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organization of basal ganglia is more complex than classically proposed.

SUPPLEMENTAL INFORMATION

Supplemental information includes two figures, supplemental statistics for Figure 1 and experimental procedures, and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.02.036.

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Encoding four gene expression programs in the activation dynamics of a single transcription factor

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Cellular signaling response pathways often exhibit a bow-tie topology [1,2]: multiple upstream stress signals converge on a single shared transcription factor, which is thought to induce different downstream gene expression programs (Figure 1A). However, if several different signals activate the same transcription factor, can each signal then induce a specific gene expression response? A growing body of literature supports a temporal coding theory where information about environmental signals can be encoded, at least partially, in the temporal dynamics of the shared transcription factor [1,2]. For example, in the case of the budding yeast transcription factor Msn2, different stresses induce distinct Msn2 activation dynamics: Msn2 shows pulsatile nuclear activation with dose-dependent frequency under glucose limitation, but sustained nuclear activation with dose-dependent amplitude under oxidative stress [3]. These dynamic patterns can then lead to differential gene expression responses [3–5], but it is not known how much specificity can be obtained. Thus, a major question of this temporal coding theory is how many gene response programs or cellular functions can be robustly encoded by dynamic control of a single transcription factor. Here we provide the first direct evidence that, simply by regulating the activation dynamics of a single transcription factor, it is possible to preferentially induce four distinct gene expression programs.

To understand how gene promoters respond to different patterns of dynamical transcription factor activation, we previously developed a chemical genetic method that allows us to control the activity of Msn2 using a small molecule, 1-NM-PP1 [3]. Combining this method with microfluidics and time-lapse microscopy, we can therefore generate any dynamical pattern of Msn2 activity and simultaneously measure induction of Msn2 target genes using yellow fluorescent protein (YFP) reporters [6]. Conceptually, two key promoter properties determine how a promoter decodes transcription factor dynamics. First, the amplitude threshold quantifies how sensitive the promoter is to the nuclear concentration (amplitude) of the transcription factor. That is, promoters may require a minimal threshold concentration of Msn2 before they can activate gene expression. Second, the activation timescale quantifies how quickly a promoter activates after Msn2 has entered the nucleus. For example, a slow promoter may be unable to respond to a sufficiently brief Msn2 pulse. In principle, therefore, four distinct extreme promoter classes should exist: a Low threshold Fast class (LF), a Low threshold Slow class (LS), a High threshold Fast class (HF), and finally, a High threshold Slow class (HS) corresponding to the four corners in Figure 1B. This raises the question of whether it is possible to differentially induce each of the four promoter classes just by regulating the activation dynamics of a single transcription factor.

To investigate this, we focused on four Msn2-specific gene promoters that represent each of the four promoter classes: *HXK1* and *SIP18* belong to the LF and HS classes, respectively; *RTN2* is a borderline HF promoter [4]; and, finally, a recently identified *SIP18* promoter mutant, mut D6, belongs to the LS class [5]. First, we considered *HXK1*. *HXK1* is significantly faster than the other promoters (Figure 1B). We therefore reasoned that it would be possible to preferentially induce *HXK1* using brief, low frequency Msn2 pulses too short to activate the other slower promoters. In agreement with our prediction, four well-separated 5-min Msn2 pulses strongly induced *HXK1* without significantly activating the other promoters (Figure 1C). Second, mut D6 has the lowest amplitude threshold and is slow (Figure 1B). Thus, we gathered that a sustained 70-min

