Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Systems Biology

Information-theoretic analyses of cellular strategies for achieving high signaling capacity—dynamics, cross-wiring, and heterogeneity of cellular states

Piotr Topolewski and Michał Komorowski

Abstract

An individual eukaryotic cell senses identity and quantity of ligands through molecular receptors and signaling pathways, dynamically activating signaling effectors. A distinct ligand often activates multiple different effectors, and a distinct effector is activated by numerous different ligands, which results in cross-wired signaling. In apparently identical cells, the activity of signaling effectors can vary considerably, raising questions about the accuracy of cellular signaling and the interpretation of heterogeneous responses, as either functional or simply noise. Cell-to-cell variability of signaling outcomes, signaling dynamics, and cross-wiring all give rise to signaling complexity, complicating the analysis of signaling mechanisms. Here, we consider a simple input–output modeling approach of information theory that is suitable to analyze signaling complexity and highlight recent studies that have advanced our understanding of the role different components of signaling complexity play in achieving effective information transfer along cellular signaling pathways.

Address

Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland

Corresponding authors: Topolewski, Piotr (p.topolewski@sysbiosig.org); Komorowski, Michał (m.komorowski@sysbiosig.org)

Current Opinion in Systems Biology 2021, **27**:100352

This review comes from a themed issue on **Theoretical approaches to analyze single-cell data**

Edited by **Olivier Gandrillon** and **Michael Stumpf**

For a complete overview see the Issue and the Editorial

Available online 12 June 2021

https://doi.org/10.1016/j.coisb.2021.06.003

Keywords

Signaling pathways, Hormones, Growth factors, or cytokines, Signaling dynamics, Cross-wired signaling, Shannon information, Fisher information.

Signaling accuracy

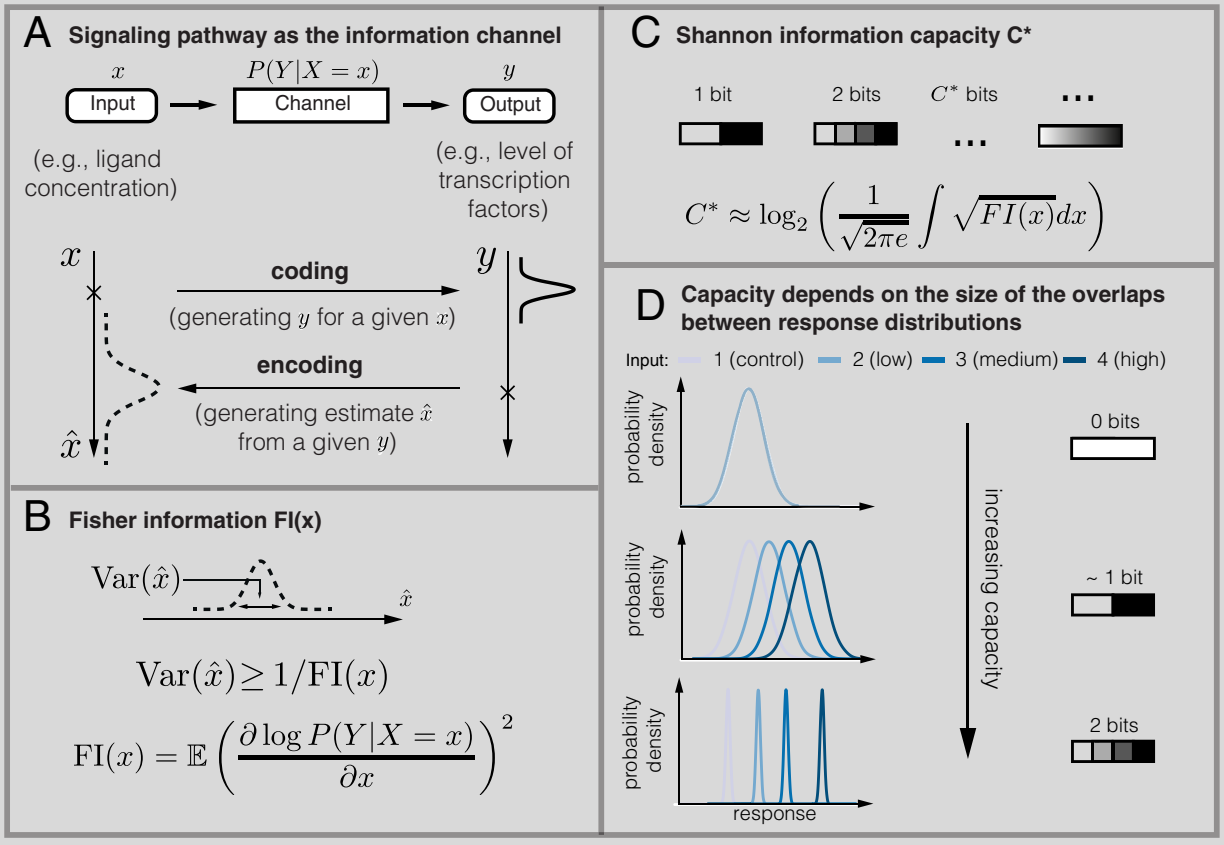
A number of factors limit the accuracy by which a cell senses ambient ligand concentrations. These include limited sensitivity to small concentration changes, the inherent stochasticity of biochemical reactions (noise), variability in copy number of signaling pathway components, and limitations in specificity of ligands, all of which may result in deterioration of the information transmitted along signaling pathways. In their seminal work, Berg and Purcell [1] established a theoretical limit of best-achievable sensing precision in the simplest instance of a single receptor. Their work indicated that signaling precision is inevitably limited by random ligand binding and unbinding events, which can be counteracted by increasing the receptor copy number and temporal averaging of random effects. It has inspired a number of questions related to experimental quantification of signaling accuracy and strategies of achieving sufficient precision to ensure reliable functioning of cellular mechanisms [2,3]. We have learned, for example, that different cellular systems may require different signaling accuracy for reliable functioning. Precise sensing of positional information is required for formation of the body plan during development [4–7] (see reviews [8–10]). On the other hand, nutrient sensing in bacterial populations can cope with less accurate signaling or potentially no signaling, that is, by different cells in the population randomly adapting strategies suitable for different states of available nutrients on a bet-hedging basis [11]. For some systems, including cytokines, hormones, or growth-factor signaling systems in mammalian cells (on which this review focuses), the level of signaling precision required for the effective function within a multicellular organism begins to be established. Here, we review an emerging line of signaling research based on information theory, a mathematical language for studying communication processes, which is addressing the mechanisms that ensure reliable signaling, and therefore is beginning to reveal the roles of different elements of signaling complexity, including variability of responses, signaling dynamics, cross-wired signaling architecture, and heterogeneity of cellular states.

