasiveness, high spatiotemporal and biochemical resolutions, and high specificity, complementing other techniques. Nowadays, we have an ever-growing palette of biosensors (Figure 3b) at our disposal that we can use to probe the activity of biochemical pathways (e.g., ERK, PI3K, AMPK), second messengers (e.g., cAMP, calcium concentration), and metabolic networks (e.g., ATP, lactate, pyruvate concentrations) in single living cells and tissues [60−62]. The use of biosensors is somewhat established and gradually reaching an increasing number of non-specialist laboratories. Integrating biochemical imaging with microfluidics also facilitates the study of cell signalling with real-time control of both stimuli and responses [63−68].

Notably, optogenetics (Figure 3c) − the capability to control biochemical reactions by light − is proving to be extremely powerful in probing biochemical networks [69−72]. For example, Wilson et al. [34] have demonstrated the dynamic and combinatorial control of genes by ERK using OptoSOS to stimulate RAS signalling. OptoSOS is a fusion of light-inducible heterodimers (Figure 3c) that facilitate the translocation of SOS as a cargo protein to the plasma membrane. The guanine nucleotide exchange factor SOS then activates RAS proteins. At the same time, the authors monitored the activation of ERK by the nuclear translocation of a fluorescently tagged ERK; the transcription of target genes using an engineered sequence within the nascent RNA that induces the localisation in sites of transcription of a second fluorescent protein; and the expression of the target protein tagged with a third genetically encoded fluorophore. Other innovations in fluorescence microscopy, such as high-content imaging, multiplexed biochemical imaging are also laying the foundation for systems level understanding of cell decisions [34,55,73−78].

Moreover, research in the study of cell-to-cell communication and how cell signalling regulates the collective behaviour of cells is intensifying [79−81]. For example, with the use of a light-inducible RAF kinase and a FRET-based sensor for ERK activity (see also Figure 3), Aoki et al. [81] characterised waves of ERK signalling that depend on ADAM17 travelling opposite to the direction of collective movement of cells. Using genetically engineered cells that express the inducible oncogene BRAF^{V600E} and translocation-based biosensors, Aikin et al. [82] have demonstrated a switch in autonomous signalling dynamics of mutant cells, that trigger a paracrine ADAM17-dependent wave of ERK signalling in its neighbourhood leading BRAF^{WT} cells to migrate towards the mutant cell. These efforts have to be matched by computational tools to aid the analysis of imaging data permitting us to monitor biochemical activities and cell fates in populations of living and interacting cells [83−94]. Further methodological innovations might be necessary, for example, in computational biology to integrate data at multiple scales and to model the emerging properties of cell populations.

Cell signalling can be described as a network of networks operating across cellular compartments and between cells. With constantly improving technologies, such as genomic editing, biochemical imaging, single-cell sequencing, in vitro organ-like cultures, and machine learning, we might soon be able to reveal how spatiotemporal dynamics of interconnected biochemical networks in a tissue cooperate to maintain both cellular and tissue homeostasis, how cell signalling might change during ageing, and how disease often stems from the corruption of cell signalling.

Conflict of interest statement

Nothing declared

References

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

- of special interest
- of outstanding interest

1. Holmes FL: **Claude bernard, the" milieu intérieur", and regulatory physiology**. *Hist Philos Life Sci* 1986.

2. McCabe Pryor M, Steinkamp MP, Halasz AM, Chen Y, Yang S, Smith MS, Zahoransky-Kohalmi G, Swift M, Xu X-P, Hanien D, *et al.*: **Orchestration of ErbB3 signaling through hetero-interactions and homointeractions**. *Mol Biol Cell* 2015, **26**: 4109−4123.

3. Citri A, Yarden Y: **EGF–ERBB signalling: towards the systems level**. *Nat Rev Mol Cell Biol* 2006, **7**:505−516.

4. Wieduwilt MJ, Moasser M: **The epidermal growth factor receptor family: biology driving targeted therapeutics**. *Cell Mol Life Sci* 2008, **65**:1566−1584.