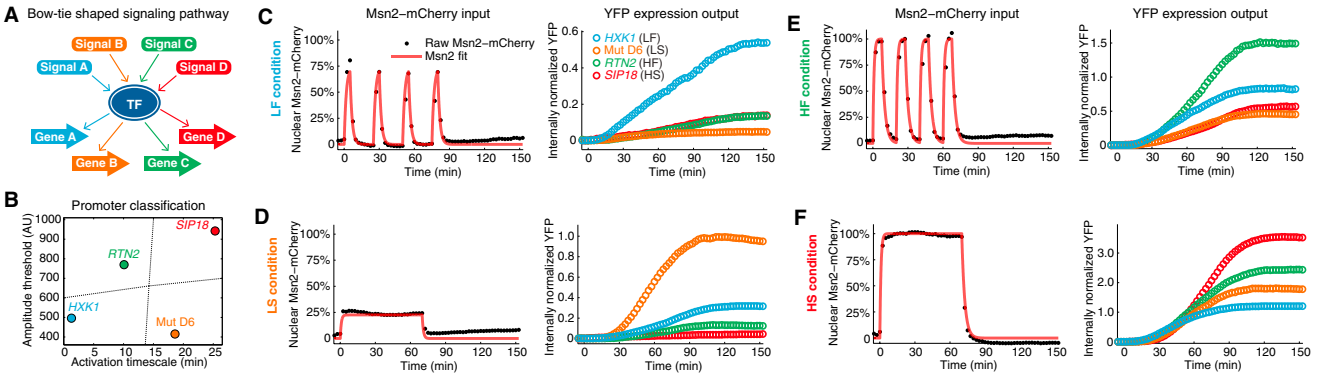

Figure 1. Preferential induction of any single one of four promoter classes by regulating Msn2 activation dynamics.
(A) A bow-tie shaped signaling pathway. Many signaling pathways exhibit a bow-tie topology, where several different stresses and signals activate the same transcription factor. In an ideal bow-tie shaped pathway each signal input preferentially activates one gene output. It is believed that one way different signals can activate the same transcription factor, but nonetheless induce different genes, is by differentially regulating the activation dynamics of the transcription factor. (B) Promoter classification. Based on the promoter amplitude threshold and activation timescale, four extreme promoter classes exist in principle. The parameters for each promoter were determined previously [4,5] and were calculated by simulating a previously described mathematical model [4]. (C–F) Encoding four gene expression programs in the dynamics of Msn2. Msn2-mCherry input is shown on the left and gene::YFP expression output is shown on the right for *HXK1*, mut D6, *RTN2* and *SIP18*. For each gene we replaced the endogenous open reading frame with a YFP reporter gene. Measurements were made every 2.5 min for 64 timepoints in single yeast cells using time-lapse microscopy and microfluidics [6]. YFP expression has been internally normalized by dividing by the average YFP expression in response to a 30 min, 40 min and 50 min Msn2 pulse at 690 nM 1-NM-PP1 for each promoter (see also Figure S1 and Supplemental Information). Internal normalization was necessary to adjust for differences in inherent promoter strength (Figure S1). 1-NM-PP1 concentrations used were 690 nM (C), 100 nM (D) and 3 μM (E–F). Each condition is an average of at least around 500 single cells.

and very low amplitude Msn2 pulse would preferentially induce mut D6. As predicted, this Msn2 input is largely filtered out by the other promoters but strongly induces mut D6 (Figure 1D). Third, we studied *RTN2*, which is the least extreme promoter (Figure 1B). Although significantly faster than mut D6 and *SIP18*, *RTN2* is still significantly slower than *HXK1*. As a result, we hypothesized that Msn2 pulses of intermediate duration (7.5 min) would be long enough to significantly activate *RTN2*, but too short to significantly activate mut D6 and *SIP18*. Furthermore, we hypothesized that pulses of maximal Msn2 amplitude would induce *RTN2* more strongly than *HXK1*. Indeed, four 7.5-min Msn2 pulses separated by 12.5-min intervals induced *RTN2* to a two-fold higher extent than even *HXK1* (Figure 1E). Fourth and finally, we considered *SIP18*. As an HS promoter, *SIP18* filters out both low amplitude and short duration Msn2 input. Accordingly, a sustained 70-min pulse of maximal Msn2 amplitude preferentially induced *SIP18* (Figure 1F).

We emphasize that each of the dynamic Msn2 inputs chosen resemble Msn2 dynamics under natural stress [3]. We note that only preferential differential expression is possible — each condition invariably induces the target gene as well as the other three to some extent (Figure 1C–F). It is not possible to induce one and only one of the Msn2 target gene classes solely through control of Msn2 dynamics. We stress that each promoter response has been internally normalized (Figure S1 in Supplemental Information, published with this article online) — this is necessary because the absolute promoter strength differs between the promoters. Thus, our current data do not show differential expression at an absolute level. Nevertheless, with appropriate tuning of promoter strengths, preferential expression (Figure 1C–F) among the four promoter classes should also be possible at an absolute level [4]. Taken together, these results demonstrate that the cell can preferentially induce any one of the four Msn2 target gene groups by regulating the nuclear translocation dynamics of Msn2.