Information formalism for cellular signaling

Within information theory, a signaling pathway can be interpreted as a communication device, which for a given stimulus level x (input), for example, ligand concent-

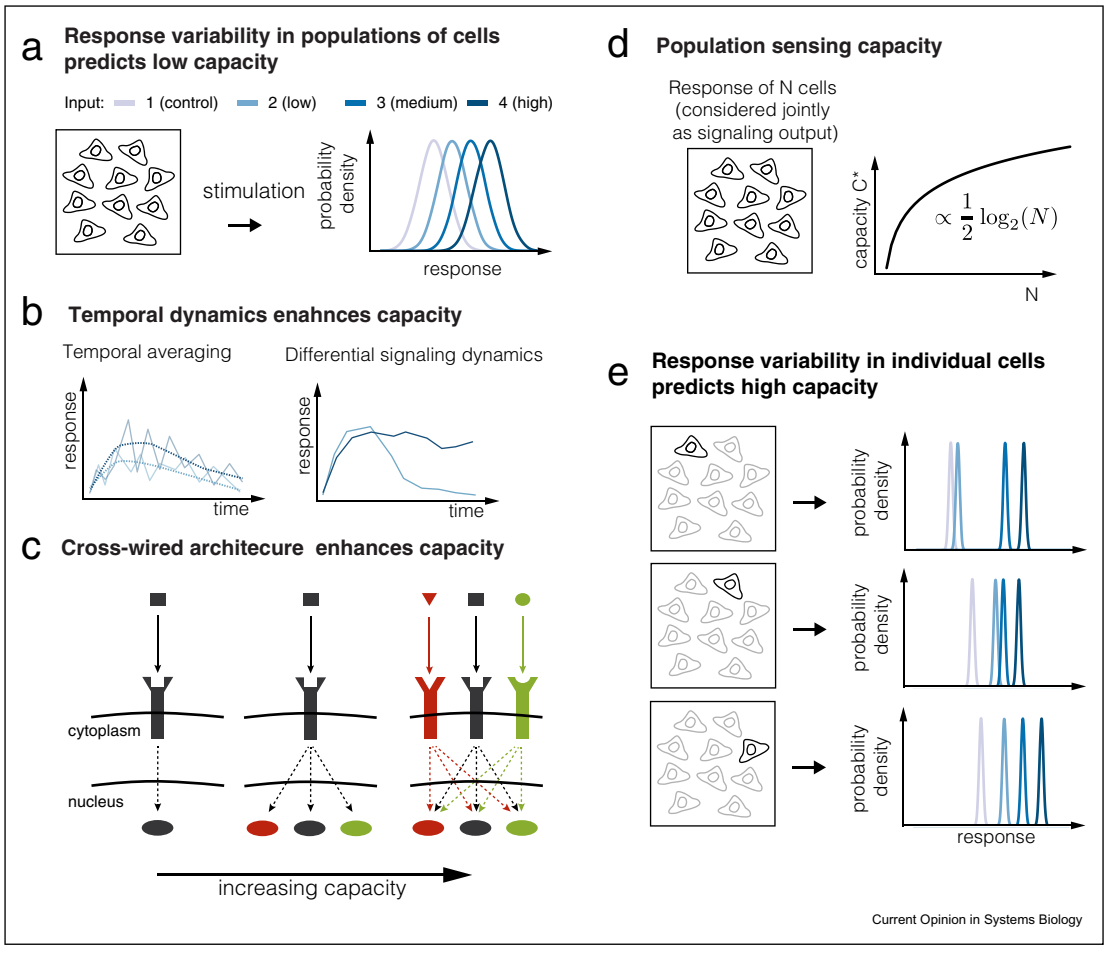
Box 1. Information formalism for cellular signaling

