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Review

Dynamic signal encoding—From cells to organisms

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ABSTRACT

Encoding information at the level of signal dynamics is characterized by distinct features, such as robustness to noise and high information content. Currently, a growing number of studies are unravelling the functional importance of signalling dynamics at the single cell level. In addition, first insights are emerging into how the principles of dynamic signal encoding apply to a multicellular context, such as development. In this review, we will first discuss general concepts of information transmission via signalling dynamics and recent experimental examples focusing on underlying principles, including the role of intracellular network topologies. How multicellular organisms use temporal modulation of specific signalling pathways, such as signalling gradients or oscillations, to faithfully control cell fate decisions and pattern formation will also be addressed. Finally, we will consider how technical advancements in the detection and perturbation of signalling dynamics contribute to reshaping our understanding of dynamic signalling in developing organisms.

Contents

Abbreviations: NGF, nerve growth factor; DSB, double strand break; SSB, single strand break; ATR, ataxia telangiectasia and Rad3 related protein; ATM, ataxia telangiectasia mutated protein; Shh, sonic hedgehog; DPP, decapentaplegic; NPC, neuronal progenitor cell; PSM, presomitic mesoderm; RA, retinoic acid.

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1. Introduction

In 1957, Conrad Waddington (1905–1975) noted that "the main respect in which the biological picture is more complicated than the physical one is the way time is involved in it" [1]. This is particularly apparent during development of a multicellular organism. Starting from a single cell, the generation of a complex organism requires not only tight regulation in space, but also in time. How a multitude of complex information (both in identity and intensity) is processed to allow development is a central question that is still not fully understood. Strikingly, this is accomplished by only a limited number of conserved signalling pathways.

Theoretical considerations implied early on that signalling dynamics might offer additional properties and layers of information encoding. Signalling dynamics, in contrast to a static "either or" perspective, describes the temporal evolution of a signalling system [2]. Excitingly, technical advancements in visualizing and perturbing intracellular signalling now enable experimental approaches to investigate the significance of dynamics at the signalling level. Indeed, experimental evidence indicates that upstream stimuli can be encoded in the dynamic properties of signals, such as delay, duration, fold-change or frequency (see Fig. 1). Here, we discuss recent experimental progress in understanding signalling dynamics, signal encoding and the implications for development of multicellular organisms. We will first review general principles of dynamic information transmission (Sections 2 and 3) before turning to specific examples in developing organisms (Section 4).

2. Properties of dynamic signal encoding

Dynamic signal encoding is characterized by robustness to noise, high information content and the possibility for temporal organization [3,4]. In recent years, it has become apparent that these general features are also an integral part of biological information encoding.

2.1. Robustness

For efficient cellular communication, the information has to be robust to noise and convey the message in identity and quantity [5]. In biology, noise is generated by random fluctuations and changes of environmental conditions such as pH or temperature, which in turn impacts on the kinetics of all cellular reactions.

In general, a digital signal is more robust to noise than an analogue one, as long as the noise amplitude is smaller than the discrete quanta of the digital signal. Several studies provide theoretical and experimental evidence that digital encoding in the signal frequency or in the fold-change of a signal is more robust and resistant to information loss by noise than detection of absolute levels [6–10]. In the human colon carcinoma cell line RKO or *Xenopus laevis* embryos a fold-change in β-Catenin levels is detected upon stimulation with Wnt [7]. Similarly, in the non-small cell carcinoma cell line H1299 a fold-change in nuclear levels of ERK2 (extracellular signal-regulated kinase 2), a mitogen-activated protein kinase (MAPK), rather than absolute ERK2 level is relevant for the downstream response upon stimulation with the epidermal growth factor (EGF) [8]. By encoding information in the fold change instead of the absolute level, cell-to-cell variations in basal signalling activity can be compensated. Indeed, perturbations of the Wnt signalling pathway, which alter the baseline but preserve β-catenin fold-change, do not interfere with the dorsoanterior development of *X. laevis* [7].