In addition to Msn2, many yeast transcription factors such as Mig1/2 and Crz1 also show stimulus-dependent pulsatile activation [7]. In mammalian cells, the tumor suppressor transcription factor p53 also shows different activation dynamics in response to different stresses [8]. Whereas sustained p53 activation is associated with terminal cell fates, p53 pulsing is associated with transient cell cycle arrest [9]. Similarly, cell fate in neural progenitor cells is under dynamic control of the transcription factor Ascl1/Mash1: sustained Ascl1 activation induces differentiation into neurons, whereas Ascl1 pulsing leads to cell proliferation [10]. For both p53 and Ascl1 it is believed that different activation dynamics preferentially induce distinct gene expression programs, although it has not been possible to dissect this hypothesis at the promoter level. Here we experimentally demonstrate that by tuning promoter threshold and activation timescale, the cell can distinguish multiple dynamic patterns of a single transcription factor and preferentially induce any one of four distinct gene expression programs. Our results provide experimental support for a dynamic coding theory [1,2], wherein cells can transmit information about multiple distinct signals by regulating the dynamics of a single shared transcription factor. This may allow a cell with a limited set of pathways to respond

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to a greater number of signals and stresses.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Experimental Procedures, one figure and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.02.058.

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Ancestral sleep

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While we do not yet understand all the functions of sleep, its critical role for normal physiology and behaviour is evident. Its amount and temporal pattern depend on species and condition. Humans sleep about a third of the day with the longest, consolidated episode during the night. The change in lifestyle from hunter-gatherers via agricultural communities to densely populated industrialized centres has certainly affected sleep, and a major concern in the medical community is the impact of insufficient sleep on health [1,2]. One of the causal mechanisms leading to insufficient sleep is altered exposure to the natural light–dark cycle. This includes the wide availability of electric light, attenuated exposure to daylight within buildings, and evening use of light-emitting devices, all of which decrease the strength of natural light–dark signals that entrain circadian systems [3].

While a change in sleep timing from pre-industrial to industrial, and from rural to urban lifestyles is generally accepted, the sleep research community has not reached consensus on whether sleep duration has changed as people moved from pre-industrial to industrial societies with indoor work in enclosed buildings and 24/7 access to electricity [3–5]. A recent study by Yetish *et al.* [6] recorded activity–rest patterns in 94 individuals from three hunter-gatherer communities living without electricity (in Tanzania, Namibia and Bolivia; near to or within 20° south of the equator). While we commend them on carrying out this difficult study, we disagree with their interpretation that "…sleep in industrial societies has not been reduced below a level that is normal for most of our species' evolutionary history", and that the recorded sleep patterns in their study "… are central to the physiology of humans living in the tropical latitudes…".

In approaching the question of how human sleep may have evolved, pre-electricity communities are of special interest, but are becoming increasingly harder to find. The effort of Yetish *et al.* to identify and study such groups is therefore important. However, to use such diverse groups (spread over two continents) for the interpretation of sleep behaviour in the context of evolution, one needs comparisons to groups of similar ethnic and sociocultural background with access to artificial light in more industrialized environments. In two recently published studies [7,8] and one ongoing one [9], the rest–activity and light exposure patterns of genetically and culturally homogeneous communities that live both in their traditional settings as well as in more modern ones were investigated. The average sleep duration of the Toba/Qom from Argentina [7], who still rely to some extent on hunting-gathering and live exposed to similar photoperiods and temperatures as the communities studied by Yetish *et al*. was longer (7.0–8.5 h) than in the latter report (5.7–7.1 h), and was significantly shortened by access to electricity (by up to one hour). Moreno *et al*. also showed that sleep (assessed by sleep diaries) is shorter with concomitant delayed melatonin onsets when Amazon rubber tappers have access to electricity [8]. These differences underline the large variability among populations and individuals (also evident from Figure 3 in Yetish *et al*. [6]).

Evidence for changes in sleep duration in modern societies is mixed [4,5]. The Munich ChronoType study, which has accumulated about 250,000 world-wide entries (using the Munich ChronoType Questionnaire, MCTQ), revealed that sleep on workdays shortened by 3.7 min/year over the past decade [2] while only that on work-free days remained the same. Overall, people sleep more than three hours less per week than ten years ago.

Yetish *et al*. reported that the standard deviation (SD) in sleep