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Opinion

Information Theory: New Look at Oncogenic Signaling Pathways

K.A. Zielińska[1] and V.L. Katanaev[1,2,*]

Sustained pro-proliferative signaling is one of the hallmarks of cancer. Although it is generally understood that the oncogenic signaling pathways are overactivated, or at least abnormally activated, in cancer cells, important mechanistic details of such abnormal activation remain unresolved. Among these details are such aspects of signaling as robustness, redundancy, signal amplification, and others, which touch upon the domain of information theory – the field of mathematics and engineering dealing with properties of information storage, encoding, and transmission. Information theory only recently has started to be applied to intracellular signaling. Here, we overview the recent advances provided by the information theory, focusing on the nuclear factor (NF)-κB, extracellular signal-regulated kinase (ERK), and G-protein-coupled receptor (GPCR) pathways, which are frequently hijacked in cancer. Furthermore, we show how viewing previously untouched mechanics of oncogenic signaling through information theory applications may evolve into novel ways of anticancer drug discovery.

Information Theory Can Help to Study Cellular Decisions

Cells are exposed in their everyday life to fluctuating environments and need to make critical decisions whether to live or die, proliferate or differentiate, migrate, or stabilize their current position [1]. Thinking about cellular decisions generates sticky questions. How do intracellular signaling networks process information from the environment? How does variability in cellular decisions reflect the performance of signaling networks? How do cells distinguish important cues from noise? Recent studies show that **information theory** (see Glossary), developed by Claude E. Shannon to study communication channels designed by humans (Box 1), emerges as a powerful mathematical tool to address these questions (Figure 1, Key Figure) and to measure the limits of signaling capacities of complex networks [2–4].

Understanding the mechanisms of cellular decisions appears particularly important in the context of diseases. Alterations in signaling networks may lead to wrong cellular decisions and have been implicated in diseases such as cancer (reviewed in [5]). In this article, we discuss the applications of information theory to cellular signaling. We specifically focus on signaling pathways involved in cancer pathogenesis exemplified by the nuclear factor κB (NF-κB), ERK, and G protein-coupled receptor (GPCR) pathways. NF-κB has a long record of promoting solid and hematological malignances, whereas abnormal activation of ERK has been reported in one third of all human cancers with pancreas, colon, and thyroid cancer being the most often associated with this abnormality [6,7]. In contrast, GPCRs play an essential but underappreciated role in cancer metastasis and progression and a recent study shows that GPCRs are highly expressed in human cancers [8]. In this opinion article, we first briefly describe the contribution of these pathways to cancer and explain the key concepts of information theory (Boxes 1–3). Second, we overview the current examples of applications of information theory to these oncogenic signaling pathways addressing their information transmission capacities and the impact of noise, feedbacks and environment. Finally, we try to generalize on the new angle of analysis of signaling the information theory offers, advertising it as a new tool to be commonly applied to signal transduction research, and speculating on the new avenues to drug discovery it may open.

NF-κB, ERK, and GPCR Signaling Pathways in a Nutshell

NF-κB

NF-κB was identified more than 30 years ago as a regulator of expression of the κB light chain in B cells [9]. The NF-κB family of transcription factors consists of five members: NF-κB1 p50, NF-κB2

Highlights

Information theory developed in the 1940s to analyze human-made communication systems can be applied to study intracellular signal transduction. Channel capacity – a key concept of information theory – provides the measure of the amount of information a given signaling pathway can reliably transmit.

NF-κB, ERK, and GPCR signaling pathways have been studied, at single-cell and cell population levels, from the perspective of information theory. Depending on the experimental setup, the pathway under study, and the cell line, individual cell channel capacity was calculated to be around 1 bit (all-or-none response) or well above 2 bits (differential response to four or more levels of the signaling input).

Information theory provides a new angle for the understanding of cell signaling, with promises to deliver breakthroughs in receptor pharmacology, mechanisms of oncogenic transformation, and drug discovery.

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p52, RELA (also called p65), RELB, and c-REL, which can homo- or heterodimerize forming up to 15 different NF-κB complexes [10–12].

When inactive, NF-κB proteins reside in the cytoplasm complexed with inhibitor of κB (IκB) (Figure 2A). An active player in the sensing of pathogens, NF-κB has further emerged as an important transcriptional regulator in the immune system and beyond, controlling cell differentiation, proliferation, and survival [10]. Moreover, its deregulation has been implicated in many diseases, as reviewed in [6,12].

In cancer, NF-κB controls multiple processes including inflammation, proliferation, angiogenesis, metastasis, and resistance to therapy [13]. Under normal conditions, NF-κB activates transcriptional programs that help cells to adapt to environmental challenges. The NF-κB response in a healthy cell is self-limiting due to the presence of negative feedback loops. They involve several NF-κB inhibitors: IκBα, IκBε, p105, and A20. However, in cancer NF-κB activity is often hijacked and tumor cells show abnormal patterns of NF-κB-induced genes [6]. NF-κB is constitutively active in tumor cells in many types of malignancies such as melanoma and breast and hematological cancers. Its inhibition blocks tumor growth in models of multiple myeloma and lung cancer [13–15]. It remains unclear how NF-κB is constitutively active in many cancers but diverse factors can be involved in its overactivation [13].

ERK

The mitogen-activated protein kinase (MAPK) pathway is evolutionarily conserved across eukaryotic cells. It controls cell proliferation and differentiation, and also becomes activated in response to tissue damage and pathogen invasion. MAPK activation results in induction of various target genes involved in proliferative and inflammatory responses. In mammals, 14 MAPKs have been identified. ERK1/2, p38α, and C-Jun N-terminal kinase (JNK)1 and JNK2 are the best-studied members in the context of disease and innate immunity [16].