(A) A biochemical signaling pathway can be represented as a probabilistic input–output relationship, P(Y|X = x), which encodes input, x, using output, y. Because of stochastic factors, decoding is possible only with a limited precision.
(B) Fisher information quantifies the inverse of the minimal variance with which a given input x can be decoded, which is known as Cramér-Rao inequality. How precisely can x be decoded depends on sensitivity of the response distribution to changes in x. Therefore, formally, Fisher information is defined as the average sensitivity of the logarithm of the probability, P(Y|X = x), to changes in the input, x.
(C) Shannon information capacity, C*, quantifies the overall signaling accuracy. It can be interpreted as $\log_2$ of a number of inputs that a signaling system can resolve and is approximated by the integration of Fisher information.
(D) Hypothetical response distributions to four inputs (e.g. ligand concentrations) in three scenarios with a different degree of overlaps. Completely overlapping distributions do not allow for information transfer and imply the capacity of 0 bits. Information capacity increases for more distinct distributions and reaches 2 bits for completely distinct distributions to four considered inputs.



ration, generates a response y (output), for example, activation of a transcription factor (TF) (Box 1A). Because of the stochasticity of cellular biochemistry, the input–output relationship is subject to random fluctuations and can be represented as a probability distribution, P(Y|X = x). A high amount of information about x contained in Y would enable accurate estimation (decoding) of x from Y, which inspired the mathematical concepts of information defined by R.A. Fisher and C. Shannon [12]. The lowest possible variance with which a specific x can be estimated is the inverse of the Fisher information, FI(x) [12] (Box 1B). High Fisher information implies the possibility of accurate decoding: $\mathrm{Var}(\hat{x}) \geq 1/\mathrm{FI}(x)$, where $\mathrm{Var}(\hat{x})$ is the variance of an estimate $\hat{x}$ of a true value x—the relationship known as Cramér-Rao inequality. Fisher information can vary for different values of x, for example, some ligand concentrations can be sensed more accurately than others, and therefore, it does not quantify the overall signaling accuracy. On the other hand, an overall measure of signaling accuracy is known as Shannon information capacity [12], C^*. Information capacity (Box 1C) is expressed in bits, and broadly speaking, 2^{C^*} represents the maximal number of different inputs that a system can effectively decode, for example, different ligand concentrations. If, for example, the derived $C^* = 2$, then 4 different ligand concentrations can be resolved with negligible error. Shannon information capacity can be expressed through integration of Fisher information over a

Figure 1



An information theory perspective of cellular strategies to achieve high signaling capacity. (**a**) Hypothetical response distributions of a population of cells exhibiting considerable overlaps between distributions corresponding to different inputs, which implies low information capacity. (**b**) The hypothetical temporally resolved responses to two inputs. Temporal averaging (left panel): responses to two inputs (solid lines) might overlap at any given time point, but their averages (dashed lines) are distinct, which allows for input discrimination and, hence, increases information capacity. Differential signaling dynamics (right panel): two inputs may induce responses that are similar over one time window but distinct over another, which, again, allows for input discrimination and increases information capacity. (**c**) Activation of several distinct signaling effectors by a single ligand enhances information transfer and provides a basis for parallel (multiplexed) information transfer about concentrations of several distinct ligands, increasing information capacity. (**d**) Responses of several cells can be jointly considered as an output of a signaling system. Information capacity of a system composed of N cells scales with $^1/_2$ $\log_2$(N), implying that a population has a considerably higher information capacity than an individual cell. (**e**) Shown are hypothetical response distributions of individual cells exhibiting low response variability compared with population responses. Limited overlaps between distributions predict considerably higher information capacity than population responses. Individual cells can be in different states, which determine signaling responses, and therefore, the cell-to-cell heterogeneity of responses is not necessarily equivalent to signaling noise.

range of input values (Box 1C) with high Fisher information implying high Shannon information capacity [13]. Intuitively, how much information can be transmitted through a signaling system depends on how distinct the response distributions corresponding to different inputs are (Box 1D). For example, four distinct response distributions imply four resolvable inputs and two bits of transferred information, whereas full overlapping distributions imply one resolvable input and zero bits transferred. In the intermediate scenarios, the information can vary between zero and two bits depending on the degree of overlaps.

Population response distributions predict low signaling accuracy

After the initial use of information theory to study developmental signaling pathways [14,15], Shannon's framework was initially deployed in the context of

Table 1

Selected most recent studies examining different mechanisms enhancing information transfer.

