Cell PRESS

A post-translational modification code for transcription factors: sorting through a sea of signals

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Cellular responses to environmental or physiological cues rely on transduction pathways that must ensure discrimination between different signals. These cascades 'crosstalk' and lead to a combinatorial regulation. This often results in different combinations of post-translational modifications (PTMs) on target proteins, which might act as a molecular barcode. Although appealing, the idea of the existence of such a code for transcription factors is debated. Using general arguments and recent evidence, we propose that a PTM code is not only possible but necessary in the context of transcription factors regulating multiple processes. Thus, the coding potential of PTM combinations should both provide a further layer of information integration from several transduction pathways and warrant highly specific cellular outputs.

Introduction

Biological responses to environmental or physiological cues rely on signal transduction pathways that must ensure discrimination among a wide panoply of signals. These pathways must also enable discrimination between noise, owing to random fluctuations of signals, and a true input. Signal transduction will ultimately induce modulation of the cellular proteome in response to a molecular effector (e.g. a hormone) or another type of signal (e.g. stress). A particular cell can be subjected to multiple environmental and physiological signals, and their integration is mandatory to elicit a coherent response. Until recently, signaling cascades were perceived as rather linear pathways. Now, these cascades have been shown to communicate, to 'crosstalk', ensuring a combinatorial regulation. Such interconnections between pathways form networks, which elicit integrated responses in a way that can be assimilated to a coherent code [1,2]. By definition, a code is a system of elements that are linked by rules so as to convert pieces of information (for instance, stimuli) into other forms of representation or response (for instance, a cellular outcome or a biological response; http://en.wikipedia.org/wiki/code). Using a code, a limited set of elements can be assorted into combinations and specify different meanings or outputs (Box 1).

The typical textbook signaling pathway is activated by the binding of a ligand to a transmembrane receptor, which in turn modulates the activity of cytoplasmic transducers. After one or more steps of signal amplification through these transducers, an endpoint is often the activation or inhibition of specific transcription factors (TFs), which ultimately modulate expression of a specific set of genes [3]. Many transducers in signaling pathways are post-translational modification (PTM) enzymes, the substrates of which can be specific amino acid residues of TFs embedded within appropriate consensus sequences. The position of TFs in signaling networks makes them, at least in theory, good candidates to act as integrators of various stimuli, especially because the activity of specific TFs can be simultaneously modulated by several signaling pathways.

The existence of a histone PTM code in which expressional information would be encoded as combinatorial nucleosomal PTMs has been under debate for years. However, as shown later, there is a growing body of evidence supporting the existence of some sort of code [4–9]. The ongoing debate about the existence of a PTM code regulating non-histone proteins is more recent and far from being settled [5,10–13]. In particular, in a recent opinion paper, Sims and Reinberg [10] have challenged the relevance of the concept of PTM code outside the realm of histones. Here, we present a different perspective. We argue that the existence of such a code is not only possible but necessary in the case of at least some (if not all) TFs, and we provide evidence supporting our claims.

The 'histone code' paradigm

Histones are the recipients of a large panel of PTMs, including serine/threonine phosphorylation, lysine acetylation, lysine or arginine methylation, glutamine ADP-ribosylation, and conjugation to ubiquitin and ubiquitin-like proteins such as small ubiquitin-related modifiers (SUMO) [4]. Distinct combinations of post-translational modifications of histone tails have been proposed to function as a molecular 'code', which is able to modulate the chromatin transcriptional status. This adds a layer of complexity to gene-expression regulation [14]. These modifications are set, maintained or removed by an ever-expanding number of enzymes [4]. Interestingly, high-throughput analyses have shown that combinations

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0962-8924/$ – see front matter doi:10.1016/j.tcb.2009.02.003 Available online 26 March 2009

of histone PTMs cluster in regions of similar transcriptional activity. For instance, acetylation of lysines H2AK7, H3K9, H3K14, H3K18, H4K5 and H4K12 correlates positively with transcription at different loci [15], whereas H3K9 lysine methylation, which is often accompanied by histone hypo-acetylation, correlates with transcriptional repression [4,6]. These correlations indicate that specific PTM marks somehow 'encode' the transcriptional status of chromatin, at least in a binary way. The existence of a 'histone code' is now supported by a large body of experimental evidence. Differentially modified residues of histone tails would provide interaction surfaces recognized by proteins (adaptors) which, upon binding, would affect chromatin structure and gene expression accordingly [4].

The fact that the same histone PTMs can be interpreted differently according to the cellular context, which is one of the arguments of Sims and Reinberg against a PTM code, does not invalidate the existence of a histone code, at least in its wider sense. In our biological framework, we must accept that the cell is somehow able to grasp the right 'contextual meaning' of the PTM-encoded message, however complicated and context-dependent the decoding process is.