Box 1. Measuring Uncertainty of Random Outputs: Entropy

Many theoretical approaches including information theory have been applied to gain insights into how cells make decisions in response to fluctuating environments [69]. Let us consider a cell that receives stimulus from the environment, for example, TNF. TNF binds to its receptor and induces signal transduction. These events result in a specific response, referred to as output, such as changes in localization of transcription factors and consequent changes in gene expression (Figure I). The output is not always identical since it is subject to a certain level of noise and thus it should be described by a random variable Y. For simplicity, we assume that Y can take on only a finite number of outcomes $y_{1,\ldots,}y_N$. The frequency with which Y assumes each outcome, that is the probability of an event $Y = y_n$, is denoted by $p(y_n)$, $n = 1,\ldots,N$. The mapping p, which associates probabilities with outcomes, is called probability mass function and it is characterized by two properties: $p(y_1)+\cdots+p(y_N) = 1$ and $p(y_n) \geq 0$ for every $n = 1,\ldots N$.

Often, we are interested in predicting the outcome of a random variable. From the mathematical perspective, it seems crucial to assess how easy or difficult it is to make such a prediction. On one end of the spectrum, we have a deterministic random variable Y that takes on one value, say y_1, with probability equal 1, that is, $p(y_1) = 1$. In this case, it is trivial to predict the outcome of Y. On the other end, we have a uniform random variable Y with equal probabilities $p(y_n) = 1/N$ of its outcomes (N is the number of values taken on by Y). In this case, it is difficult to guess the outcome since all are equally probable. The predictability is the flip side of the amount of uncertainty in the random variable. In the first example, there is no uncertainty and we can easily predict the outcome of Y, whereas in the second case the uncertainty is maximal. The uncertainty of every other random variable should fall in between these two outermost cases and we need a systematic way to quantify it. Information theory brings an answer to this problem by introducing a measure of uncertainty called entropy.

The Shannon entropy H (later referred to as entropy) of a random variable Y is defined as

$$H(Y) = -\sum_{n=1}^{N} p(y_n)\log_2\ p(y_n). \qquad [I]$$

The units of entropy depend on the base of the logarithm used in the defining formula (Equation I) and $\log_2$ indicates that entropy is measured in bits.

Glossary

Channel capacity: the highest information transmission rate over a communication channel that can be reliably achieved [64]. In biology, this concept of information theory has recently started to be applied to address questions related to signal transduction and information processing such as how many different ligand concentrations a cell can distinguish.
Extrinsic noise: reflects cell-to-cell variability in distinct starting conditions, such as protein levels of the components of a signaling cascade [43,65].
Information theory: mathematical framework for the theory of communication. Although developed by Claude E. Shannon to analyze human-made communication systems, it has been applied in many other areas including biology [3,4]. For example, in the field of gene regulatory networks, it can be applied to reconstruct the network and in signal transduction it measures the amount of information transfer [66,67]. Furthermore, information theory enables quantification of the influence of stochasticity on dose responses in cell populations and helps to determine the degree to which noise affects the fidelity of messages in biological systems [67,68].
Intrinsic biochemical noise: originates from the stochastic molecular interactions in individual cells [43,65].
Mutual information: the average amount of information that two random variables share with each other [64]. In biology, this concept of information theory has been applied to study different aspects of signal transduction such as its specificity and how information is encoded in the cell.

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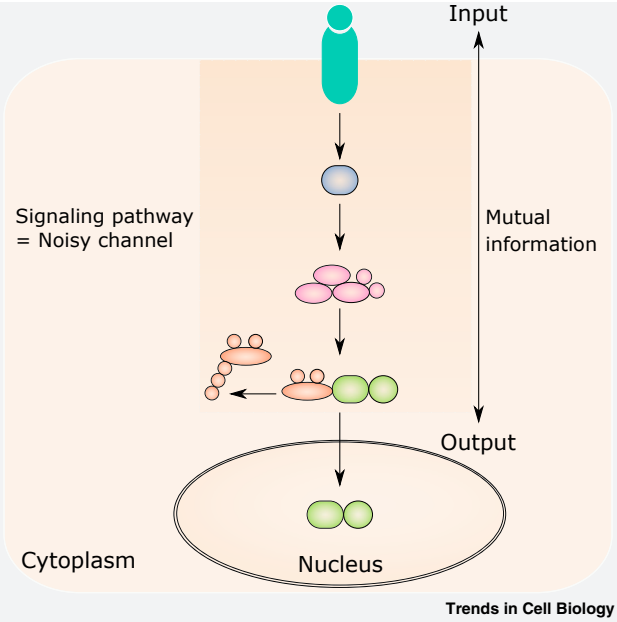

Figure I. Information Theory Can Be Applied to Model Biological Signal Transduction.

Signal transduction pathways can be viewed as noisy communication channels that become activated by diverse ligands such as tumor necrosis factor, also referred to as inputs. Binding of inputs to its receptors mediates the information transmission inside a cell through a multistep cascade leading to specific output such as nuclear translocation of a transcription factor. Mutual information measures the statistical dependence between the input and the output and quantifies the amount of information the output conveys about the input.

The ERK pathway is activated upon binding of growth factors to cell surface receptors, mainly receptor tyrosine kinases (RTKs). RTKs activate the small GTPase Ras, which in its GTP-bound state recruits Raf, the first kinase of the ERK cascade (Figure 2B) [7]. Activation of Raf involves its recruitment to the membrane, formation of the Ras–Raf complex, dimerization of Raf, and phosphorylation of different domains of Raf [17]. Downstream from Raf, the dual specificity kinases MAPK kinase (MEK)1 and MEK2 are activated. Finally, MEK1/2 activate the kinases ERK1 and ERK2 through their phosphorylation on Thr and Tyr residues (Thr202 and Tyr204 of ERK1, and Thr173 and Tyr185 of ERK2) [7]. This constitutes the three-level organization of the ERK pathway [18].

Abnormal activation of members of the MAPK pathway is of paramount importance in cancer, with the ERK pathway being the most important oncogenic pathway in humans [19]. Dysregulated ERK signaling occurs in various types of human cancers including pancreas, colon, lung, and melanoma [7]. The ERK pathway can act as a pro-oncogenic signal and an inhibitor of tumor growth; the exact outcome depends on the intensity of the signal and the context where abnormal activation occurs (reviewed in [20]. Genetic alterations in the ERK pathway have been suggested as one of the mechanisms underlying cancer initiation and progression [7]; extended kinetics of the ERK pathway results in signal misinterpretation and abnormal proliferative decisions in cancer cells [21].