5. Birtwistle MR, Hatakeyama M, Yumoto N, Ogunnaike BA, Hoek JB, Kholodenko BN: **Ligand-dependent responses of the ErbB signaling network: experimental and modeling analyses**. *Mol Syst Biol* 2007, **3**:144.

6. Maik-Rachline G, Hacohen-Lev-Ran A, Seger R: **Nuclear ERK: mechanism of translocation, substrates, and role in cancer**. *Int J Mol Sci* 2019, **20**:1194.

7. 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**:1−13.

8. Verveer PJ, Wouters FS, Reynolds AR, Bastiaens PIH: **Quantitative imaging of lateral ErbB1 receptor signal propagation in the plasma membrane**. *Science* 2000, **290**:1567−1570 (80).

9. Chen RH, Sarnecki C, Blenis J: **Nuclear localization and regulation of erk-and rsk-encoded protein kinases**. *Mol Cell Biol* 1992, **12**:915−927.

10. Batchelor E, Loewer A, Mock C, Lahav G: **Stimulus-dependent dynamics of p53 in single cells**. *Mol Syst Biol* 2011, **7**:1−8.

11. Mysore VP, Zhou Z-W, Ambrogio C, Li L, Kapp JN, Lu C, Wang Q,
• Tucker MR, Okoro JJ, Nagy-Davidescu G, *et al.*: **A structural model of a Ras-Raf signalosome**. *Nat Struct Mol Biol* 2021, **28**: 847−857.
RAS proteins slocalise at the plasma membrane in *nanoclusters* that enable signal amplification. Here, the authors use cryo-EM, molecular dynamics simulations and FRET to build a model for the RAS-RAF signalosome. This study highlights the unique superstructural features of RAS complexes providing insight of complex nanomachines dedicated to signal processing.

12. DeBruine ZJ, Xu HE, Melcher K: **Assembly and architecture of the Wnt β-catenin signalosome at the membrane**. *Br J Pharmacol* 2017, **174**:4564−4574.

13. Escors D, Gato-Cañas M, Zuazo M, Arasanz H, García-Granda MJ, Vera R, Kochan G: **The intracellular signalosome of PD-L1 in cancer cells**. *Signal Transduct Target Ther* 2018, **3**: 1−9.

14. Sharrocks AD: **Cell cycle: sustained ERK signalling represses the inhibitors**. *Curr Biol* 2006, **16**:R540−R542.

15. Du Bois-Reymond EH: *Vorläufiger Abriss einer Untersuchung über den sogenannten Froschstrom und über die elektromotorischen Fische*. 1843.

16. Turing AM: **The chemical basis of morphogenesis**. *Philos Trans R Soc Lond B Biol Sci* 1952, **237**:37−72.

17. Evans T, Rosenthal ET, Youngblom J, Distel D, Hunt T: **Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division**. *Cell* 1983, **33**:389−396.

18. Kholodenko BN, Demin OV, Moehren G, Hoek JB: **Quantification of short term signaling by the epidermal growth factor receptor**. *J Biol Chem* 1999, **274**:30169−30181.

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

20. Brightman FA, Fell DA: **Differential feedback regulation of the MAPK cascade underlies the quantitative differences in EGF and NGF signalling in PC12 cells**. *FEBS Lett* 2000, **482**: 169−174.

21. 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.

22. Cohen-Saidon C, Cohen AA, Sigal A, Liron Y, Alon U: **Dynamics and variability of ERK2 response to EGF in individual living cells**. *Mol Cell* 2009, **36**:885−893.

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

24. Ryu H, Chung M, Dobrzyński M, Fey D, Blum Y, Sik Lee S, Peter M, Kholodenko BN, Li Jeon N, Pertz O: **Frequency modulation of ERK activation dynamics rewires cell fate**. *Mol Syst Biol* 2015, **12**:866.