2.2. Increasing diversity through dynamic signal encoding

How a very limited number of conserved signalling pathways can encode a wide variety of distinct downstream responses is a longstanding question in the signalling field. Dynamic information encoding can be seen as powerful strategy to provide additional versatility: one and the same pathway can elicit different outcomes depending on its dynamics. Information can, for example, be encoded at the level of signal delay, duration, fold-change or periodic signalling frequency.

This concept can be exemplified using signalling gradients in development: the classical (static) view emphasizes the *amount* of stimulus as key determinant [11]. This view is currently undergoing drastic change. Accordingly, gradients can encode information in versatile ways, including the rate at which a signal changes in time [12]. In this *dynamic* view, two gradients with greatly differing absolute amounts that decay with the same kinetics over time, could elicit similar downstream responses (Fig. 1).

2.3. Temporally ordered cells as basis for higher-order spatiotemporal patterning

Within assemblies of cells, signalling dynamics, such as oscillations, have an additional, critical potential: whenever coupling between cells enables temporal synchronization, it can form the basis for subsequent patterning that requires spatial, but also temporal coherence. Development of multicellular organisms has been one context in which such coherent spatiotemporal signalling dynamics have been identified [3,11,13–16] and we will review specific examples (see Section 4).

In the following, we will discuss specific modes of dynamic information encoding in more detail as well as recent experimental work that provided novel insights into underlying mechanisms.

3. Dynamic information transmission

In engineering a message is sent by a source, encoded (by the *transmitter*), transferred and decoded (by the *receiver*) before reaching the destination [5,17]. Similar principles apply to biological information transmission, in which encoding and decoding are accomplished by complex signalling networks. The topology of the network defines signal dynamics and the consequent output. To allow information encoding in signalling dynamics, more complex molecular networks are necessary than for encoding information only in the absolute signal [4]. Interestingly, the signalling topologies that have been identified so far involve recurrent motifs, such as feedback or feedforward loops [4]. For instance, a steady stimulus can lead to oscillatory signalling based on a limited set of topological requirements, such as a delayed negative feedback loop [18].

3.1. Network topology matters

The importance of the network topology for the resulting dynamics of a signalling pathway can be illustrated at the level of p53 signalling in response to DNA breaks (Fig. 2): whereas double strand breaks (DSBs) result in *oscillations* of p53 levels with a period of 4–7 h, single strand breaks (SSBs) induce a single pulse of signalling [19–21]. The underlying mechanism that generates these different dynamic outcomes involves subtle changes in the topology being employed, in particular an additional negative feedback present in the DSB-induced network, but lacking in SSB-induced signalling (Fig. 2). While double strand breaks are digitally encoded in the *number* of p53 oscillations (damage correlates with number of oscillations), the extent of SSBs is encoded in signal *duration* and *amplitude* [22]. Interestingly, in the context of p53, the functional relevance of the different dynamic responses has been addressed. Purvis et al. [23] used a small molecule inhibitor to change p53 dynamics upon DSBs from oscillations to sustained activity reflecting SSB-specific signalling dynamics. Instead of only promoting cell