Mechanism	Reference	Signaling model	Main conclusion
Temporal averaging & Differential signaling dynamics	Selimkhanov et al. [19]	TNF-α → NF-κB, EGF → Erk, ATP→Ca^{2+}; live imaging	Dynamic responses increase signaling accuracy.
	Jetka et al. [20]	TNF-α → NF-κB; live imaging	Dynamic response improves discrimination of high TNF-α doses and introduces an R-package SLEMI for calculation of Shannon information based on multivariate experimental data: https://github.com/sysbiosig/SLEMI
	Nandagopal et al. [21]	Dll1 or Dll4, Notch; live imaging	Ligand identity is encoded in pulsatile or sustained Notch activation dynamics.
	Sampattavanich et al. [22]	Growth factors → FOXO3; live imaging	FOXO3 dynamics can encode growth factor identities and concentrations.
	Harper et al. [23]	TNF-α → NF-κB; live imaging	NF-κB dynamics and target gene expression are modulated by temperature and can transmit multidimensional information.
Cross-wiring	Komorowski and Tawfik [25]	Theoretical model	Duplication of cross-reactive receptors doubles information capacity even with minimal divergence.
	Pope et al. [26]	Insulin, EGF → AKT, ERK; immunofluorescence	Joint activity of two effectors carries tangibly more information than each effector considered individually.
Differential signaling dynamics of cross-wired effectors	Granados et al. [27]	Environmental stress → multiple transcription factors; live imaging (yeast)	Dynamics of several transcription factors constitutes a precise representation of extracellular environments.
	Lane et al. [29]	Bacterial infections → NF-κB and MAPK; live imaging	NF-κB and MAPK (monitored through JNK) dynamics vary with bacterial location, pathogenicity, and replication.
	Jetka et al. [13]	IFN-α, IFN-λ1 → STAT1, STAT2; theoretical model	Information about identity and quantity of IFN-α and IFN-λ1 can be transmitted despite activating the same signaling effectors.
Population sensing	Suderman et al. [18]	TRAIL → Casp-3, Casp-8; flow cytometry	High cell-to-cell heterogeneity is advantageous when a system needs to regulate the behavior of populations of cells.
	Wada et al. [30]	Electrical pulse stimulation → Ca^{2+}, live imaging	Variable binary activation of individual myotubes leads to better discrimination of stimulation intensity in skeletal muscles.
	Jetka et al. [13]	Theoretical derivation	Expressing Shannon information in terms of Fisher information predicts information capacity of cellular populations.
Heterogeneity of cellular states	Yao et al. [36]	ATP → Ca^{2+}; live imaging	Most of calcium cell-to-cell response variability can be explained by variability in intracellular receptor activity.
	Guilbert et al. [37]	Heat stress → nuclear stress bodies; immunostaining	Most of heat shock response cell-to-cell variability can be explained by variability of the basal level of heat shock proteins.
	Voliotis et al. [38]	GnRH → NFAT; live imaging	Based on the single-cell response to a first stimulation, the response to the second stimulation can be accurately predicted.
	Keshelava et al. [39]	Acetylcholine → Ca^{2+}; live imaging	Each individual cell has distinct dynamic range and a high signaling capacity.
	Gross et al. [40]	IGF → FOXO1; live imaging	Single cells exhibit reproducible responses to repetitive stimulations.
	Spencer et al. [41]	TRAIL → apoptosis; live imaging	Nongenetic determinants of death probability can be passed from mother to daughter cells.
	Phillips et al. [42]	Transcriptional activity; live imaging	Transcriptional activity is strongly correlated in sister cells as well as in daughter–mother pairs.

cytokine signaling to examine the overall fidelity of tumor necrosis factor α (TNF-α) and platelet-derived growth factor (PDGF) responses [16]. Mouse embryonic fibroblasts were stimulated with a range of TNF-α or PDGF doses, considered as input, x. Single-cell responses of signaling effectors, nuclear factor κB (NF-κB) and activating transcription factor-2 (ATF-2), were measured at a single time point, using immunostaining, and considered as output, Y. The response distributions in cellular populations stimulated with different doses were then used as distributions of signaling output, Y, and analyzed using the Shannon information formula. Quantification revealed ∼1 bit of information was transferred, which was interpreted as cells having the potential to discriminate between the presence and absence of the stimulants but lacking the ability to resolve intermediate concentrations. Examining other signaling systems using an analogous framework yielded similar conclusions regarding signaling accuracy. In particular, quantification of information transmission from the gonadotropin-releasing hormone (GnRH) to extracellular signal-regulated kinase (Erk) or to the nuclear factor of activated T-cells (NFAT) in GnRH-sensitive HeLa cells both yielded < 1 bit [17]. In addition, measured activities of caspase-8 and caspase-3, in response to the TNF-related apoptosis-inducing ligand (TRAIL) in HeLa cells, gave ∼1 and ∼0.5 bits of information, respectively [18]. These studies showed that if cell-to-cell heterogeneity of responses is interpreted as signaling noise, the predicted capacity is low because response distribution of population cells to different doses exhibited considerable overlaps (Fig. 1a). Strongly responding cells stimulated with a low dose of a ligand had responses higher than weakly responding cells stimulated with a high dose, which does not allow for errorless discrimination between low and high doses based on the measured response alone and resulted in low capacity.

The overall low signaling fidelity, which would significantly hinder effective discrimination between different ligand concentrations, appears difficult to reconcile with the seemingly reliable, synchronized activities of individual cells observed in many physiological processes, such as stress responses, immunity, circadian rhythms, muscle contraction, or wound healing. Several different mechanisms have been postulated that aim to resolve this paradox, Table 1, and we discuss each one in the following.

Signaling dynamics and cross-wiring enhance information transfer

Temporal averaging is a simple strategy to reduce noise as the average of multiple measurements taken over time has lower variability than a single time-point measurement (Fig. 1b). Following this intuition, temporal integration of response signals was shown to provide additional information compared with single time-point measurements [19]. It was achieved by live imaging of human and murine cell lines stimulated with epidermal growth factor (EGF), ATP, or lipopolysaccharides (LPS) and measuring the fluorescence-based responses of Erk, calcium (Ca^{2+}), or NF-κB, respectively. Comparison of information flow between static and dynamic responses revealed that signaling dynamics provides up to 0.5 bit of additional information, which, broadly speaking, enables discrimination of an additional intermediate dose. Besides, by providing a simple algorithm for effective evaluation of Shannon information for multivariate experimental data, it was demonstrated that dynamic NF-κB responses only improved discrimination of high TNF-α concentrations, having limited impact on discrimination of low doses [20]. In addition to noise averaging, signaling dynamics can enhance information transfer via stimuli that control duration, amplitude, onset, or other characteristics of response so that information can be encoded in differential signaling dynamics, that is, in the shape of temporally resolved responses (Fig. 1b).