25. Loewer A, Lahav G: **p53 dynamics control cell fate**. *Science* 2012, **336**:13−16 (80-).

26. Purvis JE, Lahav G: **Encoding and decoding cellular information through signaling dynamics**. *Cell* 2013, **152**:945−956.

27. De S, Campbell C, Venkitaraman AR, Esposito A: **Pulsatile MAPK signaling modulates p53 activity to control cell fate decisions at the G2 checkpoint for DNA damage**. *Cell Rep* 2020, **30**:2083−2093.

28. Hormoz S: **Cross talk and interference enhance information capacity of a signaling pathway**. *Biophys J* 2013, **104**: 1170−1180.

29. Makadia HK, Schwaber JS, Vadigepalli R: **Intracellular information processing through encoding and decoding of dynamic signaling features**. *PLoS Comput Biol* 2015, **11**.

30. Madsen RR, Vanhaesebroeck B: **Cracking the context-specific PI3K signaling code**. *Sci Signal* 2020, **13**:1−13.

31. Uhlitz F, Sieber A, Wyler E, Fritsche-Guenther R, Meisig J, Landthaler M, Klinger B, Blüthgen N: **An immediate−late gene expression module decodes ERK signal duration**. *Mol Syst Biol* 2017, **13**:928.

32. Roesch A: **Tumor heterogeneity and plasticity as elusive drivers for resistance to MAPK pathway inhibition in melanoma**. *Oncogene* 2015, **34**:2951−2957.

33. Jena SG, Yu C, Toettcher JE: **Dynamics and heterogeneity of Erk-induced immediate-early gene expression**. *bioRxiv* 2021.

34. Wilson MZ, Ravindran PT, Lim WA, Toettcher JE, Wilson MZ, Ravindran PT, Lim WA, Toettcher JE: **Tracing information flow from erk to target gene induction reveals mechanisms of dynamic and combinatorial control**. *Mol Cell* 2017, **67**: 757−769.

35. Min M, Rong Y, Tian C, Spencer SL: **Temporal integration of**
•• **mitogen history in mother cells controls proliferation of daughter cells**. *Science* 2020, **368**:1261−1265 (80-).
Cells are believed to sense mitogenic signals in the G1 phase of the cell cycle. With the use of high-throughput live-cell imaging, the authors show that contrarily to the canonical model, cells integrate mitogenic signals throughout the cell cycle. Moreover, the stability of proteins, such as cyclin D, modulated by MAPK signalling, constitutes cellular memory for past mitogenic signalling. Interestingly, this memory is inheritable and influences cell cycle decisions also in daughter cells.

36. Zwang Y, Sas-Chen A, Drier Y, Shay T, Avraham R, Lauriola M, Shema E, Lidor-Nili E, Jacob-Hirsch J, Amariglio N, *et al.*: **Two phases of mitogenic signaling unveil roles for p53 and EGR1 in elimination of inconsistent growth signals**. *Mol Cell* 2011, **42**:524−535.

37. Loewer A, Batchelor E, Gaglia G, Lahav G: **Basal dynamics of p53 reveal transcriptionally attenuated pulses in cycling cells**. *Cell* 2010, **142**:89−100.

38. Gillies TE, Pargett M, Minguet M, Davies AE, Albeck JG: **Linear integration of ERK activity predominates over persistence detection in fra-1 regulation**. *Cell Syst* 2017, **5**:549–563. e5.

39. Nandagopal N, Santat LA, LeBon L, Sprinzak D, Bronner ME, Elowitz MB: **Dynamic ligand discrimination in the notch signaling pathway**. *Cell* 2018, **172**:869–880.

40. Sender R, Milo R: **The distribution of cellular turnover in the human body**. *Nat Med* 2021, **27**:45–48.

41. Iwamoto K, Shindo Y, Takahashi K: **Modeling cellular noise underlying heterogeneous cell responses in the epidermal growth factor signaling pathway**. *PLoS Comput Biol* 2016, **12**, e1005222.