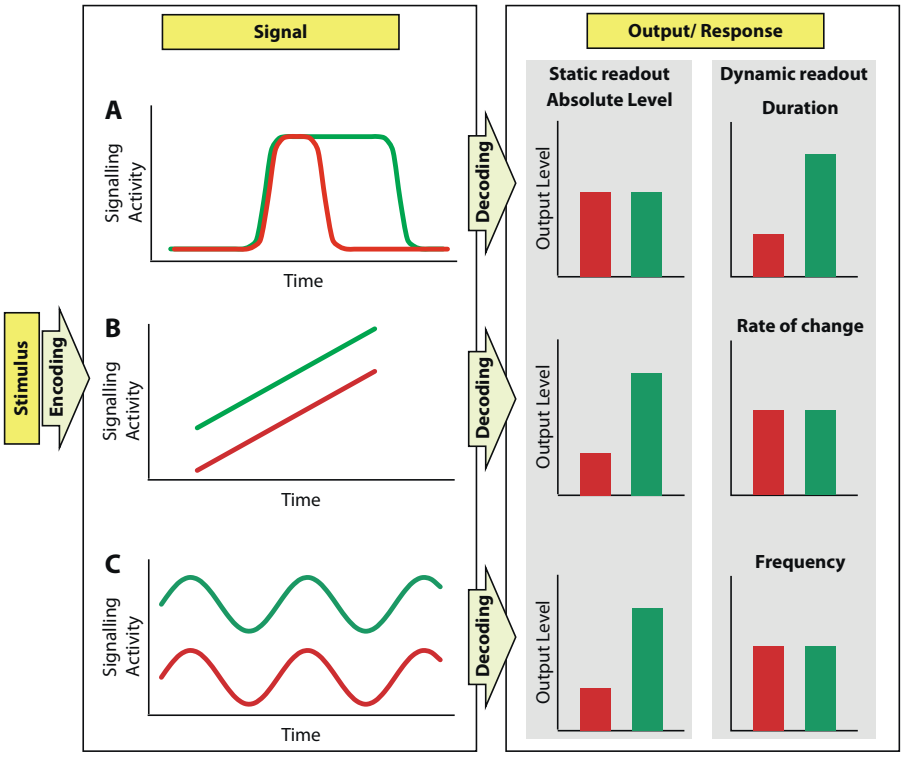

Fig. 1. Principles of dynamic information transmission. An upstream stimulus is encoded in a specific intracellular signalling event. The signal is then decoded to induce a cellular response. In these examples, either absolute signalling levels or signalling dynamics, e.g. signal duration, rate of change or frequency, can be read out. Decoding of different features of a dynamic signal can result in quantitatively (and qualitatively) different responses. For instance, two signals with the same amplitude but different durations induce the same response, if absolute levels are taken into account, but induce different responses, if signal duration is critical (A). Signals that show different absolute levels but increase, i.e. ramp, with an identical slope can induce the same response, if the rate of change is taken into account (B). Information can be encoded in oscillation frequency and hence signals at different absolute levels, but with identical frequency, result in the same cellular response (C).

cycle arrest, this prolonged activity led to changed gene expression and the induction of senescence and pro-apoptotic factors.

In the following sections, we will address encoding of information in certain features of a dynamic signal and corresponding decoding mechanisms. Whenever known, relevant network motifs will be discussed.

3.2. Signal duration

Certain network topologies have been implicated in encoding information in the *duration* of a signal. For instance, coherent feed-forward loops (e.g. X activates Y, both X and Y are then necessary to activate Z [4]) can integrate stimulus duration, since these topologies ensure that a signal is only generated by a prolonged stimulus and hence function as persistence detectors. As such, noise in the form of short stimulus fluctuations are buffered and do not lead to a downstream signalling event [4].

Several molecular examples for duration encoding have been identified. Besides p53 signalling in response to SSBs (see Section 3.1), ERK signalling in PC12 cells is an example for encoding stimulus identity in signal duration. In PC12 cells, NGF induces persistent ERK activity via the receptor tyrosine kinase (RTK) TrkA and leads to differentiation, whereas EGF binds to the EGF receptor (EGFR) and induces only transient activation and results in proliferation [24–26]. As in the examples above, the differences in ERK dynamics can be traced back to different network topologies being employed upon NGF or EGF stimulation [27].

Artificially mimicking persistent activation of ERK by expression of a constitutively active MAPK kinase was sufficient to promote neurite formation in unstimulated cells [24]. Toettcher et al. [28] used optogenetics to investigate the effect of different ERK signal durations on NIH3T3 cells (mouse fibroblast cells). By inducing ERK signalling with varying durations they identified fast responding genes, activated by ERK pulses of 20 min each, and other genes, such as STAT3, that were only activated after a longer activity period of 120 min.