Extending the 'PTM code' hypothesis to other proteins

A recent phospho-proteomic study in HeLa cells has revealed that phosphorylation is a major modification of a vast repertoire of cellular proteins (>6000 phosphorylation sites identified in >2000 proteins), and that a good proportion of these modifications are modulated in response to extra-cellular signals (i.e. 14% of those phosphorylations changed at least twofold in abundance after EGF treatment). Interestingly, at least 46 known transcriptional regulators were found among those phosphoproteins [16]. It is tempting to propose that extensive PTM of proteins, leading to several co-existing isoforms, compensates for the relative paucity of genes in vertebrate genomes by enabling a single gene product to accomplish different roles according to their PTMs. Interestingly, PTMs have been found to cooperate with one another, either positively (by priming or enhancing other modifications) or negatively (by inhibiting other modifications). This can generate robust dynamic responses [13,17]. Positive PTM interactions occur, for instance, in the case of the Forkhead TF FOXO1 (Box 2). Indeed, phosphorylation of one particular serine of FOXO1 by protein kinase B (PKB, also known as AKT) creates a casein kinase I (CKI) phosphorylation consensus, which, after modification, creates another CKI consensus. The presence of these PTMs eventually leads to a robust and quick nuclear exclusion [18].

The existence of many proteins, besides histones, displaying multiple PTM isoforms therefore calls for an extension of the histone code paradigm. Naturally, the use of the term 'PTM code' for non-histone proteins does not imply that 'one particular site modification equals one particular downstream event'. A PTM code is more likely to be combinatorial and context-dependent in nature. For instance, phosphorylation of Thr28, Ser193 and Ser258 of FOXO4, stimulated by Insulin and/or IGF1 (Insulin/IGF1) signaling, promotes nuclear exclusion but stress-induced phosphorylation of Thr447 and Thr451 by Jun N-terminal kinase (JNK) is sufficient to promote nuclear import, whatever the PTM status of Thr28, Ser193 and Ser258 [11]. However, in any given environmental or physiological context, cellular outputs should be similar, so particular PTM protein isoforms emerging in response to this context might help encode these 'stereotyped' responses.

The existence of a combinatorial code would stem from the very nature of signal transduction, which is also combinatorial. Thus, to ensure signal discrimination, the response to a particular signal must be encoded by the triggering of a combination of signaling cascades. This combinatorial use of signaling pathways can be regarded as an evolutionary strategy (and necessity), thereby increasing the number of possible outputs using a restricted number of cascades. This should lead to the emergence of some sort of 'pathway code' (Figure 1a,b). Just try to figure out how a cell can distinguish between extracellular signals S1, S2...Sn, when they all activate the same modifying enzyme (e.g. PKA or AKT): clearly, other concurrent pathways must be specifically activated or inhibited conjointly according to the signals to ensure their discrimination.

An extension of the 'PTM code' hypothesis has already been proposed for tubulin [12]. Tubulin is a highly post-

Box 1. Modular construction and the concept of code

What is a code? A code is a system of elements (signs, words, sequence motifs and/or modules) that involves a rule for converting a piece of information (e.g. the codon AUG, or a biological stimulus) into another form of representation or response. With the use of a code, a limited set of elements can be assorted to obtain a large set of combinations that convey different meanings or biological responses. The use of generic modules as a way to generate new functional elements is a well known evolutionary strategy. Here, we show how combinations of a few DNA motifs (TATA-box, GC-box, CAAT-box, Octamer motif) separated by specific intervening distances give rise to the backbone of very different promoters (simian virus 40 [SV40] early, HSV thymidine kinase or histone H2B promoters) [45] (Figure Ia). A similar mechanism seems to have led to the emergence of TFs (Figure Ib). The minimal TF requires the presence of a DNA-binding domain (of which several types exist, defining TF families, such as the HOX or FOX TFs) and a transactivation or repression domain (here, the yeast TFs Gal4 and Gcn4 and the mammalian SP1) [46]. Of course, other domains (e.g. protein–protein interaction domains, dimerization domains, nuclear localization or exclusion sequence) can increase the 'coding' potential of this fruitful combinatorial strategy. Finally, Figure Ic displays a schematic representation of three FOXO TFs and their conserved sites that have been shown to be PTM acceptor sites (as well as the modifying enzymes responsible for these PTMs) [11,33,47]. To date, 17 possible PTM acceptor residues have been described in FOXO3a alone [11,33]. By analogy with the modular architecture of promoters and of TFs themselves, it is very likely that a particular combination of PTMs will specify the subset of target promoters that must be modulated according to specific input signals. In the case of FOXO3a, single and multiple modifications in a binary way could theoretically give rise to 131 072 (i.e. 2^{17}) different PTM isoforms.