GPCRs

GPCRs regulate diverse aspects of human physiology, constituting the largest class of cell surface receptors encoded in the human genome with approximately 850 GPCR genes identified [22,23]. Roughly half of all currently marketed drugs target GPCRs and their signaling pathways [24]. Among

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Key Figure

Information Theory Provides New Perspectives on Studying Cellular Signaling

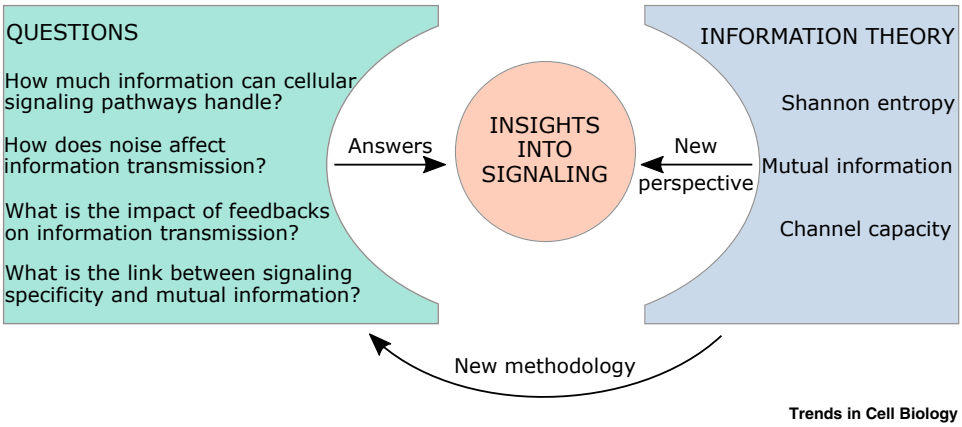

Figure 1. Information theory treats signaling pathways as noisy communication channels. Measuring of the theory's key parameters such as mutual information and channel capacity in individual cells or cell populations provides new insight into the basic features of cellular signal transduction. Information theory thus provides a new methodology to study cellular signaling, focusing on its previously unappreciated aspects.

many other diseases, GPCRs are involved in cancer where they may regulate different steps of cancer progression. Tumor cells often take over GPCR signaling in order to evade clearance by the immune system, to proliferate, and to spread to other organs. Abnormal expression and activation of GPCRs remain the most frequently used mechanisms by tumor cells to hijack the GPCR signaling pathway [25].

Box 2. Reducing Uncertainty Measurements: Conditional Entropy

Cells need to react and choose a particular phenotype or engage in a specific process in response to fluctuating environmental signals. These signals, referred to as inputs, are also described by a random variable X with outcomes $x_1,\ldots,x_M$ and the probability mass function $p(x_m)$, $m = 1,\ldots,M$. The system in which the output Y depends probabilistically on the input X is called a communication channel (later referred to as channel; Figure I). Although channels in biology have complex structures, they can be easily characterized by the conditional probability $p(y|x)$ of the output given the input. We can understand these conditional probabilities as follows: when we know beforehand that X took on the value x_m, it changes the probabilities of the outcomes of Y now given by $p(y_n|x_m)$, $n = 1,\ldots,N$. In this case, we can quantify the uncertainty by noticing that the conditional probability is the probability mass function of the random variable $Y \mid X = x_m$, which describes how our guess about the outcome of Y changes when we know the input value was x_m. Thus, according to (see Equation I in Box 1), the conditional entropy is equal to

$$H(Y|X = x_m) = -\sum_{n=1}^{N} p(y_n|x_m)\log_2\ p(y_n|x_m). \quad [I]$$

Since the output Y depends on the input X, knowing that $X = x_m$ can decrease the uncertainty of Y and the conditional entropy updates the measurement of this uncertainty [70].

In the experimental setup, we typically select a range of inputs and measure the output value for each input. For example, we choose a range of TNF concentrations and for each concentration we measure the nuclear translocation of its target transcription factor NF-κB. Such a setup, via Equation I, allows us to calculate entropy

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conditioned on each input value x_m separately. Taking into consideration all possible inputs, we can measure the average uncertainty in Y as

$$H(Y|X) = -\sum_{m=1}^{M} p(x_m)\, H(Y|X = x_m)$$

$$= -\sum_{m=1}^{M} p(x_m) \sum_{n=1}^{N} p(y_n|x_m) \log_2\, p(y_n|x_m). \quad [II]$$

Conditional entropy Equation II, viewed in terms of a channel with input X and output Y, corresponds to our uncertainty in Y upon challenge with a range of stimulus concentrations described by X (see chapter 2 in [70]).

All GPCRs consist of seven α-helical transmembrane domains, an extracellular N terminus, three extracellular loops, an intracellular C terminus, and three intracellular loops [22]. GPCRs bind to different ligands including photons, ions, vitamins, lipids, peptides, hormones, and neurotransmitters [23,26]. GPCRs transmit the signal via heterotrimeric G proteins, composed of α, β, and γ subunits. In the resting state, the Gα subunit is bound to GDP. Agonist binding promotes a conformational change in the GPCR, unleashing its guanine nucleotide exchange factor (GEF) activity towards the G proteins. As a result, GDP dissociates from the Gα subunit and is replaced with GTP, further causing dissociation of the heterotrimer into Gα-GTP and Gβγ. Both parts are then active as intracellular signal transducers launching specific signaling pathways [22,23,25]. In mammals, the Gα subunits can be divided into four subfamilies: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_q$, and $G\alpha_{12,13}$. Each Gα protein activates specific downstream pathways (Figure 2C). Members of the $G\alpha_s$ subfamily mainly activate adenyl cyclase and increase the levels of cAMP [25]. This increase leads to activation of protein kinase A (PKA) and phosphorylation of the CREB transcription factor [27]. In contrast, the $G\alpha_{i/o}$ members decrease the levels of cAMP inhibiting the enzyme [28]. $G\alpha_q$ activates phospholipase C (PLC)β, which cleaves phosphatidylinositol bisphosphate into diacylglycerol (DAG) and inositol phosphate (IP_3), leading to Ca^{2+} release from the endoplasmic reticulum and stimulation of NF-κB or MAPK signaling [27,29]. Members of the $G\alpha_{12/13}$ family activate the small GTPase RhoA via the RhoGTPase nucleotide exchange factors [30]. Gβγ subunits activate diverse signaling molecules including kinases, for example, PI3Kβ and voltage-dependent Ca^{2+} and GIRK ion channels [31,32]. Moreover, Gβγ subunits stimulate MAPK signaling via Ras and Raf upon ligand binding to GPCRs [33].