In 2002, Murphy et al. proposed a molecular explanation for the decoding of ERK signal duration. ERK signalling, transient or sustained, induces the expression of immediate early genes such as the transcription factor c-Fos. However, only upon sustained ERK signalling, c-Fos is additionally phosphorylated by ERK, leading to the stabilization of c-Fos. In contrast, in transient ERK signalling c-Fos is upregulated but remains unstable at the protein level [29]. Therefore, proteins like c-Fos can function as persistence sensors for induction of downstream effects such as the neuronal differentiation in PC12 cells in situations of prolonged signalling.

3.3. Frequency encoding

Periodic signals are abundant in biology, showing a wide spectrum of forms, from sharp pulses similar to a relaxation-type oscillator to sinusoidal oscillations [2], and covering largely varying time scales, such as the circadian clock [30] with a period of approximately 24 h or calcium oscillations with periods in the milliseconds range [18,31,32]. Periodic signals offer particularly diverse possibilities for information encoding: amplitude, frequency, phase, oscillation form, number of oscillations are some of the levels at which information can be encoded.

Frequency encoding indicates that the quantity and quality of an upstream stimulus controls the frequency of a signal (which is hence frequency modulated, FM) and downstream responses are decoded based on these signal frequencies. Numerous examples for frequency encoding are known, including classic examples from neurophysiology, such as encoding of stimuli at the level of

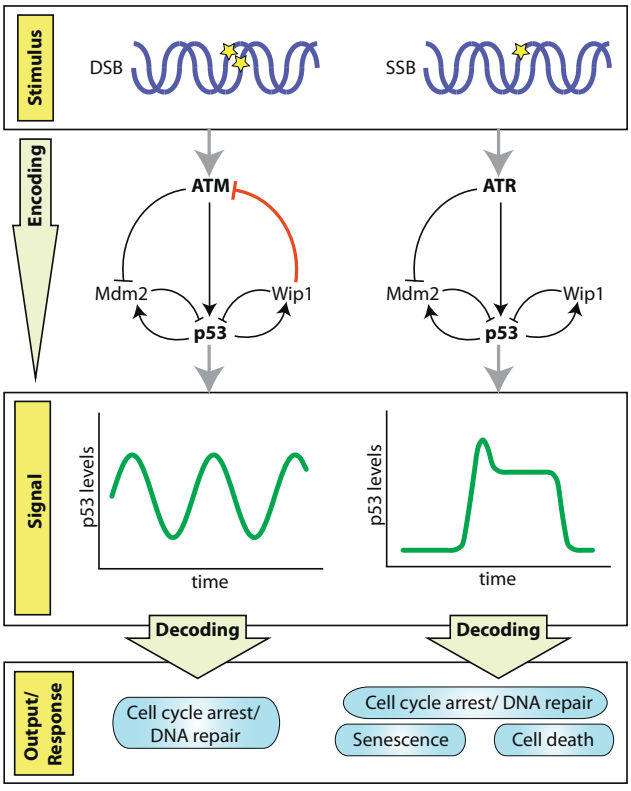

Fig. 2. Network topology defines p53 signalling dynamics upon DNA strand breaks. Whereas DNA DSBs lead to the activation of the kinase ATM, SSBs induce the activation of the kinase ATR. Due to differing signalling networks, p53 signalling either oscillates (in the case of ATM activation) or is transiently activated (in the case of ATR activation). p53 induces the phosphatase Wip1, which inhibits ATM, thus forming an additional negative feedback loop, which results in the formation of oscillations. In contrast, ATR is not inhibited by Wip1 [21]. The signal is then decoded to induce particular cell responses.

action potential frequency [33]. More recently, several signalling pathways have been shown to employ frequency modulation and encoding as well [34,35].