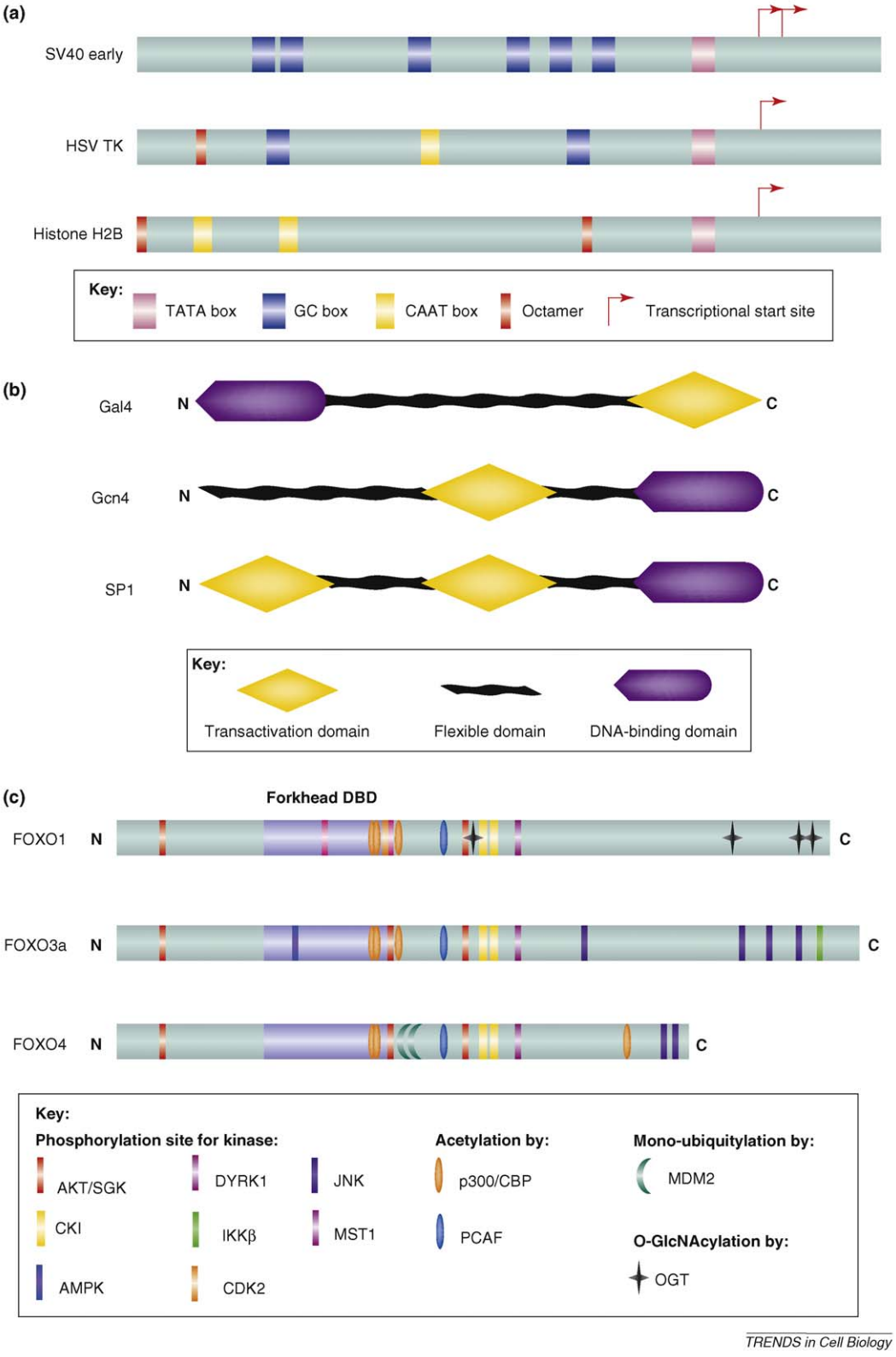

Figure I. Modularity at the level of DNA sequences, TF architecture or PTM acceptor sites.

translationally modified protein because it can be modified by acetylation, phosphorylation, glutamylation, glycylation, palmitoylation and detyrosination [12]. Interestingly, its various PTM isoforms do not participate equally in all microtubule-based functions (e.g. mitotic spindle or cilia formation, or cellular trafficking), which supports the existence of a 'tubulin code' [12]. The existence of a PTM code for TFs might represent a simple and swift means of multiple input integration, and such a possibility has already been suggested in the particular case of FOXO factors [11]. We believe that they are an example of a more general phenomenon (Box 1).