42. Alcolea MP, Greulich P, Wabik A, Frede J, Simons BD, Jones PH: **Differentiation imbalance in single oesophageal progenitor cells causes clonal immortalization and field change**. *Nat Cell Biol* 2014, **16**:612–619.

43. Frede J, Greulich P, Nagy T, Simons BD, Jones PH: **A single dividing cell population with imbalanced fate drives oesophageal tumour growth**. *Nat Cell Biol* 2016, **18**:967–978.

44. Doupé DP, Perrimon N: **Visualizing and manipulating temporal signaling dynamics with fluorescence-based tools**. *Sci Signal* 2014, **7**:re1.

45. Risques RA, Kennedy SR: **Aging and the rise of somatic cancer-associated mutations in normal tissues**. *PLoS Genet* 2018, **14**, e1007108.

46. Martincorena I, Fowler JC, Wabik A, Lawson ARJ, Abascal F, Hall MWJ, Cagan A, Murai K, Mahbubani K, Stratton MR, et al.: **Somatic mutant clones colonize the human esophagus with age**. *Science* 2018, **362**:911–917 (80-).

47. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, et al.: **Mutational landscape and significance across 12 major cancer types**. *Nature* 2013, **502**:333–339.

48. Muñoz-Maldonado C, Zimmer Y, Medová M: **A comparative analysis of individual ras mutations in cancer biology**. *Front Oncol* 2019, **9**.

49. Ostrow SL, Simon E, Prinz E, Bick T, Shentzer T, Nagawkar SS, Sabo E, Ben-Izhak O, Hershberg R, Hershkovitz D: **Variation in KRAS driver substitution distributions between tumor types is determined by both mutation and natural selection**. *Sci Rep* 2016, **6**:21927.

50. Zafra MP, Parsons MJ, Kim J, Alonso-Curbelo D, Goswami S, Schatoff EM, Han T, Katti A, Fernandez MTC, Wilkinson JE, et al.: **An in vivo kras allelic series reveals distinct phenotypes of common oncogenic variants**. *Cancer Discov* 2020, **10**:1654–1671.

51. M.Haigis K: **KRAS alleles: the devil is in the detail**. *Trends in Cancer* 2017, **3**:686–697.

52. Haigis KM, Cichowski K, Elledge SJ: **Tissue-specificity in cancer: the rule, not the exception**. *Science* 2019, **363**:1150–1152 (80-).

53. Li S, Balmain A, Counter CM: **A model for RAS mutation patterns in cancers: finding the sweet spot**. *Nat Rev Cancer* 2018, **18**:767–777.

54. Spencer SL, Gaudet S, Albeck JG, Burke JM, Sorger PK: **Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis**. *Nature* 2009, **459**:428–432.

55. Yang C, Tian C, Hoffman TE, Jacobsen NK, Spencer SL: **Mela-
•• noma subpopulations that rapidly escape MAPK pathway inhibition incur DNA damage and rely on stress signalling**. *Nat Commun* 2021, **12**:1–14.
A role for cell-to-cell variability of non-genetic origin in therapeutic resistance is disputed. By combining single-cell RNA sequencing, and live single-cell imaging, the authors demonstrate that melanoma cells harbouring a BRAFV600E treated with MAPK inhibitors are capable to periodically reactivate the MAPK pathway. This mechanism, dependent on stress response pathways, permits cells to escape treatment. Escapees are prone to DNA damage but - being capable to cycle - outgrow non-escapees mediating the generation of pools of genetically diverse clones that can fix genetically, drug resistance. The authors thus suggest that the use of inhibitors targeting mTORC1 and ATF4 - or other mechanisms of non-genetic adaptation to drug treatment - could reduce the probability of recurrence of disease after treatment.