Applications of Information Theory to Oncogenic Signaling Pathways

Noise, Dynamics, and Environment Impact Information Transmission by the NF-κB Pathway

One of the pioneering studies in the field of information processing in cells was conducted using tumor necrosis factor (TNF) as a ligand [34]. Surprisingly, the authors found that the TNF–NF-κB pathway transmits information sufficient only for binary decisions [34]. The **channel capacity** (Box 3) in a single cell was measured as 0.91 bits, meaning that a cell can at best distinguish if TNF is present or not. TNF activates NF-κB and JNK pathways resulting in nuclear translocation of NF-κB and activating transcription factor (ATF)-2. NF-κB alone provided 0.92 bits of information about TNF concentration while ATF-2 provided 0.85 bits. The authors developed a tree model that predicted approximately 1 bit for both pathways matching experimental results. This model suggests that the network possesses an information bottleneck and can process at most 1.26 bits of information. The biology of the TNF pathway indicates that the bottleneck occurs at TNF receptor activation, which includes ligand binding, receptor trimerization, and ligand–receptor complex formation [34]. Negative feedback mediated by A20, a TNF inhibitor induced by NF-κB, exerts a time-dependent effect on the amount of information at the bottleneck (it increases the information after 30 min but decreases it after 4 h) [34].

Although the binary channel capacity of the TNF pathway seems surprising, for cell populations maximization of **mutual information** (Box 3) may be a suboptimal strategy. Higher mutual information is

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associated with increased metabolic costs. In line with the previous results, Gillespie simulations of a model of the TNF– NF-κB pathway provide 0.9 bits for channel capacity and 0.6 bits for mutual information. This supports the idea that low values of mutual information might be beneficial in biological systems. The authors also show that fractional bit differences in mutual information can lead to significant differences in capabilities of a gene network to encode the input signal distributions [35].

In contrast, when the NF-κB dynamics and gene expression profile are studied together, the capacity of the NF-κB pathway to transmit information may increase. NF-κB dynamics show oscillations upon presence of external stimuli and plays a key role in the regulation of survival and apoptosis. Furthermore, it is often deregulated in cancer [36]. A recent study showed that NF-κB dynamics contain sufficient information to encode various TNF-dependent responses in cancer cell lines [37]. Thus, cells can grade their responses to different concentrations of TNF. Information transmission capacity was 1.2 or 1.4 bits for single cell time courses or as fold change of nuclear RELA, respectively. The real channel capacity may be obscured by the fraction of nonresponder cells. Upon removal of the nonresponder fraction, channel capacity calculated using RELA fold change increased to 1.7 bits. These results suggest that cells can process information about distinct TNF concentrations and elicit a graded response to further fine tune their responses to cytokines [37].

When studying the NF-κB pathway signal transmission capacity, various aspects of the system should be considered, including the ligand identity, changes in temperature and modifications of NF-κB subunits, and interactions among them. For example, physiologically relevant temperature changes shift NF-κB dynamics in single cells upon TNF challenge [38]. Also, different NF-κB ligands induce distinct NF-κB dynamics. In mouse fibroblasts, challenges with Toll-like receptor (TLR)2 or TLR4 ligands (synthetic triacylated lipopeptide Pam3CSK4 (PAM) and lipopolysaccharide (LPS)] elicit diverse NF-κB responses over time. Coactivation of TLR2 and TLR4 induces ligand-specific responses, called nonintegrative processing, rather than a mixed response on a single cell level. The cell-to-cell variability determines if the cells respond to PAM or LPS. This underlies the cooperation between different cell populations in fighting infections [39].

Timing is another factor that affects NF-κB dynamics and cell fate decisions. In individual human neuroblastoma cells, TNF pulses spaced at least by 100 min result in a high probability of NF-κB activation. However, pulse intervals below 100 min activate fewer cells. This suggests a refractory state

Box 3. Quantifying Channel Noisiness: Mutual Information and Capacity

Several research questions might be addressed with the experimental setup described in Boxes 1 and 2. How are the inputs encoded intracellularly? How is the information read and how is the internal representation of the extracellular changes involved in cellular decision making? Information theory provides a way to address these issues by introducing mutual information (Figure I). Mutual information can be applied to estimate how much information the output contains about the input signal (see chapter 2 in [3]). In other words, mutual information measures the dependence between random variables and how accurately the input value can be determined when we know the output value [3]. The mutual information between X and Y is defined as

$$I(X;Y) = H(Y) - H(Y|X). \quad [I]$$

On the right hand side of Equation I, we first calculate the entropy of Y and subsequently subtract the conditional entropy considering the knowledge of X. This means that mutual information measures the reduction of uncertainty in the output Y (see chapter 2 in [70]).

In biology, channels are often noisy meaning that the output is not completely specified by the input. Information theory enables the quantification of channel noisiness via capacity. Channel capacity is defined as the maximal mutual information over all possible distributions of the input $p(x)$ (see chapter 2 in [3]) (Box 2)

$$C = \max_{p(x)} I(X;Y). \quad [II]$$

Channel capacity has recently been applied to study biological signal transduction [37,52,57]. For example, it enables the measurement of the signaling accuracy. Specifically, 2^C is the largest number of different inputs, such as ligand concentrations, a cell can reliably distinguish.