Box 2. Forkhead box (FOX) transcription factors

The superfamily of Forkhead box (FOX) transcription factors is a conserved family of transcriptional regulators, which comprises >100 different members, found in evolutionarily distant species ranging from yeast to humans. Their main common feature is their highly conserved DNA-binding domain (DBD), the Forkhead box or 'winged helix' domain. According to phylogenetic analyses, FOX factors have been classified into 19 subfamilies (A–S) [48]. Apart from the high conservation of their DBD, FOX factors are highly divergent in their N- and C-terminal sequences, which contain protein–protein interaction, repressor and transactivation domains. Distinct family members have been shown to be able to activate or inhibit transcription of target genes [49,50].

FOX factors exhibit a wide range of expression patterns, regulation mechanisms and physiological functions. They have been involved in processes ranging from eye organogenesis (FoxC1–2) to language acquisition (FoxP2) [51], ovarian determination and female fertility (FoxL2) [52,53], and immunity (FoxN1, FoxP3) [49]. Interestingly, FOX factors have also been involved in longevity regulation (FoxO, FoxA) [22,23,25,26,54] and in cancer progression regulation [50].

Interestingly, FOX factors have been shown to be subjected to many PTMs and to be controlled by various signaling pathways [22,33,49,50].

Regulating a transcriptional output without a PTM code

Of course, several mechanistic possibilities exist to regulate the global activity of a TF without the intervention of a PTM code (Figure 1c). First, the expression levels of a TF can be regulated by simply increasing or decreasing its transcription or translation in response to a signal. Second, degradation of a TF can also be modulated. Third, a TF can be stored (e.g. outside the nucleus) as an inactive form awaiting translocation or final maturation. All these mechanisms have been shown to occur in particular cases, and are likely to contribute to the regulation of signal-induced modulation of TF activity. For instance, FOXO factors levels are increased in response to stress, whereas Insulin/IGF1 signaling promotes their degradation by the ubiquitin-proteasome system [19]. Moreover, FOXO factors are excluded from the nucleus in the presence of growth factors [19]. Another example is the heat shock factors (HSF), which are stored in the cytoplasm as monomeric inactive forms in complexes with chaperones. Under heat-shock, they are released and form active trimers, which are actively imported to the nucleus [20]. Even if global, mainly indiscriminate TF activation has been shown to occur, it cannot fully account for all the integrative potential of signaling networks. Global activation or inhibition is expected to lead to a general modulation of all the target genes of a TF. This is compatible with the action of TFs that are specialized in the regulation of either a single process or several closely related processes. For instance, HSF1 is mainly involved in the regulation of the unfolded-protein and heat-shock response, and its activation is triggered globally when chaperones are captured by misfolded client proteins, thus releasing it in an active form [20].

There are also examples of transcriptional outputs resulting from activation of parallel signaling pathways (in response to a single or multiple inputs) eliciting binding of distinct TFs to the same target promoters. Thus, in such cases, integration of the effects of several signaling pathways into a transcriptional output occurs directly at the target promoters, as a function of the presence of a particular combination of TFs (Figure 1a). This is, for instance, the case of the activation of the *even-skipped* gene in *Drosophila* muscle precursor cells, which depends on the Wingless, TGFβ and RTK signaling cascades activating their specific effector TF (Tcf, Mad and Pnt) [3]. A similar mechanism is responsible for the activation of the flocculin *FLO11* gene in *Saccharomyces cerevisiae*, which can only occur if the target of rapamycin (TOR), MAP kinase (MAPK) and PKA pathways are activated and enable activation of TFs Tec1 and Flo8 [21]. Here, the use of distinct TFs in distinct combinations can ensure a specific response as a function of the activation of a particular combination of signaling pathways. The mechanism of signal integration at the promoter level, as depicted here, is not very flexible because it supposes that the various TFs involved in the response must be constitutively expressed in an active state for combinatorial activation to occur. Moreover, this might call for a multiplicity of effector TFs to obtain all the possible responses to environmental or physiological cues. Thus, owing to the principle of cellular economy, other mechanisms that are able to integrate the effects of multiple signaling cascades are also likely to exist.

The existence of a 'PTM code' for transcription factors can be necessary

Some TFs have the ability to regulate several seemingly unrelated processes, and an indiscriminate modulation of their targets, as induced by a global activation or inhibition, would be problematic. This is the case of FOXO factors and FOXL2, which are all TFs of the Forkhead family (Figure 1d; Box 2). Indeed, FOXO factors regulate functions as diverse as glucose metabolism, cell differentiation, longevity, neuropeptide secretion, stress resistance and apoptosis, cancer progression, and female fertility [19,22–26]. FOXL2 is involved in processes such as ovarian determination or early differentiation, oxidative stress response, cholesterol homeostasis and steroid hormone production [27–30]. For instance, it would be conflicting to induce apoptosis by activating FOXO in a situation in which cell survival should be promoted. How can such TFs properly modulate specific cellular processes in response to different signals? We propose that for signaling pathways to mediate precise effects, the 'molecular behavior' of these TFs (i.e. interaction with partners and targets) can change specifically as a function of their PTMs. Thus, we predict that TFs involved in multiple physiological processes can behave as direct 'integrators' of the signals they receive from multiple pathways (Figure 1b). Consider, for instance, that FOXO factors receive input from the Insulin/IGF1, MAPK, AMPK, TGFβ, TNFα, oxidative stress stimuli and other signals that elicit different responses [19,31–34] (Figure 1d).