Differential signaling dynamics have been explored, for example, in the context of myogenesis [21], where authors demonstrated that cells use the dynamics of Notch activity to discriminate between two types of ligands (Dll1 and Dll4), which led to distinct cellular fates. Similarly, Sampattavanich et al. [22] have shown that the nuclear-to-cytosolic pulsatile dynamics of the forkhead box O3 transcription factor (FOXO3) help to discriminate the type and concentration of growth factor treatment. Furthermore, live-cell imaging of human neuroblastoma cells was used to show that NF-κB signaling dynamics can simultaneously encode information about the temperature of the ambient environment and TNF-α dose [23], which has been expanded into a theoretical framework to study analogous phenomena [24].

Cross-wiring of signaling pathways involves distinct ligands activating multiple different signaling effectors, which raises the question of the role the different signaling effectors play in encoding information about quantity and identity of the ligands (Fig. 1c). In a theoretical work [25], using a simple receptor model, authors hypothesized that the only way of circumventing the loss in signaling capacity due to molecular noise is by the emergence during evolution of paralogs that signal the presence of the cross-reactive noncognate ligand. Such a mode of expansion explains the highly cross-wired architecture of signaling pathways. The information gain from activating several signaling effectors was also examined experimentally in the study by Pope et al. [26], which measured responses to combinations of insulin and EGF in terms of activated kinase pAKT or the activated kinase ppERK. Individually, pAKT and ppERK carried ∼0.75 and ∼1.0 bit of information, respectively. Accounting for both signaling

effectors jointly increased information transfer to ~1.75 bits.

A combination of differential signaling dynamics and cross-wired signaling further boosts information transfer in terms of both the identities and quantities of stimuli. Using a yeast system, in the study by Granados et al. and Cepeda-Humerez et al. [27,28], authors examined encoding of the type and intensity of extracellular stresses in the nuclear translocation of multiple TFs. Taking the dynamics of several TFs into accounting enhanced signaling information, enabling nearly perfect discrimination of the type and intensity of stress treatment (four TFs carried information about almost all the seven states of the stress environment, leading to ~2.5 bits). On the other hand, translocation of mitogen-activated protein kinases (MAPKs), and NF-κB was measured in murine macrophages responding to bacterial infection [29]. Cells exposed to different types of bacteria, either pathogenic *Salmonella typhimurium* or nonpathogenic *Escherichia coli*, exhibited different temporal response profiles, which revealed that integrating information of NF-κB and MAPK signaling over time enables the response that is specific to the infection type. A theoretical approach to analyze models of cross-wired differential signaling dynamics was proposed in the study by Jetka et al. [13] by integrating Fisher and Shannon information measures. The framework quantified how the differential signaling dynamics of signaling effectors STAT1 and STAT2, as represented by phosphorylation state, enables discrimination between type I and type III interferon variants.

Cellular population as a sensory system

Several works examined the sensing capacity of cellular populations by considering the signaling output composed of the responses of several cells jointly, as opposed to one cell alone (Fig. 1d). As demonstrated in the study by Suderman et al. [18], cellular populations exhibiting higher cell-to-cell heterogeneity are responsive over a broader dynamic range of ligand concentrations than populations with lower cell-to-cell heterogeneity, implying that high cell-to-cell heterogeneity might increase the overall information capacity of a cellular population. Indeed, the information capacity of a population was found to scale as $^1/_2 \log_2$ of the number of cells, a relationship also demonstrated in a general setting by expressing Shannon information in terms of Fisher information [13]. The impact of cell-to-cell heterogeneity on population signaling capacity was further expanded in the study by Wada et al. [30], where Ca^{2+} signaling in myotubes was studied. Individual cells were activated by an electrical pulse. Cells exhibited binary responses with considerable variability in the current required for activation. Some cells were induced by low current, whereas others required sixfold higher current. The variability between cells enabled the cell population to achieve high signaling sensitivity over a broad range of currents, with almost perfect discrimination between the ten levels of stimulation tested.

Cellular state as a determinant of cell responses

Measurement of different parameters of single cells has revealed that specific characteristics of individual cells correlate with their responses to stimuli. For instance, levels of the signaling effector, NF-κB, before and after TNF-α stimulation, have been shown to be correlated [31], resulting in fold changes, that is, the ratio between nuclear NF-κB levels before and after stimulation, exhibiting considerably lower variability than absolute levels after stimulation. Similarly, the fold changes observed in individual cells exhibited a significantly higher correlation with downstream gene expression than the absolute response levels. This observation, when analyzed in the context of information theory [32], demonstrated a higher information transfer for fold changes versus absolute levels of the signal effector. In addition, examination of NF-κB responses to changes in cytokine concentration showed that negative feedback loop inhibitory proteins provide a resettable short-term memory of previous cytokine exposure, shaping cell-specific states [33].