3.3.1. ERK signalling

A major signalling pathway using frequency encoding is the MAPK cascade. Continuous stimulation of MCF-10A cells (human mammary epithelial cells) with EGF results in multiple ERK activity pulses, in which the duration and frequency of ERK pulses correlate with EGF stimulus concentration [36]. Toettcher et al. [28] used optogenetics to investigate the role of frequency modulation in ERK signalling. By inducing ERK signalling pulses with varying stimulus frequencies, they found that the ERK signalling network functions as a low-pass, high-bandwidth filter: ERK was not activated at high frequencies (hence low-pass), but at the same time, could encode a wide range of frequencies, i.e. from 4 min to 2 h (high bandwidth). Evidence for frequencies indeed encoding information was obtained by analyzing the effect of varying frequencies on the ability to induce proliferation: quantitative response curves indicated that proliferative stimuli correlated with signal frequency, strongly supporting the presence of frequency encoding [36]. How these network dynamics are transmitted on the molecular level to induce the cell cycle remains to be determined.

3.3.2. NFκB signalling

NFκB signalling has also been implicated in frequency encoding. This signalling pathway oscillates with NFκB periodically shuttling between nucleus and cytoplasm upon stimulation by the inflammatory cytokine TNFα [37,38]. After its activation, NFκB shuttles to the nucleus, where it activates the transcription of various genes, among them IκBα [39], which inhibits NFκB and thus forms a delayed negative feedback. Ashall et al. [40] induced nuclear localization pulses with varying frequencies by periodically stimulating cells with high doses of TNFα. Remarkably, the expression of late genes, like the chemokine RANTES, is only induced with high frequency pulses, whereas early or middle genes are also expressed at lower frequencies.

3.4. Number of signal oscillations

Periodic signals also allow digital information encoding in the *number* of oscillations. For example, p53 signalling in response to DSBs has been shown to employ oscillation-number encoding (see Section 3.1) [22].

NFκB signalling, which has been implicated in frequency encoding (see Section 3.3), has additionally been suggested to rely on signal encoding based on the *number* of induced nucleocytoplasmic NFκB oscillations [41]. Using a microfluidic system to investigate single-cell dynamics of NFκB shuttling the authors found that increasing the dose of TNFα was followed by increasing number of oscillations (while no change of frequency was seen in this context). Accordingly, while during the first oscillation wave only early genes like IκBα were induced, the activation of late genes, such as pro-apoptotic factors, was only seen after multiple nuclear localization peaks [41].

Very recently, a counting mechanism underlying the classic example of cAMP oscillations in *Dictyostelium discoideum* has been revealed to connect stimulus (i.e. starvation) to output (i.e. induction of gene expression) [10].

3.5. Fold-change detection

Another type of dynamic signal encoding employs fold-change detection of either stimulus or signal. Hence, instead of detecting absolute levels, the relative change is relevant for the downstream response. In fact, the perception of a stimulus relative to the baseline is a common feature in physiological sensing and was first described in 1834 by Ernst Weber for the perception of tactile impulses [33].

At the signalling level, it has been found that Wnt and EGF signalling pathways induce a fold-change response in downstream signalling in certain cell lines. Accordingly, perturbations that change the overall signalling intensity but leave the fold change intact do not change the downstream response (as discussed in Section 2.1) [7,8]. Similarly, in TNF-induced nucleocytoplasmic shuttling of NFκB in HeLa cells not absolute nuclear NFκB level is detected, but rather its fold change, thus compensating for stochastic fluctuations of signalling components [42].

Fold-change detection can be accomplished by network topologies capable of adaptation. Adaptation or perfect adaptation indicates that – despite continuous stimulation – signal duration is limited to a transient activation period before returning to a steady state or even the pre-stimulus level, respectively. Two network motifs have been proposed to account for adaptation: (a) an incoherent feedforward loop (incoherent feedforward loop type 1 (I1-FFL): factor X activates both Y and Z and then Y inhibits Z [4]) or (b) an integral feedback circuit including a buffering node (e.g. X activates Z, which in turn activates Y, which then inhibits Z) [43]. In both cases, the downstream target is first activated and inhibited after a delay. In the above-mentioned examples of Wnt, EGF and NFκB signalling an incoherent feed-forward loop has been suggested to execute the fold-change detection [42,44], whilst it has not been shown whether a network motif containing a buffering node can also allow fold-change detection [45]. It will be interesting to find out whether such a detection mechanism is common

in signalling pathways employed during development, as recent findings indicate ([12], see Section 4.1).