The existence of a PTM code for TFs is expected to have several non-exclusive ways of action: distinct PTM isoforms could have: (i) distinct DNA-binding specificities; (ii) different DNA-binding affinities with the same sequence specificity; and/or (iii) distinct protein partners (altering sequence specificity or not). Such protein partners could help as adaptors in the 'decoding' activity [4]. Indeed,

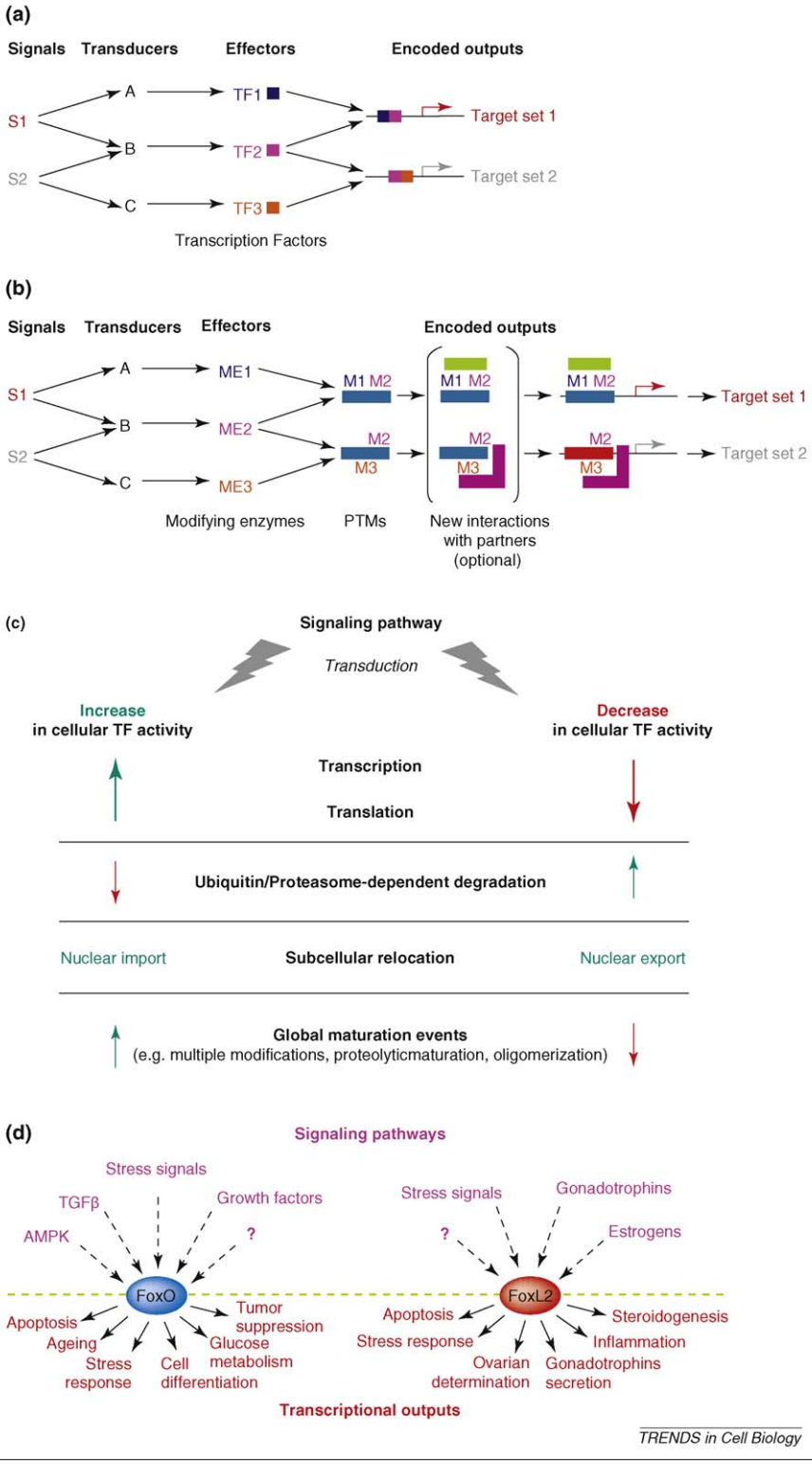

Figure 1. Coding transcriptional output specificity in response to multiple signaling pathways. **(a)** Decoding transcriptional output specificity at the promoter level. S1–2 are cellular environmental signals; A, B and C represent cytoplasmic transducers from signaling pathways of S1–2; TF1–3 are TFs targeted by the signaling pathways of S1–2. Activation of subsets of TFs enables activation of distinct promoter sets (target sets 1–2), specific to the original signal. Notice that in this theoretical example, TF2 can be activated by both signaling pathways but that output specificity is obtained by the combinatorial occupancy of target promoters by TF2 and the other TF specifically activated by the relevant pathway. Biological examples are provided in the main text. **(b)** Coding transcriptional output specificity at the transcription-factor level. S1–2 are environmental signals; A, B and C represent cytoplasmic transducers from signaling pathways of S1–2; ME1–3 are modifying enzymes (such as kinases and acetyl-transferases) activated by the signaling pathways of S1–2. Activation of subsets of modifying enzymes enables addition of specific PTM marks on the target TF. The combination of the PTMs on the TF is specific to the original signal and would constitute a molecular code. These PTMs can either modify interactions with protein partners of the TF (co-activators or co-repressors) or just alter DNA-binding specificity. The final outcome is the specific regulation of distinct promoter sets (target sets 1 or 2) in response to the original signal. **(c)** Mechanisms of global activation of a transcription factor activity, without a PTM code. Color code: green, activation; red, inhibition. **(d)** Two examples of master transcriptional regulators, FOXO factors and FOXL2, regulated by various unrelated signals and regulating various unrelated biological processes. The plethora of unrelated functions regulated by these TFs depends on several signaling pathways. To ensure some degree of specificity of the transcriptional output (no activation of ectopic functions), coding the signals on FOXO and FOXL2 is mandatory.