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downstream of the TNF receptor, which depends on the level of the negative NF-κB regulator A20 [40]. Several seconds of exposure to TNF activate NF-κB in HeLa cells and induce apoptosis. However, a 1-min pulse seems more effective at killing than 1-h stimulation [41].

Noise also needs to be considered when investigating NF-κB pathway signal transmission. Although, noise in signal transduction was previously considered harmful, current research shows that noise helps cells to achieve robust gene expression in constantly changing environments and improves signal quality [42]. In fibroblasts under periodic cytokine challenges, NF-κB dynamics synchronizes with the oscillating TNF input and becomes entrained. Entrainment increases the NF-κB oscillation amplitude and the transcriptional output. The **intrinsic biochemical noise** at a single cell level increases the NF-κB oscillation entrainment, while the **extrinsic noise** creates the cell-to-cell variability in the NF-κB natural period and enables robust response at the population level. Crosstalk between oscillations and noise enables the establishment of an efficient transcriptional profile in the cells experiencing the changing environment [43].

Moreover, both extrinsic and **intrinsic noise** affect the apoptosis/survival decisions [44]. The effects of noise on the signaling pathway differ at the population and individual cellular levels. When a population of cells needs to behave in a coordinated manner, noise increases information transfer capacity at the population level. The channel capacity in single cells, measured between the dose of TNF-related apoptosis-inducing ligand (TRAIL) and the activation of the initiator caspases is approximately 1 bit, while the channel capacity between TRAIL and the effector caspases is 0.56 bits. In contrast, at the population level, channel capacity is 3–3.4 bits depending on the population size [45]. Therefore, when we consider a signaling system with a binary output (live or die decision), heterogeneity within the population generated by noise allows the precise control of behavior of the fraction of cells above or below the decision threshold. In the control of apoptosis, the crucial variable is the fraction of cells responding at the particular dose. When behavior of a single cell plays a key role (e.g., in chemotaxis or mating), high levels of information are transferred meaning that noise is likely suppressed [46]. For example, in chemotaxis, higher channel capacities (around 2 bits) have been found [45].

Feedbacks and Dynamics Enhance Information Transfer by ERK While Noise Generates Cellular Heterogeneity

The information theory framework has also been applied to study signal transmission by the ERK pathway. A study conducted in PC12 cells evaluated the transmission of information for growth-factor-mediated gene expression using phosphorylated ERK1/2, phosphorylated CREB, and protein concentrations of c-FOS and early growth response protein 1 (EGR1) in cell populations as readouts [47]. Upon challenge with nerve growth factor (NGF), the mutual information between NGF and pCREB measured in the experiment reached 1 bit. Moreover, the mutual information between NGF and immediate response early genes (*cFOS* and *EGR1*) was also 1 bit, suggesting that those genes receive enough information for binary decisions from NGF. Upon the application of specific MAPK inhibitors, signaling pathways showed robustness and compensation at the cell population level [47]. In HeLa cells, the mutual information between gonadotropin-releasing hormone receptors and ERK was below 1 bit indicating that individual cells cannot distinguish even two input concentrations (such as no input and some input) [48]. In line with these findings, the channel capacity of ERK2 in human lung cancer cells upon challenge with epidermal growth factor (EGF) reached approximately 1 bit [34].

Both negative and positive feedback affect biological signal transduction. A systems biology study investigated the effects of cell-to-cell variability and basal network activity (propensity for activation in the absence of stimulus) on the information transfer, comparing the systems with and without negative feedback [49]. In line with the data discussed above, mutual information between stimulus concentration and nuclear activated ERK at a single cell level was measured as approximately 1 bit. Negative feedback protects information transfer from the effects of noise and increases mutual information between the signal and the phosphorylated substrate concentration [49]. Negative feedback from

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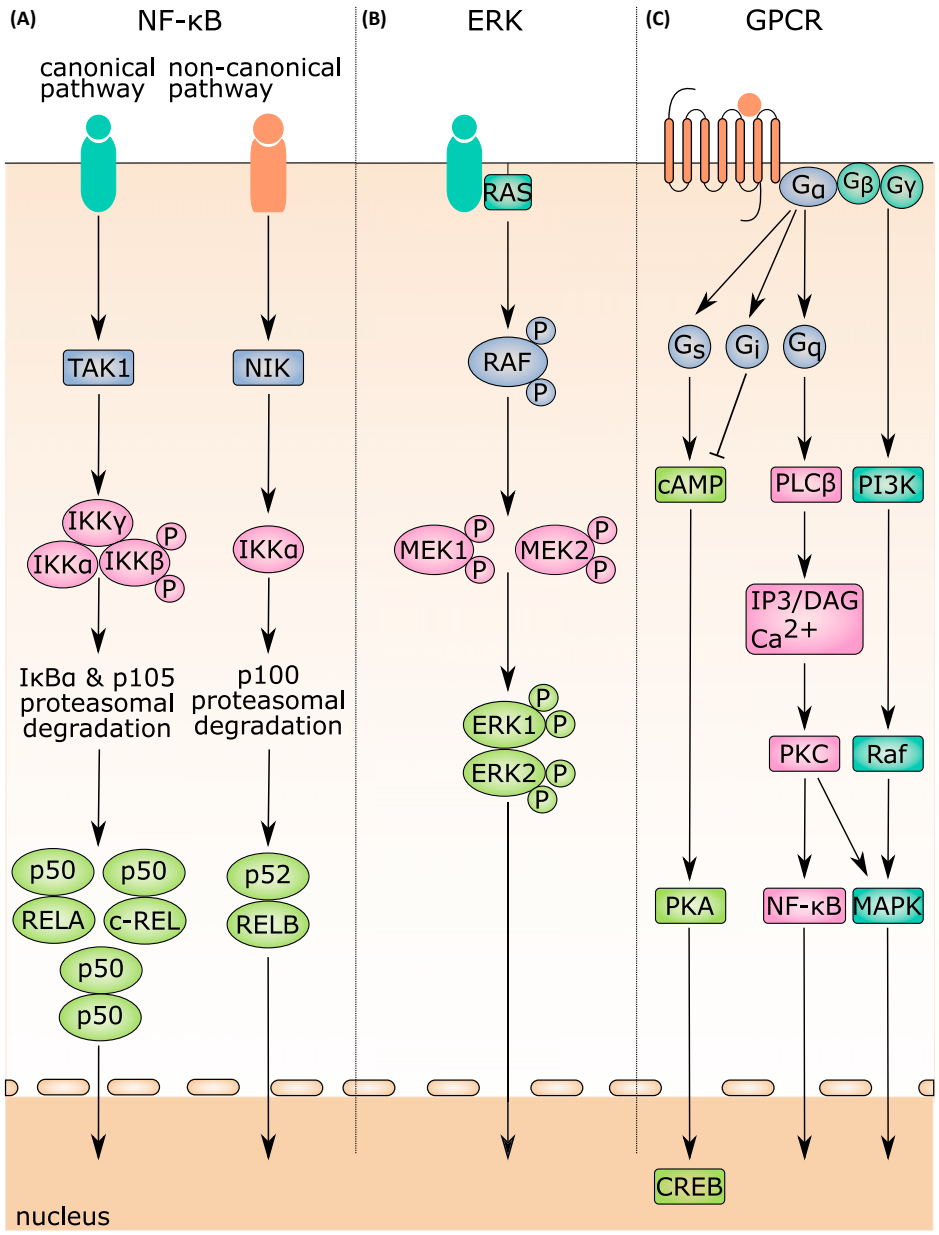