4. Signalling dynamics during development

So far, most studies on signalling dynamics addressed in previous sections have been performed with single cells. However, similar mechanisms probably govern aspects of development in multicellular organisms. With technical advancements in real-time imaging researchers have started to quantitatively and functionally analyze signalling dynamics in multicellular organisms. For proper development pattern formation is essential, which requires organization of the whole cell population in time and space. While theoretical studies provided the conceptual framework how higher-order signalling dynamics could form the basis of patterning [13,14,46–48], more recently, experimental evidence for the significance of signalling dynamics is emerging.

4.1. Dynamic readout of signalling gradients

During development gradients of signalling molecules provide long-range spatial stimuli for embryonic patterning [11,47,49]. Classically, these signalling molecules, termed morphogens, were proposed to induce concentration-dependent cell specification [31,50]. In contrast to the classical view, it has become increasingly clear in recent years that firstly, morphogen gradients are dynamic and can change over time [51–53]. Secondly, gradients cannot only be read out in absolute levels but also by signalling duration or fold change within each cell [12,53–55]. Interestingly, during plant development the flux of the morphogen Auxin across cells has also been proposed to encode information [56].

In the developing neural tube, a gradient of sonic hedgehog (Shh) is essential for the development and specification of distinct neuron types [57–59]. The gradient of Shh originates from the ventrally located notochord and floor plate and is highly dynamic, as gradient amplitude and range increase over time [60,61]. Importantly, both morphogen concentration and also duration of exposure were found to determine differentiation in the neural tube and the Shh inhibitor patched 1 (PTC1) has been implicated in the molecular mechanism encoding signal-duration [53]. Decoding of the Shh gradient (in level and duration) in combination with the activity of SoxB1 transcription factors determines the sequential expression of downstream transcription factors for the specification of neural tube cells [53,62–64].

In addition, morphogen gradients can also be decoded by a fold-change-detection mechanism, such as in the case of the decapentaplegic (Dpp) gradient during *Drosophila* wing disc development [12]. Dpp is produced along the anteroposterior compartment boundary in the centre of the wing disc [65–68] and a spatial Dpp gradient is found across the wing disc. In addition, Dpp levels increase over time during growth of the wing disc, forming a temporal Dpp gradient [12,51,69,70]. Interestingly, despite different absolute levels of Dpp, cells experience similar temporal gradients, i.e. a similar fold-change of Dpp over time [12]. Decoding this fold-change of Dpp is, in turn, the basis enabling homogenous cell proliferation throughout the imaginal wing disc [12].

Thus, dynamic readout of signalling gradients – for instance in the form of signal duration or signalling fold-change – can underlie spatiotemporal patterning in developing organisms.

4.2. Signal oscillations in development

Periodic signal dynamics are a common feature of developing organisms and recently, approaches relying on real-time imaging have provided insights into the role of signalling oscillations in development.

4.2.1. Signalling oscillations and neuronal fate decisions

Several transcription factors have been identified to govern cell fate decisions during neuronal differentiation in mouse embryogenesis [71–76], such as Hes1, Olig2 and Ascl1, which oscillate in undifferentiated neuronal progenitor cells (NPCs) with a period in the range of a few hours [77–79]. More recently, both the mechanism controlling these oscillations (77) and, importantly, their functional relevance (78) have been addressed. In an elegant study, the group of Kageyama succeeded in using optogenetic tools to control expression dynamics of a selected transcription factor. This revealed that *oscillatory* AscII expression leads to NPC maintenance and proliferation, while *sustained* AscII expression promotes neuronal fate determination. This exemplifies the role of signalling dynamics in controlling specific downstream responses in a developmental context (see review article by Harima et al., in this issue).