Transforming growth factor beta (TGF-β)—induced Smad signaling was shown to have similar properties, with the temporal dynamics of the fold change in Smad nuclear levels containing more information than absolute levels [34]. Similarly, the fold change of the early and late EGF responses carries more information than absolute levels [35].

The idea that certain cellular characteristics—which define an internal 'state' of the cell not revealed by measuring signaling responses alone—determine a cell's signaling response and, therefore, cell-to-cell heterogeneity is not necessarily equivalent to noise was explored broadly in the study by Yao et al. [36]. By combining experimental measurements of ATP-activated Ca^{2+} signaling with mathematical modeling, the authors showed that 70% of the variability of the response could be explained by intracellular receptor activity, indicating that the signaling processes inside the cell may be much more precise than predicted by the cell-to-cell heterogeneity of observed responses. Similarly, Guilbert et al. [37] showed that most of the observed cell-to-cell heterogeneity of responses to heat could be explained by the basal intracellular level of heat shock proteins.

Analogous results have been observed in research exploring the responses of individual cells exposed to repeated stimulation. Cells stimulated twice with increasing doses of TNF-α revealed that the second response is proportional to the first, demonstrating that the cellular response is specific to individual cells, which show lower variability than predicted by population responses [32]. Similarly, GnRH responses measured in the study by Voliotis et al. [38] exhibited variability that yielded Shannon information capacity of less than 0.5 bits, whereas the variability of repeatedly measured responses predicted an information capacity of ~1 bit. Furthermore, human kidney embryo cells stimulated with different doses of acetylcholine, resulting in Ca^{2+} intracellular influx, were measured as the signaling output in individual cells [39]. Repeated acetylcholine stimulation of the same cells with a range of doses enabled the acquisition of dose–response distributions not only for population of cells but also for individual cells. Information transfer, calculated based on the population response distribution, quantified an information capacity of ~1 bit. Using dose–response distributions of individual cells, on the other hand, revealed ~2 bits of information transfer. The considerably higher information capacity of individual cells indicated that not all cell-to-cell heterogeneity can be interpreted as noise that results in information loss within individual cells. Before stimulation, individual cells might be in different states that determine their response levels, such that variability of response is considerably smaller than the cellular population (Fig. 1e). Similar conclusions have been drawn in the study by Gross et al. [40], where HeLa cells showing the highest responses to small doses of insulin-like growth factor 1 (IGF-1) also tend to be in the top percentiles of cells responding to subsequent, higher doses. In addition, when cellular lineages were tracked, the siblings' responses to IGF were moderately correlated ($R^2 = 0.31$), suggesting that factors influencing the response level can be passed to daughter cells. Inheritance of cellular states had previously been shown for TRAIL signaling, where the time from treatment to apoptosis was more similar in daughter cells [41], as also seen for transcriptional activity [42], demonstrating highly correlated gene expression profiles for daughter cells.

Conclusions and outlook

Information theory is beginning to provide a clearer understanding of signaling pathways—via analysis of the level of precision that can be achieved through molecular signaling. Studies examining the vulnerabilities of signaling pathways to signaling noise stimulated research into the mechanisms that ensure accurate, high-information content signaling, and several mechanisms have been demonstrated to enhance information transfer, Table 1. The temporal dynamics of stimulation has the potential to filter out noise, and stimuli may also control the onset, duration, amplitude, and/or other characteristics of the response, such that information can be encoded in temporally resolved responses. In addition, activation of several signaling effectors by a single stimulus can further increase information capacity, for instance, by extending signaling sensitivity over a broader range of concentrations with, for example, low concentrations activating one effector and high concentrations activating another. Activation of the same signaling effectors by different ligands through cross-wired architecture enables parallel (multiplexed) signaling, strongly amplifying information transfer, especially when combined with differential signaling dynamics. Finally, substantial cell-to-cell heterogeneity was initially interpreted as signaling noise leading to information loss. However, recent research has identified cell populations as collections of cells in different states that determine individual cell responses. Therefore, signaling may be much more precise than predicted by cell-to-cell heterogeneity—and future research will reveal how much of the observed cell-to-cell heterogeneity results from genuine noise leading to information loss. Future studies will also lead to more accurate predictions of how much information signaling pathways transmit to the inside of the cell. A combination of multiplexed measurement techniques, live imaging, and single-cell transcriptomics are providing opportunities to address these questions in new ways. Adaptation of information-theoretic tools to understand multiplexed and composite data could, therefore, offer further insights into the origins, functions, and consequences of different aspects of signaling complexity.

Conflict of interest statement

Nothing declared.

Acknowledgements

P.T. and M.K. were supported by the Foundation for Polish Science within the First TEAM (First TEAM/2017-3/21) program co-financed by the European Union under the European Regional Development Fund. P.T also was supported by Polish National Science Centre under grant PRELUDIUM 2019/33/N/NZ2/02691. We thank Guy Riddihough for helpful comments during the preparation of this manuscript.

References

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

* of special interest
** of outstanding interest

1. Berg HC, Purcell EM: **Physics of chemoreception**. *Biophys J* 1977, **20**:193–219.

2. Wolde PR ten, Becker NB, Ouldridge TE, Mugler A: **Fundamental limits to cellular sensing**. *J Stat Phys* 2016, **162**:1395–1424.

