A protein kinase A–regulated network encodes short- and long-lived cellular memories

Yanfei Jiang1*, Zohreh AkhavanAghdam1*, Yutian Li1*, Brian M. Zid2, Nan Hao1†

INTRODUCTION

Cells survive rapidly changing environments through adaptation mediated by sophisticated signaling and gene regulatory networks. How these networks operate dynamically to process complex extracellular signals and elicit appropriate responses remains a challenging question (1–3). Advances in microfluidics and single-cell imaging technologies allow us to track the responses of individual living cells in a precisely controlled changing environment, providing a unique opportunity to elucidate the underlying principles for dynamic signal processing in cells (4). In this study, we exploited these technologies to systematically probe the regulatory network that enables cells to encode memory of prior environmental cues to modulate their adaptive responses to future challenges.

History-dependent cellular behaviors have been found in many organisms (5–11). For instance, plant cells obtain enhanced resistance to various future diseases and abiotic stresses, once primed by mild stresses or chemical compounds (12, 13). In another example, pretreatment of human macrophages with interferon-γ substantially boosts subsequent responses triggered by lipopolysaccharide against various pathogens and in tumor cell killing (14, 15). In this study, we referred these history-dependent responses in single cells as “cellular memory,” which is of course fundamentally different from the neuronal memory in animals.

In the yeast Saccharomyces cerevisiae, a given stress can activate its specific response pathway and a common signaling pathway shared by several different stresses, called the general stress response (GSR) pathway (16). This pathway is primarily mediated by protein kinase A (PKA). In response to stresses, PKA is rapidly inhibited, leading to activation of downstream transcription factors, such as Msn2 and Msn4, and the induction of hundreds of stress-responsive genes (17–20). The GSR pathway is not required for survival against intermediate stress threats but, instead, is required for resistance against future stressful conditions (21–23). However, the mechanisms that mediate memory encoding of environmental changes remain unclear.

In this study, we used GSR as a model to quantitatively analyze how PKA-dependent regulatory processes operate dynamically to encode the memory of environmental changes. We combined microfluidics with time-lapse microscopy to precisely control the dynamics of priming inputs and to quantify the memory effect on stress adaptation in single cells. We found that cellular memory shows two phases, a fast-decaying phase mediated by trehalose metabolism and a long-lasting phase mediated by stress-activated transcription factors and messenger ribonucleoprotein (mRNP) granules. Moreover, the memory dynamics could be modulated by priming inputs. Whereas a high-amplitude transient input specifically induced the fast-decaying phase of memory, a prolonged input was needed to elicit the long-lasting memory effect. We further developed a computational model on the basis of the molecular processes identified experimentally. Our model quantitatively revealed the regulatory scheme that encodes the information of previous environmental inputs into distinct memory dynamics, implying a general strategy to optimize resource allocation and prepare for future challenges under rapidly changing environments.

RESULTS

PKA encodes biphasic cellular memory

Probing the effect of cellular memory has long been challenging due, in part, to the difficulty in generating well-controlled sequential environmental changes in cell cultures. We took advantage of advances in microfluidic technologies that allow the precise control of extracellular conditions and tracking of the responses of single cells over extended periods (24, 25). In this study, we modified the channel design of an existing microfluidic device (18) to include separate control of three media inlets, one each for the priming input, the normal growth medium, and the stress treatment (Fig. 1A). To increase the experimental throughput, we also aligned four individual channels into a single device to enable simultaneous running of multiple experiments, each with its own cell population and stimulus condition. Using this device, we first exposed the cells to a pulse of priming input followed by a “break time” with the normal growth medium. We then treated these primed cells with a sustained environmental stress and evaluated their adaptation responses. The device was mounted on a time-lapse microscope to track the responses of a large number of single cells throughout the entire experiment.
For the priming input, we used a chemical genetics strategy in which we introduced analog-sensitive mutations into the PKA isoforms so that they remain fully functional but can be specifically inhibited by the small molecular inhibitor 1-NM-PP1 (26). We have previously used this strategy to control PKA activity, mimicking an upstream signaling event that specifically activates GSR but not other stress-signaling pathways (Fig. 1B) (18, 27–29). Moreover, combined with time-lapse microscopy and microfluidics, it enabled us to generate precisely controlled temporal patterns of PKA inhibition as priming inputs to evaluate their effects on cells’ adaptation to the subsequent environmental stress.