4.2.2. Spatiotemporal dynamics and signalling oscillations

Ultradian oscillatory signalling activities have been identified during the process of embryo segmentation, first in vertebrates [80–83] and excitingly, also in several arthropod species [84–86]. In all these species, the hallmark of oscillatory activity in the segmentation context is the striking degree of synchronization within a cell population, generating higher-order temporal coherence in signalling activity.

This temporal coherence is commonly thought to provide temporal control over the segmentation process. Hence, the oscillations have been implicated to function as segmentation clock. Several excellent reviews have covered the molecular details and possible role of a segmentation clock in somitogenesis [87,88]. Nevertheless, the precise function of oscillations per se during somite formation is not entirely clear [89–91].

Here, we focus on a potential mechanism that highlights the functional relevance of spatiotemporal synchrony between oscillations within a population of cells. In vertebrates, i.e. mouse and zebrafish embryos, the ability to reveal oscillations using reporter strategies and real-time imaging has allowed novel and direct insights into this synchronization [92–94]. Neighbouring oscillating cells show a slight degree of phase-shift, i.e. peaks of oscillations are not reached at the same time, but rather with a shift of a few minutes (Fig. 3A). As this phase-shift is orderly distributed across the cell population, periodic activity waves are generated that sweep through the population of cells. These spatiotemporal activity patterns have received considerable attention with regard to how synchrony is established [94–99].

Recently, our group addressed the significance of spatiotemporal synchronization in the context of segment scaling, a property that ensures that patterning remains proportional, even when the overall size of the to-be-patterned field of cells has been altered experimentally [100,101]. By employing a novel 2-dimensional ex vivo assay, we found that the phase-shift between cells is the single predictor for future segment size during patterning and scaling (Fig. 3B) [101]. While the mechanism that leads to scaling of phase-shift between cells awaits discovery, these findings allowed us to place oscillatory activity in a distinct perspective: oscillations are not limited to a clock or timer function, conversely, the temporal order (i.e. the phase-shift between neighbouring oscillators) carries temporal and importantly also *spatial* information. Intriguingly, this first evidence supports – at the abstract level – a theoretical model put forward decades ago, in which Goodwin and Cohen proposed a patterning model which relies on information encoded in phase-shifted periodic activities [14]. How this information is encoded in and decoded from the signal dynamics is a central question that is currently being addressed. In any case, the context of