3. Endres RG, Wingreen NS: **Accuracy of direct gradient sensing by single cells**. *Proc Natl Acad Sci Unit States Am* 2008, **105**: 15749–15754.

4. ** Petkova MD, Tkačik G, Bialek W, Wieschaus EF, Gregor T: **Optimal decoding of cellular identities in a genetic network**. *Cell* 2019, **176**. 844-855.e15.

In the early *Drosophila* embryos expression patterns of four *Gap* genes differ along the spatial axes of the embryo encoding the position of single nuclei with high precision allowing reproducible formation of the body plan.

5. Nitzan M, Karaiskos N, Friedman N, Rajewsky N: **Gene expression cartography**. *Nature* 2019, **576**:132–137.

6. Johnson HE, Toettcher JE: **Signaling dynamics control cell fate in the early Drosophila embryo**. *Dev Cell* 2019, **48**. 361-370.e3.

7. Desponds J, Vergassola M, Walczak AM: **A mechanism for hunchback promoters to readout morphogenetic positional information in less than a minute**. *Elife* 2020, **9**, e49758.

8. Graham SJL, Zernicka-Goetz M: **The acquisition of cell fate in mouse development how do cells first become heterogeneous?** *Curr Top Dev Biol* 2016, **117**:671–695.

9. Fiorentino J, Torres-Padilla M-E, Scialdone A: **Measuring and modeling single-cell heterogeneity and fate decision in mouse embryos**. *Annu Rev Genet* 2020, **54**:167–187.

10. Čapek D, Müller P: **Positional information and tissue scaling during development and regeneration**. *Development* 2019, **146**, dev177709.

11. Kussell E, Leibler S: **Phenotypic diversity, population growth, and information in fluctuating environments**. *Science* 2005, **309**:2075–2078.

12. Cover TM, Thomas JA: *Elements of information theory*. Wiley; 2012.

13. Jetka T, Nienałtowski K, Filippi S, Stumpf MPH, Komorowski M: **An information-theoretic framework for deciphering pleiotropic and noisy biochemical signaling**. *Nat Commun* 2018, **9**: 4591.

14. Gregor T, Tank DW, Wieschaus EF, Bialek W: **Probing the limits to positional information**. *Cell* 2007, **130**:153–164.

15. Tkačik G, Callan CG, Bialek W: **Information flow and optimization in transcriptional regulation**. *Proc Natl Acad Sci Unit States Am* 2008, **105**:12265–12270.

16. Cheong R, Rhee A, Wang CJ, Nemenman I, Levchenko A: **Information transduction capacity of noisy biochemical signaling networks**. *Science* 2011, **334**:354–358.

17. Garner KL, Perrett RM, Voliotis M, Bowsher C, Pope GR, Pham T, Caunt CJ, Tsaneva-Atanasova K, McArdle CA: **Information Transfer in Gonadotropin-releasing Hormone (GnRH) Signaling: extracellular signal-regulated kinase (ERK)-mediated feedback loops control hormone sensing**. *J Biol Chem* 2016, **291**:2246–2259.

18. Suderman R, Bachman JA, Smith A, Sorger PK, Deeds EJ: **Fundamental trade-offs between information flow in single cells and cellular populations**. *Proc Natl Acad Sci Unit States Am* 2017, **114**:5755–5760.

19. Selimkhanov J, Taylor B, Yao J, Pilko A, Albeck J, Hoffmann A, Tsimring L, Wollman R: **Accurate information transmission through dynamic biochemical signaling networks**. *Science* 2014, **346**:1370–1373.

20. Jetka T, Nienałtowski K, Winarski T, Błoński S, Komorowski M: **Information-theoretic analysis of multivariate single-cell signaling responses**. *PLoS Comput Biol* 2019, **15**, e1007132.

21. ** 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.e19.
Dll1 and Dll4 ligands can activate distinct targets through the same Notch receptor with their identity being encoded in pulsatile or sustained Notch activation dynamics. The ability of the pathway to utilize ligands as distinct communication channels has implications for diverse Notch-dependent processes.

22. Sampattavanich S, Steiert B, Kramer BA, Gyori BM, Albeck JG, Sorger PK: **Encoding growth factor identity in the temporal dynamics of FOXO3 under the combinatorial control of ERK and AKT kinases**. *Cell Syst* 2018, **6**. 664-678.e9.

23. Harper CV, Woodcock DJ, Lam C, Garcia-Albornoz M, Adamson A, Ashall L, Rowe W, Downton P, Schmidt L, West S, *et al.*: **Temperature regulates NF-κB dynamics and function through timing of A20 transcription**. *Proc Natl Acad Sci Unit States Am* 2018, **115**. 201803609.

24. Minas G, Woodcock DJ, Ashall L, Harper CV, White MRH, Rand DA: **Multiplexing information flow through dynamic signalling systems**. *PLoS Comput Biol* 2020, **16**, e1008076.

25. ** Komorowski M, Tawfik DS: **The limited information capacity of cross-reactive sensors drives the evolutionary expansion of signaling**. *Cell Syst* 2019, **8**:76–85.e6.
The findings suggest that expansion of signaling components via duplication and enlistment of promiscuously acting cues is virtually the only accessible evolutionary strategy to achieve overall high signaling capacity despite overlapping specificities and molecular noise.