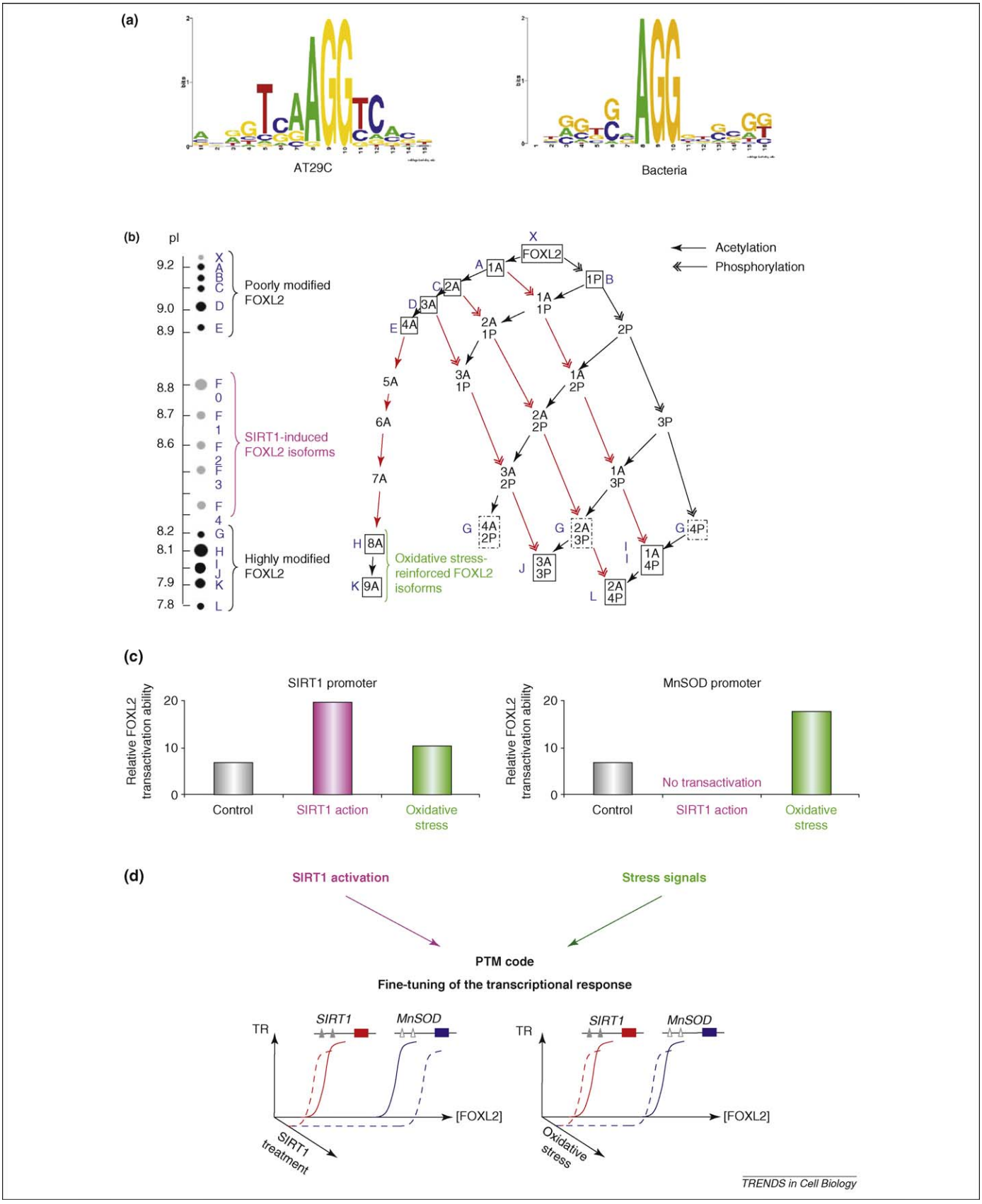

Figure 2. Evidence for the existence of a 'PTM code' in the case of the Forkhead transcription factor FOXL2. **(a)** Logo representation of high-affinity DNA-binding sites for FOXL2 in a highly modified form (AT29C cells endogenous FoxI2) and with no natural PTM (FOXL2 expressed in bacteria). Data were reanalyzed from Ref. [36]. Even if the sites share common features, DNA-binding specificity is different when the PTM content of FOXL2 is low. **(b)** Parallel modification pathways of FOXL2, and their alterations under SIRT1 activation or oxidative stress. In KGN cells (granulosa-like cells derived from an ovarian tumor [30]), at least 11 distinct PTM isoforms of FOXL2 coexist (ranging from the naked FOXL2 form 'X' to highly modified ones). In the figure, the various forms of FOXL2 are located according to their predicted isoeletric points (pI). The number of potentially acetylated residues (nA) and phosphorylated ones (nP) is displayed (n = number of modifications). Solid boxes correspond to forms for which several lines of biochemical evidence point towards a consistent prediction of the PTMs (name of the corresponding spot displayed next to the box); dashed boxes indicate spots or forms

some PTMs can give rise to new protein-binding abilities by creating new interaction surfaces. For instance, Insulin/IGF1-signaling-induced phosphorylation of FOXO factors creates a specific interaction surface with 14-3-3 proteins and mediates nuclear export [35]. New partners could also be co-activators or co-repressors, modulating TF activity without cellular redistribution. Modulation of the PTM status of TFs provides a layer of swift signal integration enabling fine-tuning of cellular responses to environmental or physiological cues.

Evidence for the existence of functionally distinct PTM isoforms of TFs