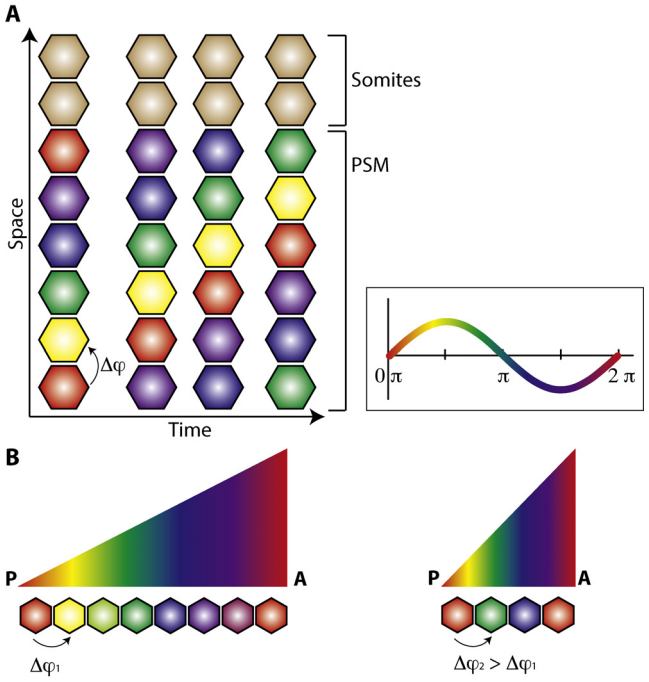

Fig. 3. Tissue-wide signalling dynamics govern pattern formation during somitogenesis. (A) The segmenting PSM, represented by a row of cells, shown at four timepoints within a somite formation cycle. Each cell displays signalling pathway oscillations (in Notch, Wnt and FGF signalling in the mouse PSM). In this scheme, Notch signalling oscillation phases ranging from 0 to 2π (colour-coded) are shown. Signalling waves are generated by a phase shift $\Delta\varphi$ between neighbouring oscillators, leading to the generation of tissue-wide signalling dynamics, which have been implicated in governing the segmentation process. (B) Phase shift between neighbouring oscillators correlates with PSM size, i.e. the signalling phase gradient (colour-coded as in A) scales with PSM size. In a larger PSM the phase shift between neighbouring oscillators is smaller than that in a shorter PSM ($\Delta\varphi_1 < \Delta\varphi_2$).

signalling oscillations, which show complex dynamics and synchronization in time and space, offers an ideal experimental set up to study the role of signalling dynamics in encoding information during development.

5. Discussion

Given the widespread identification of dynamic signal encoding at the cellular level, a future challenge will be to address and transfer these findings to more complex systems, such as developing embryos, organogenesis or disease. As the underlying signalling machineries are highly conserved and recurrent network topologies have been identified, it is likely that similar concepts will be revealed in such systems. Indeed, initial studies have provided evidence for utilization of dynamic signal-encoding principles in developing embryos. Still, the mechanism of generation and function are in most cases not fully understood.

For the study of signalling dynamics several challenges are faced: first, the pathways have to be quantitatively described in space and time. To this end, live cell reporters enabling the quantification of signalling dynamics have to be generated and in addition, in vivo real-time imaging has to be further improved. For instance, selective plane illumination microscopy (SPIM) allows real-time imaging of multicellular organisms with low phototoxicity and high spatiotemporal resolution [102,103]. Additionally, other imaging techniques such as FRET (fluorescence resonance energy transfer), FRAP (fluorescence recovery after photobleaching) and FCS (fluorescence correlation spectroscopy) have high potential for studying the kinetics of signalling pathways [52]. In parallel to advancements in real-time imaging, methods available to generate high throughput proteomics, transcriptomics or metabolomics are increasingly sensitive, requiring less input material. This will enable novel approaches using developing organisms and will likely lead to the identification of more dynamic pathways relevant for metazoan development. Concordantly, periodic expression of thousands of genes (with a period of 8 h) has recently been found in the developing worm *Caenorhabditis elegans* by high throughput, genome-wide analyses [104].

Second, the biological function of signal dynamicity can only be addressed by perturbing signal dynamics. However, instead of disrupting a pathway completely, the effect of subtle changes of the dynamics on cellular behaviour should be analyzed. Reversibly activating or inhibiting a pathway using for example optogenetics can achieve this goal. Recently, optogenetics was employed to investigate signalling dynamics of the Ras-MAPK pathway in single cells [28] or the dynamics of the transcription factor Ascl1 during brain development [79]. In addition, signalling dynamics can be changed by temporally controlled treatment with agonists and/or inhibitors [23,40].

Finally, dynamic systems show complex and non-intuitive behaviours and hence quantitative experimental approaches need to be combined with theoretical and in silico modelling in order to gain fundamental insight [105]. Modelling enables to formalize dynamic behaviours using mathematical and physical principles and to predict the outcome of specific (time-resolved) perturbations at the systems level. In turn, the ability to experimentally verify these predictions and to derive quantitative, dynamic data is critical and can lead to an iterated cycle between experimental and theoretical work. This synergy has already been illustrated in several recent publications (e.g. [12,21,36,41,43,44,61,96,101,106]) and holds great promise for the future study of dynamic signalling and systems.

To extend on Waddington's words, we see that complementing the "biological picture" with the "physical one" will help to unravel "how time is involved" in development of multicellular organisms.

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