Figure 2. Overview of Nuclear Factor (NF)-κB, Extracellular Signal-Regulated Kinase (ERK), and G-Protein-Coupled Receptor (GPCR) Signaling Pathways.

(A) The canonical NF-κB pathway is triggered by inputs from a variety of immune receptors that activate TAK1 and in consequence the trimeric IKK complex. IKK phosphorylates and targets for proteasomal degradation IκBα and p105. Subsequently, dimers of canonical NF-κB family members translocate to the nucleus. The activation of the noncanonical NF-κB pathway depends on the NF-κB-inducing kinase (NIK), which phosphorylates IKKα. Next, IKKα phosphorylates p100 leading to its degradation, generation of p52, and nuclear translocation of p52/RELB dimers. (B) The ERK pathway is activated by growth factors mostly via receptor tyrosine kinases (RTKs). RTKs activate Ras leading to Raf recruitment and MAPK kinase (MEK)1/2 activation. Finally, MEK1/2 phosphorylate ERK1 and ERK2 leading to their nuclear translocation. (C) Ligand binding to the GPCRs activates the G protein signaling mediated by the heterotrimeric complex consisting of Gα, Gβ, and Gγ subunits. Each of the subunits

(See figure legend continued at the bottom of the next page.)

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activated ERK plays a crucial role in the adaptation of the ERK nuclear oscillation characteristics to a broad range of ligand concentrations [50]. Moreover, both positive and negative feedback loops translate EGF stimulation into ERK pulses of constant amplitude but concentration-dependent frequency and duration [51]. A positive feedback loop with Ras and its activator SOS allows switch-like responses to emerge. This positive feedback is nested within a negative one with Ras, Raf, MEK, and ERK that phosphorylate and inhibit SOS. The negative feedback operates on a longer time scale and translates the switch-like response into oscillations with at least a 1-h period determined by the signal strength. The feedbacks considered in this study encode a graded input into constant-amplitude pulses, therefore the concentration of the stimulus is translated into the language of the frequency of oscillation and the pulse duration. The authors also suggest that the amplitude-to-frequency coding might decrease uncertainty in signal interpretation and increase the channel capacity [51].

Although many experimental applications of information theory to signal transduction pathways have shown that different signaling systems can handle approximately 1 bit of information at the single-cell level, computational models suggest significantly higher capacities of information transmission in cells. The majority of signaling modules can encode significantly more information (3–6 bits) than previously observed in experiments [52]. Similarly, analysis of the levels of noise in gene expression suggests that in signaling leading to transcriptional regulation, it should be feasible to transmit more than 1 bit of information [53].

Simple signaling motifs, such as ligand–receptor interactions or post-translational modification cycles are predicted to handle more than 5 bits of information [52]. As the complexity of the network increases, the amount of information it can contain decreases; however, the authors predict that signaling cascades can still transmit more than 3 bits of information. Clearly, more experimental work – and perhaps more adequate experimental models for single-cell signaling analysis – should be applied to assess whether the cellular signaling systems can reach these computationally obtained limits.

Low experimental values of channel capacity might be due to the extrinsic noise present in cells [45]. Extrinsic noise (in particular that upstream from MEK) plays a key role in the generation of cell-to-cell variability in ERK phosphorylation. Simulations of the system and estimation of the mutual information between the total amount of phosphorylated MEK and ERK at different time points show that the presence of extrinsic noise decreases the expected information flow between MEK and ERK [54]. The authors suggest that extrinsic noise plays a crucial role in cell-to-cell variability in the MEK–ERK system. Although at the first glance MEK–ERK might seem improperly tuned, this feature is predicted to prevent response to biologically irrelevant signals [54].

Cellular heterogeneity related to intrinsic and extrinsic noise plays an important role in drug resistance, cell survival, chemotaxis, and other cellular activities and can be both harmful and beneficial. Cellular noise also affects the output of EGF and ERK signaling. A mathematical model of EGF signaling includes a crosstalk between ERK and the nuclear pore complex that plays an essential role in the switch-like activation of ERK nuclear activities. Results obtained from simulations show that extrinsic noise contributes to the cellular heterogeneity of nuclear ERK responses. Moreover, variability of the EGF receptor, MEK, Ras, and Raf mediates cell-to-cell heterogeneity and their levels can be used to predict cellular responses. These proteins act as sensitive nodes and their deregulation might play an important role in the pathogenesis of diseases [55].

elicits specific downstream effects. G_s increases cAMP levels and activates protein kinase A (PKA) Aresulting in phosphorylation of CREB. In contrast, G_i decreases cAMP levels. G_q activates phospholipase C ((PLCβ), leading to the generation of inositol phosphate (IP3) and diacylglycerol (DAG) and increase of Ca in the cytosol. Subsequently, PKC activates NF-κB and MAPK signaling. The Gβγ complex also activates MAPK signaling. Abbreviation: MAPK, mitogen-activated protein kinase.