56. Kreso A, O'Brien CA, van Galen P, Gan OI, Notta F, Brown AMK, Ng K, Ma J, Wienholds E, Dunant C, et al.: **Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer**. *Science* 2013, **339**:543–548 (80-).

57. Huang S: **Non-genetic heterogeneity of cells in development: more than just noise**. *Development* 2009, **136**:3853–3862.

58. Paek AL, Liu JC, Loewer A, Forrester WC, Lahav G: **Cell-to-Cell variation in p53 dynamics leads to fractional killing**. *Cell* 2016, **165**:631–642.

59. Kholodenko BN, Hancock JF, Kolch W: **Signalling ballet in space and time**. *Nat Rev Mol Cell Biol* 2010, **11**:414–426.

60. Ponsioen B, Post JB, Buissant des Amorie JR, Laskaris D, van
•• Ineveld RL, Kersten S, Bertotti A, Sassi F, Sipieter F, Cappe B, et al.: **Quantifying single-cell ERK dynamics in colorectal cancer organoids reveals EGFR as an amplifier of oncogenic MAPK pathway signalling**. *Nat Cell Biol* 2021, **23**:377–390.
The authors demonstrate the fundamental role of EGFR in amplifying signalling from downstream oncogenic mutant drivers such as KRAS, NRAS and BRAF. This study highlights how signaling 'cascades' are in fact highly plastic and non-linear resulting in powerful mechanisms of drug resistance. The authors also showcase the vast potential for combining patient-derived organoid cultures with live single-cell biochemical imaging enabled by FRET sensors.

61. Gelles JD, Mohammed JN, Santos LC, Gelles J: *Real-time integration of cell death and proliferation kinetics at the single-cell and population-level using high-throughput live-cell imaging*. 2018. *Available SSRN 3261823*.

62. Greenwald EC, Mehta S, Zhang J: **Genetically encoded fluorescent biosensors illuminate the spatiotemporal regulation of signaling networks**. *Chem Rev* 2018, **118**:11707–11794.

63. Ryu H, Chung M, Song J, Sik Lee S, Pertz O, Li Jeon N: **Integrated platform for monitoring single-cell MAPK kinetics in computer-controlled temporal stimulations**. *Sci Rep* 2018, **8**: 1–7.

64. Tweedy L, Thomason PA, Paschke PI, Martin K, Machesky LM,
• Zagnoni M, Insall RH: **Seeing around corners: cells solve mazes and respond at a distance using attractant breakdown**. *Science* 2020, **369**:1–10 (80-).
This study demonstrates a unique mechanism for cellular information processes. While chemotaxis is a well-known cellular process, with the use of microfluidic mazes, the authors show that cells can self-generate gradients of diffusible molecules that support interactions with neighbouring cells and their local environment. The authors show cells migrate over short distances thanks to chemoattractant gradients. Cells acquire the capability to sense environmental properties over a long range and migrate towards the exit of microfluidic mazes.

65. Wei W, Zhang M, Xu Z, Li W, Cheng L, Cao H, Ma M, Chen Z: **A microfluidic array device for single cell capture and intracellular Ca2+ response analysis induced by dynamic biochemical stimulus**. *Biosci Rep* 2021:41.

66. Heijden M Van Der, Miedema DM, Waclaw B, Veenstra VL, Lecca MC, Nijman LE, Dijk E Van, Neerven SM Van, Lodestijn SC, Lenos KJ, et al.: **High-resolution dose—response screening using droplet-based microfluidics**. *Proc Natl Acad Sci U S A* 2012, **109**:378–383.

67. Blum Y, Mikelson J, Dobrzyński M, Ryu H, Jacques M-A,
•• Jeon NL, Khammash M, Pertz O: **Temporal perturbation of Erk dynamics reveals network architecture of FGF2-MAPK signaling**. *Mol Syst Biol* 2019, **15**:1–17.
Using a microfluidic device and a FRET-based sensor for ERK activity, the authors show that PC-12 cells treated with fibroblast growth factor responds with vast heterogeneity in ERK signalling dynamics. This study demonstrates that genetically identical cells can exhibit variability in feedback mechanisms resulting in cell-to-cell heterogeneity of non-genetic origin in signalling dynamics.