Recent data indicate that when FOXL2 is devoid of its natural PTMs (i.e. when expressed in bacteria), its consensus binding site is different from the one that is recognized by its highly modified isoforms (Figure 2a, reanalyzed from Ref. [36]). Interestingly, structural data on FOXO factors indicate that PTMs can interfere with DNA-binding or regulate the DNA-binding affinity of the Forkhead domain [37,38]. Moreover, distinct PTM isoforms of particular TFs can coexist or, more interestingly, be induced by different signals [11,39,40]. For instance, the PTM profile of FOXL2 in ovarian granulosa cells is particularly rich and changes in response to oxidative stress and to variations of the expression level of its regulator, the SIRT1 deacetylase [30,39]. The existence of parallel modification pathways that lead to various mutually exclusive combinatorial sets of FOXL2 PTM is likely to reflect the impact of various signaling pathways on FOXL2 function, and the resulting coexisting PTM isoforms should therefore be functionally different (Figure 2b).

TFs can acquire novel target specificities in response to signal-induced PTMs. For instance, the activity of FOXO factors shifts from apoptosis induction to cell survival promotion, DNA-damage repair and cell-cycle arrest in response to the activation of SIRT1 [41]. This can be linked to changes in their PTMs, which alter their interaction properties with DNA, with other partners, or with both. Along similar lines, recent findings show that: (i) FOXL2 transactivation ability is differentially affected by SIRT1; and (ii) promoter recognition efficiency shifts as a consequence of PTM changes [30]. Specifically, FOXL2 is able to upregulate the activity of the promoters of *SIRT1* and of the manganese mitochondrial superoxide dismutase (*MnSOD*) genes (Figure 2c, grey bars). Interestingly, this trend, which results at least partly from FOXL2 acetylation, is markedly enhanced upon oxidative stress (Figure 2c, pink bars). On the contrary, SIRT1-deacetylated FOXL2 is unable to activate *MnSOD* transcription, but is more efficient than 'normal' FOXL2 at activating *SIRT1* transcription (Figure 2c, green bars). This clearly shows that highly acetylated or deacetylated FOXL2 forms are not functionally interchangeable. A mechanistic explanation for this is provided in Figure 2d.