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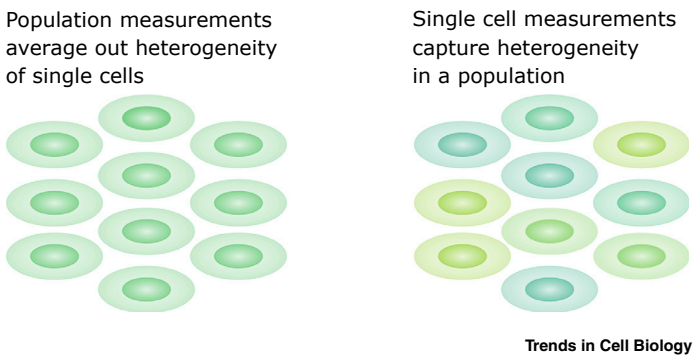

Figure 3. Measurements at Different Biological Scales, Population, and Single Cells, Provide Dissimilar Estimates of the Information Transmission Capacities of Signaling Pathways.
Population measurements obscure cell-to-cell variability and may underestimate information transmission capacities of single cells. In contrast, single cell measurements reveal heterogeneity among cells that emerges due to their different biological states and can capture their accurate information transmission properties.

Mechanisms of robust functioning of signaling pathways under noisy conditions have attracted the attention of systems biologists. A recent systems biology study analyzed if signaling dynamics could reduce noise-related information loss. The response dynamics in ERK, as in Ca^{2+} and NF-κB pathways, increased the measured information transmission capacities when compared with nondynamic responses [46]. Previous measurements of information transmission in the ERK pathway under varying signal-to-noise ratios also suggested that signaling dynamics can compensate for noise-induced information loss [56]. Other studies, which applied information theory-based approaches to study signaling networks used scalar measurements taken at a single time point [34,47]. However, the information on ligand concentrations is usually encoded by a multivariate vector of single cell responses at different time points. The channel capacity of the dynamic response in MCF10a (mammary gland) cells for all three signaling pathways was significantly higher than for static scalar responses (below 1 bit) [46]. Since the accurate transmission of information by cellular signaling networks plays a crucial role in ensuring proper function of the cell, it seems likely that evolution maximized the ability of cellular systems to decode multidimensional dynamic signals from the environment [46].

Single Cell Approach Reveals High Channel Capacity of GPCR Signaling

Single cell measurements help to distinguish between extrinsic and intrinsic noise (which influences the performance of signaling networks) and remain indispensable when evaluating information transmission capacities of signaling pathways. We studied channel capacity at a single cell level in HEK293 cells focusing on the M3R GPCR receptor [57]. In our experimental setup, cells were challenged with multiple pulses of the M3R agonist acetylcholine at various concentrations. This setup permitted us to obtain the estimates of channel capacity above 2 bits. Therefore, the M3R signaling system can reliably distinguish between at least four different concentrations of the ligand [57]. These results remain in contrast with some previous studies, which provided lower values for the information transmission capacity of the GPCR pathways. For example, in RAW264.7 macrophages upon activation of P2Y GPCRs with uridine diphosphate, channel capacity was estimated approximately at 1 bit [34]. In these studies, cell populations were challenged with different concentrations of the ligand; however, an individual cell was never exposed to more than one concentration of a stimulus [34,47]. As significant variability in GPCR (P2Y and M3R) signaling among individual cells exists, caused by long-lived cell state differences in the levels of the receptor and downstream signal transducers [57,58], the averaged measurements from a cell population are likely to obscure the real channel capacity of a single cell (Figure 3). These considerations bring us again to the issue central to the applications of information theory tools to signaling in individual cells – the necessity to design an experimental setup adequate to assess the channel capacity in the presence of both extrinsic and intrinsic noise. We will elaborate more on this issue in the next section.