68. Sonnen KF, Lauschke VM, Uraji J, Falk HJ, Petersen Y, Funk MC, Beaupeux M, François P, Merten CA, Aulehla A: **Modulation of phase shift between Wnt and Notch signaling oscillations controls mesoderm segmentation**. *Cell* 2018, **172**:1079–1090.

69. Singh AP, Wu P, Ryabichko S, Raimundo J, Swan M, Wieschaus E, Gregor T, Toettcher JE: **Optogenetic control of the Bicoid morphogen reveals fast and slow modes of gap gene regulation**. *bioRxiv* 2021.

70. Dessauges C, Mikelson J, Dobrzyński M, Jacques M-A, Frismantiene A, Gagliardi PA, Khammash M, Pertz O: **Opto-genetic actuator/biosensor circuits for large-scale interrogation of ERK dynamics identify sources of MAPK signaling robustness**. *bioRxiv* 2021.

71. Farahani PE, Reed EH, Underhill EJ, Aoki K, Toettcher JE: **Signaling, deconstructed: using optogenetics to dissect and direct information flow in biological systems**. *Annu Rev Biomed Eng* 2021:23.

72. Duplus-Bottin H, Spichty M, Triqueneaux G, Place C, Mangeot PE, Ohlmann T, Vittoz F, Yvert G: **A single-chain and fast-responding light-inducible Cre recombinase as a novel optogenetic switch**. *Elife* 2021, **10**, e61268.

73. Pargett M, Albeck JG: **Live-cell imaging and analysis with multiple genetically encoded reporters**. *Curr Protoc Cell Biol* 2018, **78**:4.36.1–4.36.19.

74. Fries MW, Haas KT, Ber S, Saganty J, Richardson EK, Venkitaraman AR, Esposito A: **Multiplexed biochemical imaging reveals caspase activation patterns underlying single cell fate**. *bioRxiv* 2018, https://doi.org/10.1101/427237.

75. Haas KT, Fries MW, Venkitaraman AR, Esposito A: **Single-cell biochemical multiplexing by multidimensional phasor demixing and spectral fluorescence lifetime imaging microscopy**. *Front Physiol* 2021, https://doi.org/10.3389/fphy.2021.637123.

76. Trinh AL, Ber S, Howitt A, Valls PO, Fries MW, Venkitaraman AR, Esposito A: **Fast single-cell biochemistry: theory, open source microscopy and applications**. *Methods Appl Fluoresc* 2019, **7**: 44001.

77. Goglia AG, Wilson MZ, Jena SG, Silbert J, Basta LP,
• Devenport D, Toettcher JE: **A live-cell screen for altered erk dynamics reveals principles of proliferative control**. *Cell Syst* 2020, **10**:240–253.e6.
The authors of this study demonstrate the power of assays that combine live single-cell biochemical imaging and optogenetics with high-throughput screening of kinase inhibitors. With control and analysis of single-cell biochemistry at high temporal resolution, the authors could identify small molecule inhibitors that alter ERK signalling dynamics, including RTKs different from the canonical ERBB signalling.

78. Lin J-R, Fallahi-Sichani M, Sorger PK: **Highly multiplexed imaging of single cells using a high-throughput cyclic immunofluorescence method**. *Nat Commun* 2015, **6**:1–7.

79. Davies AE, Pargett M, Siebert S, Gillies TE, Choi Y, Tobin SJ,
•• Ram AR, Murthy V, Juliano C, Quon G, et al.: **Systems-level properties of EGFR-RAS-ERK signaling amplify local signals to generate dynamic gene expression heterogeneity**. *Cell Syst* 2020, **11**:161–175.e5.
Heterogeneity is often observed in cancer and its presence is commonly linked to malignant-type phenotypes. Here the authors study the effects of the microenvironment on signaling dynamics and how these result in heterogeneous gene expression. The latter is a consequence of paracrine communication between two different cell types and specifically relies on signaling amplification of paracrine signals and on gene-dependent kinetic filtering.