Specific combinations of PTMs with specific outcomes also seem to be induced in response to cellular signals in the case of FOXO factors. Indeed, growth-factor signaling induces phosphorylation of one threonine and two serines of FOXO factors by AKT or SGK, promoting their nuclear export and transcriptional inactivation and, thus, stimulating cell growth [11,35]. However, in the case of FOXO4, cellular stress induces phosphorylation of Thr447 and Thr451 by JNK and mono-ubiquitylation on Lys199 or Lys211 by double minute 2 E3-ubiquitin ligase (MDM2), which induces nuclear import and increased transactivation capacity and, thus, cell cycle arrest and stress resistance [22,34,42,43]. Interestingly, a recent high-throughput RNAi study to identify FoxO activators in *Drosophila* cells further illustrated the multiplicity of modifying enzymes involved in the activation of FoxO activity [44]. These results also indicated some degree of specificity in the action of these activators because the amplitude of transactivation changes that they induced was different at comparable degrees of FoxO nuclear localization [44].

Concluding remarks

Because PTMs rely on rapidly acting transduction pathways, imposing modification on a pre-existing TF is expected to be swift process. This contrasts with other possibilities of information integration discussed in the text that require lengthy processes, such as transcription and/or protein synthesis.

Speaking of a 'PTM code' for non-histone proteins and specifically for TFs could be misleading if one expects a perfect colinearity between the PTMs and their cellular effects, as between codons and amino acids for the genetic code. The emergence during evolution of master transcriptional regulators involved in several cellular processes and the requirement of specificity in the activation of transcriptional programs pleads in favor of the existence of a code. A 'PTM code' is an appealing possibility and is expected to enrich the regulatory capabilities of the cell through the generation of a 'molecular barcode' that would enable fine-tuning of transcriptional responses beyond a mere global modulation of TF concentration. However, most probably, integrated regulation should require the use of both global and specific or targeted modulations.

with strong, yet less confident, prediction (no boxes: intermediates required but evidence for their individual existence is unavailable). In a 2D western blot, there is a leap between the basic or poorly modified FOXL2 forms and the more acidic or highly modified ones [39]. Indeed, F0–F4 isoforms are virtually undetectable in the steady state in KGN cells and *in vivo* (mouse ovary). However, overexpression of the deacetylase SIRT1 induces strong deacetylation and 'alkalinisation' of FOXL2 isoforms (the phantom F spots, more basic than the highly modified ones, appear [39]). On the contrary, oxidative stress favors hyperacetylation [30]. Thick red arrows outline possible parallel modifications pathways. This suggests the co-existence of several PTM isoforms that are likely to be functionally non-equivalent (see later). **(c)** Transactivation ability of FOXL2 on the promoter of its targets *SIRT1* and *MnSOD* in distinct signaling contexts. In both cases, oxidative-stress-induced PTM isoforms have a higher transactivation ability. However, SIRT1-deacetylated forms have no transactivation ability on the *MnSOD* promoter, whereas they have increased transactivation abilities on the *SIRT1* promoter [30]. **(d)** Model of alterations of the transcriptional response of FOXL2 in response to distinct signal-induced PTMs. On mechanistic grounds, the transcriptional response (TR) of a promoter to a TF is expected to follow a sigmoidal curve, where an increase in active TF concentrations induces a dramatic increase of TR [55]. The minimum concentration of active TF necessary to induce a significant transactivation depends on the number and the affinity of its cognate binding sites in the target promoters [36,55]. The fact that the alterations of transactivation ability of FOXL2 under SIRT1 action and under oxidative stress are different on two distinct target promoters indicates that its PTMs do modulate either the specificity or the affinity of FOLX2 for its target promoters in a signal-specific manner. High-affinity binding sites are represented in promoters by solid triangles and low-affinity binding sites by empty triangles. The promoter of *SIRT1* seems to be a higher-affinity FOXL2 target than that of the *MnSOD* [30]. We represent on the graphs the putative consequences of the PTM modifications induced by the SIRT1 and oxidative stress signals on the alterations of the TR. The two promoters respond differentially to both signals through FOXL2, thus indicating that the PTM isoforms favored in each case code for a specific response.

We have mainly focused on the cases of FOXO and FOXL2, which are well documented, but we believe that this paradigm can be extended to a wide repertoire of master regulators, and even other types of proteins, including modifying enzymes themselves. Recent advances in high-throughput technologies including mass spectrometry are likely to facilitate the decryption of the 'code'. However, the key remaining challenge will be the isolation and characterization of specific PTM combinations of existing protein isoforms, much like haplotypes in DNA, as well as their functional implications. We believe that recent research advances lend credence to the 'PTM code' hypothesis and that this code, however complex, will eventually be deciphered.

Acknowledgements

The authors thank D. L'Hôte and A. Dipietromaria for helpful comments on the manuscript. B.A.B. is supported by an AMN and Université Diderot-Paris VII fellowship. R.A.V. is supported by the INSERM, CNRS, and the University Diderot-Paris VII, the Institut Universitaire de France and the Association pour la Recherche contre le Cancer (ARC).

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