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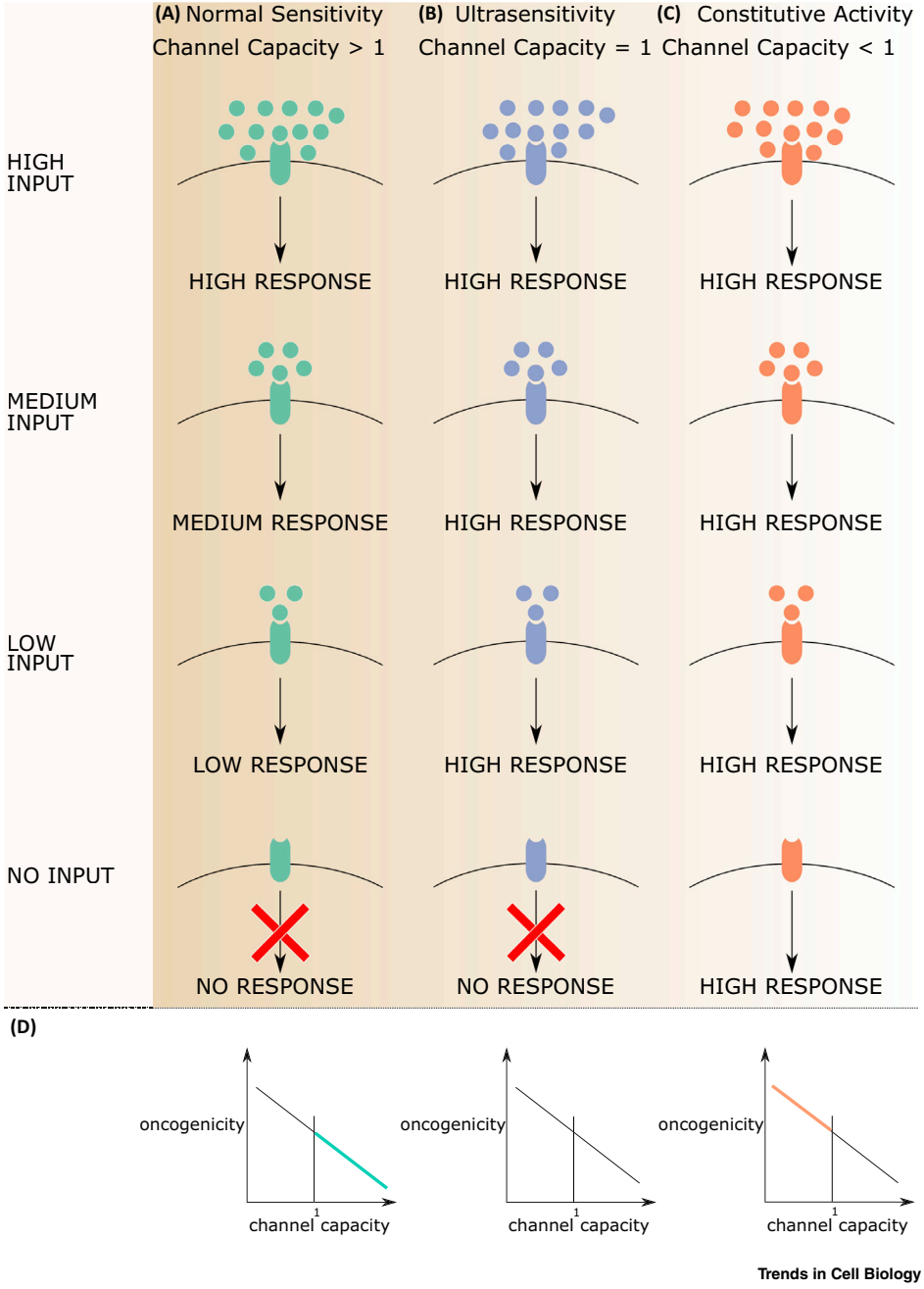

Figure 4. Low Channel Capacity Might Underlie Cancer Development.

(A) Healthy cells possess high channel capacity and are able to distinguish different input levels and grade their responses. High input elicits strong activation of the pathway which decreases with the concentration of input. In the absence of stimulus, the pathway remains inactive. (B) When channel capacity decreases to 1 leading to ultrasensitivity, the cell fails to distinguish different concentrations of the ligand and elicits all-or-none response. (C) Under pathological conditions channel capacity further drops below 1 resulting in constitutive activity. Therefore, the signaling system produces a uniform response. (D) Channel capacity of a signaling system is expected to correlate with oncogenicity. Both ultrasensitivity (middle graph) and constitutive activity (left graph) are likely to induce oncogenic properties in a cell such as proliferation and migration.

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Concluding Remarks

A plethora of 'wet' and 'dry' studies shows that information theory constitutes an excellent tool to study biological signal transduction. Three major questions emerge from the applications of the information theory to intracellular signal transduction performed so far (see Outstanding Questions).

The first question relates to the fundamental properties of signal transduction by single cells, underlying the importance of experimental design to address the channel capacity of cell signaling. For example, cell population assays may obscure responses of single cells whereas single cell measurements capture the heterogeneity within a population (Figure 3).

The second question highlights the potential of information theory to receptor pharmacology and drug sensitivity studies. It is conceivable that a code can be proposed to translate the basic findings on signal transduction delivered by information theory. Full agonist/antagonist may engage full channel capacity, with partial agonist/antagonist engaging decreased channel capacity. Well-designed experiments are required to address whether such a code may exist.

The third question addresses how the fundamental properties of cellular signaling change in pathology. It has recently been proposed that certain pathological conditions such as cancer can be viewed as information diseases [59]. We suggest that it is crucial to compare the information transmission capacity of cancerous and healthy cells. If we start from a hypothetical healthy cell, armed with a high channel capacity of, for example, approximately 2 bits, such a cell will be able to differentiate among and respond differently to four different levels of the activating stimulus: no pathway activation in the absence of the stimulus; low activation in response to low input; and intermediate and high levels of activation in response to intermediate and high concentrations of the stimulus (Figure 4A). If we suppose that in a pathological condition, the channel capacity of this cell decreases to 1 bit, the cell will no longer be able to discriminate between different levels of the stimulus, but will simply provide an all-or-none response (Figure 4B). This is the scenario of ultrasensitivity in the signaling response, and is often associated with a carcinogenic state, when a cell goes into proliferation no matter how high or low the growth factor input is [60]. We can consider further aggravation of the system, when the channel capacity drops below 1 bit, associated with constitutive activation of the oncogenic pathway, independent of the presence or absence of the external stimulus (Figure 4C). Deletion mutations in the negative components of a signaling pathway or gain-of-function mutations in the positive components of a pathway may promote such ligand-independent signaling(reviewed in [61–63]). Of note, channel capacity below 1 bit may also mean complete lack of signaling, which may occur through mutational inactivation of the pathway, and can lead to pathological states.

Shedding light on the fundamental principles of signal transduction in the context of cancer may provide a novel means for therapy development. Indeed, current logic is to develop drugs wiping out a given signaling pathway – with inevitable adverse effects to healthy tissues depending on certain levels of activation of the same pathway [7]. In contrast, pharmacological tuning of the pathway may instead be searched for, such that the reduced channel capacity of an oncogenic pathway is brought back to normal levels. Such an approach may work against constitutive activation or ultrasensitivity of the pathway, reducing the oncogenic properties of tumor cells (Figure 4D), without elimination of signaling in healthy counterparts. Experiments in this direction will show how far-fetched or realistic these ideas and approaches could be. Although these applications of the information theory to cell signaling are challenging tasks, we believe that this new branch of systems biology will continue to expand and that exciting discoveries are ahead.

Outstanding Questions

Channel capacity of intracellular signaling: all-or-none or multibit pathways?

Information theory – a new look at receptor pharmacology?

Channel capacity in pathological states – is cancer an information disease?

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