80. Aoki K: **Visualization of intracellular signaling with fluorescence resonance energy transfer-based biosensors**. *Protein Modif Pathog Dysregulation Signal* 2015, **4**:31–41.

81. 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.

82. Aikin TJ, Peterson AF, Pokrass MJ, Clark HR, Regot S: **MAPK**
•• **activity dynamics regulate non-cell autonomous effects of oncogene expression**. *Elife* 2020, **9**:1–24.
The authors demonstrate how oncogene-induced MAPK signaling dynamics exhibit significant cell-to-cell variability that correlates with specific phenotypes, for example, pulsatile ERK dynamics to proliferation, and sustained ERK activity to cell cycle arrest. The authors show that oncogenic-induced signalling can also propagate through a paracrine axis involving ADAM17-EGFR. This signal is elicited by sustained ERK activity that triggers a wave of ERK activity emanating from the mutant cell that results in the net migration of wild-type cells towards the mutant cell.

83. Fantham M, Kaminski CF: **A new online tool for visualization of volumetric data**. *Nat Photonics* 2017, **11**:69.

84. Berg S, Kutra D, Kroeger T, Straehle CN, Kausler BX, Haubold C, Schiegg M, Ales J, Beier T, Rudy M, et al.: **ilastik: interactive machine learning for (bio)image analysis**. *Nat Methods* 2019, **16**:1226–1232.

85. Dobrzyński M, Jacques M-A, Pertz O: **Mining single-cell time-**
• **series datasets with time course inspector**. *Bioinformatics* 2020, **36**:1968–1969.
This study presents an intuitive and feature-rich R-based tool for the analysis of single-cell time-series of kinase activity measurements. This tool also provides the capability to cluster heterogeneous signaling responses in sets of homogeneous classes to better investigate cell-to-cell variability in signalling dynamics.

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

87. Haubold C, Schiegg M, Kreshuk A, Berg S, Koethe U, Hamprecht FA: **Segmenting and tracking multiple dividing targets using ilastik**. In *Focus on bio-image informatics*. Edited by De Vos WH, Munck S, Timmermans J-P, Cham: Springer; 2016:199–229.

88. Schmidt U, Weigert M, Broaddus C, Myers G: **Cell detection with star-convex polygons**. In *International conference on medical image computing and computer-assisted intervention*; 2018: 265–273.

89. Weigert M, Schmidt U, Haase R, Sugawara K, Myers G: **Star-convex polyhedra for 3d object detection and segmentation in microscopy**. In *Proceedings of the IEEE/CVF winter conference on applications of computer vision*; 2020:3666–3673.

90. Stringer C, Wang T, Michaelos M, Pachitariu M: **Cellpose: a generalist algorithm for cellular segmentation**. *Nat Methods* 2021, **18**:100–106.

91. Hu T, Xu S, Wei L, Zhang X, Wang X: **CellTracker: an automated toolbox for single-cell segmentation and tracking of time-lapse microscopy images**. *Bioinformatics* 2021, **37**: 285–287.

92. Ulicna K, Vallardi G, Charras G, Lowe A: **Automated deep lineage tree analysis using a Bayesian single cell tracking approach**. *Front Comput Sci* 2021:3.

93. Napari contributors: **napari: a multi-dimensional image viewer for python**. *GitHub repository* 2019, https://doi.org/10.5281/zenodo.3555620.

94. Haase R, Royer LA, Steinbach P, Schmidt D, Dibrov A, Schmidt U, Weigert M, Maghelli N, Tomancak P, Jug F, et al.: **CLIJ: GPU-accelerated image processing for everyone**. *Nat Methods* 2020, **17**:5–6.