For the subsequent stress treatment, we chose hyperosmotic stress (0.75 M KCl) because the stress adaptation process in individual cells can be reliably quantified using a specific reporter, the stress-activated protein kinase Hog1, which we tagged with yellow fluorescent protein (YFP). In response to osmotic stress, Hog1 rapidly translocates to the nucleus to induce an increase in intracellular osmolyte; once the osmolyte balance is restored and the cell recovers from the stress, Hog1 exits the nucleus (30). The timing of Hog1 nuclear export strongly correlates with the restoration of cell volume (reflecting the turgor pressure recovery and cellular adaptation) (31). Thus, the duration of Hog1 nuclear localization serves as a proxy for the time needed for the cell to recover from a stress treatment. A shorter duration represents a faster adaptation, whereas a longer duration represents a slower adaptation. We note that other pathways besides Hog1 that respond and adapt to hyperosmotic stress can also be used as indicators for stress recovery (32, 33). We used Hog1 nuclear localization in this study because it has been well characterized and is easy to quantify with time-lapse microscopy (31, 34, 35). The priming input of PKA inhibition did not trigger Hog1 nuclear localization (fig. S1).

Using Hog1 nuclear localization as a reporter, we observed that a 45-min priming input with 3 μM PKA inhibitor (Fig. 1C), which...
causes full PKA inhibition [based on the level of Msn2 translocation (18, 27, 29)], shortened the time needed to recover from hyperosmotic stress. Furthermore, the effect of this priming input decayed with increasing break times (Fig. 1D). To quantify the effect of priming input and the dynamics of its decay, we measured and compared the durations of Hog1 nuclear translocation with and without the priming input ($T_{\text{primed}}$ and $T_{\text{unprimed}}$) for each break time. For instance, the priming input with a 10-min break time decreased the adaptation time by 34.7% from 40 to 26 min (Fig. 1E). We defined this percentage decrease in recovery time ($\frac{T_{\text{unprimed}} - T_{\text{primed}}}{T_{\text{unprimed}}} \times 100\%$) as a quantitative measure of the "memory effect" and used it throughout our report (data processing workflow shown in fig. S2).

When we plotted the memory effect as a function of break time, we observed that the decay of memory effect was biphasic. About half of the memory effect was lost rapidly within 30 min, whereas the remaining memory effect plateaued until 90 min and then declined slowly (Fig. 1F; Hog1 time traces shown in fig. S1). These biphasic memory dynamics were in contrast with the scenario where the memory is primarily mediated by the expression of stress resistance genes (21, 22), which would follow an exponential decay trajectory because gene products are degraded during the break time (Fig. 1F; exponential decay curve). We termed the fast-decaying component as "short-lived" memory and the longer-lasting component as "long-lived" memory. We also evaluated the effect of priming inputs on Hog1 adaptation in single cells but did not observe overt changes in cell to cell variability (fig. S3, A and B). As a control, we performed the same experiments in wild-type (WT) cells without PKA analog-sensitive mutations and did not observe a memory effect from the 1-NM-PP1 input, confirming that analog treatment affected stress recovery specifically by inhibiting PKA activity (fig. S4).

To test whether the memory effect showed two phases with a natural stress as priming input, we used 0.5 M KCl as the priming input and observed similar biphasic memory dynamics (fig. S5, A and B, and fig. S6), indicating that the memory pattern we found was not specific to chemical inhibition of PKA. In addition, to determine whether similar memory dynamics were observed with a different secondary stress, we evaluated the effect of PKA inhibition as the priming input on the cells' adaptation to glucose limitation. The stress-activated transcription factor Msn2 displays an adaptive nu-

short-lived memory is mediated by trehalose synthesis and metabolism

To determine the molecular process that mediated short-lived memory, we deleted five known or putative PKA phosphorylation targets (Gph1, Ctt1, Nth1, Gcy1, and Tps1) involved in metabolic or stress-response pathways (37–39). Among these PKA targets, deletion of the trehalose synthase Tps1 (40, 41) abolished short-lived memory (Fig. 3A). Trehalose is a simple carbohydrate produced in many organisms that acts as a membrane protectant and protein stabilizer to enhance cell survival under stressful conditions (42). We used the $tps1\Delta hxk2\Delta$ strain because $tps1\Delta$ is unable to grow on fermentable carbon sources, such as glucose, but this growth is restored in a $tps1\Delta hxk2\Delta$ double mutant (43). Because the deletion of HXK2 alone did not affect the memory effect (Fig. 3A), the effect of the double mutant was primarily because of the deletion of TPS1. We further confirmed that intracel-

ular trehalose level was rapidly increased in response to the PKA inhibition input and that this increase was lost with Tps1 deletion (Fig. 3B). Because trehalose plays an important role in cellular protection against osmotic stress (42), the increased level of trehalose by the priming input could temporarily enhance the acquired resistance to the subsequent osmotic stress, accounting for the observed short-lived memory.