26. * Pope RJP, Garner KL, Voliotis M, Lay AC, Betin VMS, Tsaneva-Atanasova K, Welsh GI, Coward RJM, McArdle CA: **An information theoretic approach to insulin sensing by human kidney podocytes**. *Mol Cell Endocrinol* 2020, **518**. 110976.
Insulin acts via noisy communication channels with more information flow to Akt than to ERK. Information capacity was increased by consideration of joint sensing (ERK and Akt) and response trajectory (live cell imaging of FOXO1-clover translocation).

27. ** Granados AA, Pietsch JMJ, Cepeda-Humerez SA, Farquhar IL, Tkačik G, Swain PS: **Distributed and dynamic intracellular organization of extracellular information**. *Proc Natl Acad Sci Unit States Am* 2018, **115**:6088–6093.
The paper demonstrates that dynamics of nuclear translocation of multiple TFs gives accurate decoding of several abiotic stresses. Changes in the dynamics of the localization of transcription factors constitute a precise, distributed internal representation of extracellular change. The work suggests that such multidimensional representations are common in cellular decision-making.

28. Cepeda-Humerez SA, Ruess J, Tkačik G: **Estimating information in time-varying signals**. *PLoS Comput Biol* 2019, **15**, e1007290.

29. ** Lane K, Andres-Terre M, Kudo T, Monack DM, Covert MW: **Escalating threat levels of bacterial infection can Be discriminated by distinct MAPK and NF-κB signaling dynamics in single host cells**. *Cell Syst* 2019, **8**. 183-196.e4.
Dynamics of NF-κB and MAPK signaling enables precise discrimination between different variants of bacterial location, pathogenicity, and replication.

30. ** Wada T, Hironaka K, Wataya M, Fujii M, Eto M, Uda S, Hoshino D, Kunida K, Inoue H, Kubota H, *et al.*: **Single-cell information analysis reveals that skeletal muscles incorporate cell-to-cell variability as information not noise**. *Cell Rep* 2020, **32**:108051.
The authors quantified the information transmission of human facial muscle contraction during intraoperative neurophysiological monitoring and found that information transmission of muscle contraction is comparable to that of a multiple-cell channel. The data showed that intracellular response variability is small, whereas the cell-to-cell variability is large. Besides, the latter can increase the information capacity of tissues.

31. Lee REC, Walker SR, Savery K, Frank DA, Gaudet S: **Fold change of nuclear NF-κB determines TNF-induced transcription in single cells**. *Mol Cell* 2014, **53**:867–879.

32. Zhang Q, Gupta S, Schipper DL, Kowalczyk GJ, Mancini AE, Faeder JR, Lee REC: **NF-κB dynamics discriminate between TNF doses in single cells**. *Cell Syst* 2017, **5**. 638-645.e5.

33. Son M, Wang AG, Tu H-L, Metzig MO, Patel P, Husain K, Lin J, Murugan A, Hoffmann A, Tay S: **NF-κB responds to absolute differences in cytokine concentrations**. *Sci Signal* 2021, **14**. eaaz4382.

34. Frick CL, Yarka C, Nunns H, Goentoro L: **Sensing relative signal in the Tgf-β/Smad pathway**. *Proc Natl Acad Sci Unit States Am* 2017, **114**:E2975–E2982.

35. Benary M, Bohn S, Lüthen M, Nolis IK, Blüthgen N, Loewer A: **Disentangling pro-mitotic signaling during cell cycle progression using time-resolved single-cell imaging**. *Cell Rep* 2020, **31**:107514.

36. Yao J, Pilko A, Wollman R: **Distinct cellular states determine calcium signaling response**. *Mol Syst Biol* 2016, **12**:894.

37. * Guilbert M, Anquez F, Pruvost A, Thommen Q, Courtade E: **Protein level variability determines phenotypic heterogeneity in proteotoxic stress response**. *FEBS J* 2020, **287**:5345–5361.

Microscopy measurements combined with modeling indicated that a basal level of heat shock proteins explains the majority of the observed heterogeneity of responses.

38. Voliotis M, Garner KL, Alobaid H, Tsaneva-Atanasova K, McArdle CA: **Gonadotropin-releasing hormone signaling: an information theoretic approach**. *Mol Cell Endocrinol* 2018, **463**: 106–115.

39. * Keshelava A, Solis GP, Hersch M, Koval A, Kryuchkov M, Bergmann S, Katanaev VL: **High capacity in G protein-coupled receptor signaling**. *Nat Commun* 2018, **9**:876.
Different doses of acetylcholine were used to activated the muscarinic acetylcholine receptor M3R (GPCR – family) resulting in Ca^{2+} intracellular influx. An individual cell could resolve higher numbers of doses than predicted by the population response distributions.

40. ** Gross SM, Dane MA, Bucher E, Heiser LM: **Individual cells can resolve variations in stimulus intensity along the IGF-PI3K-AKT signaling Axis**. *Cell Syst* 2019, **9**. 580-588.e4.
The analysis shows that cells respond to repetitive IGF doses with high accuracy and reproducibility, indicating that intracellular noise is relatively small compared to the observed cell-to-cell heterogeneity.

41. 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.

42. Phillips NE, Mandic A, Omidi S, Naef F, Suter DM: **Memory and relatedness of transcriptional activity in mammalian cell lineages**. *Nat Commun* 2019, **10**:1208.