To validate the central role of trehalose in short-lived memory, we manipulated its degradation. The activity of the trehalose-degrading enzyme Nth1 is also regulated by PKA-dependent phosphorylation (38, 44). We observed that, whereas short-term memory was abolished
PKA-regulated stress-responsive transcription factors (Gis1, Sko1, Hot1, Yap1, and Msn2/4) (17, 45–49). Whereas the deletion of Msn2/4 abolished long-lived memory in response to the 45 min, 0.75 μM 1-NM-PP1 priming input (fig. S11A), the mutant showed only a partial loss of memory in response to the 3 μM 1-NM-PP1 priming input (fig. S11B), suggesting that another transcription factor might play a compensatory role under this condition. Because the deletion of Yap1 also diminished long-lived memory in response to the 0.75 μM 1-NM-PP1 priming input (fig. S11A), we generated the msn2Δ msn4Δ yap1Δ triple mutant and observed a complete loss of long-lived memory in the triple mutant (fig. S11B). These results identified Msn2/4 and Yap1 as the primary transcription factors that mediate the transcriptional response generating long-lasting memory. Moreover, we observed that the short- and long-lived memories are both abolished in the triple mutant (Fig. 4, A and B). This loss of short-lived memory in the triple mutant was consistent with previous studies (29, 50), which showed that the expression of TPS1, required for short-lived memory, depends on the transcription factors deleted in the triple mutant.

We then investigated the mechanism that stabilizes long-lived memory underlying the plateau phase of memory that is maintained up to 90 min after the removal of priming inputs (Fig. 1F). In response to stress or PKA inhibition, cells accumulate various stress-responsive
gene mRNAs in cytoplasmic mRNP granules, called processing bodies (PBs) and stress granules (SGs), which regulate mRNA translation, decay, and storage (51–54). PKA regulates the formation of these mRNP granules by phosphorylating the PB-scaffolding protein Pat1 (51), which is essential for the formation of PBs (51, 55). We observed that the plateau phase of long-lived memory was abolished in the pat1Δ strain, and the memory effect exhibited a continuous decay (Fig. 4C; compare the red to the dark pink curves). We also observed a similar change in memory dynamics in edc3Δ lsm4Δ cells, which are defective in PB formation (Fig. 4D)(56). These results indicate that PBs are required for maintaining the plateau phase.

PBs and SGs are discrete but functionally interacting compartments. Some mRNAs in PBs can be stored in a translationally silenced state during stress and then return to translation through SGs upon stress removal (53, 55). To determine the role of SGs in memory, we examined memory dynamics in the absence of the SG component Pub1 (55). Similar to pat1Δ and edc3Δ lsm4Δ cells, pub1Δ cells no longer exhibited the plateau phase of memory (Fig. 4E; compare the red and dark pink curves). We note that pub1Δ and WT cells grew at a similar rate throughout the experiments, indicating that the plateau phase observed in WT cells was not related to cell growth rate. These results suggested that the plateau phase of long-lived memory depended on the PB- and SG-mediated mRNA storage pathway. pat1Δ, edc3Δ lsm4ΔC, and pub1Δ showed higher memory levels than those of WT for shorter break times (Fig. 4, C to E; 15 to 30 min), in agreement with the role of PBs and SGs in mRNA translational silencing in addition to storage.

Furthermore, to confirm the localization of the mRNAs of stress-responsive genes to mRNP granules, we used the MS2 coat protein (MS2-CP) fused to green fluorescent protein (GFP) (57, 58) to visualize the mRNAs of two well-characterized PKA-regulated stress-responsive genes, DCS2 and SIP18 (28, 29) in living cells. In response to the 3 μM 1-NM-PP1 input, DCS2 and SIP18 mRNAs formed foci that colocalized with PBs as indicated by the PB marker Dcp2-mCherry (59) and some distinct granules (fig. S12). We have previously observed a similar localization pattern for Msn2/4 targets, such as GLC3 and HXK1, during glucose starvation (54). This localization pattern coincided with poor protein production from these mRNAs during stress, suggesting that these stress-induced mRNAs were translationally silenced and stored in mRNP granules to confer long-lasting cellular memory. Together, these findings uncovered that two processes, gene transcription and mRNA storage by mRNP granules, are operated together to generate long-lived memory (Fig. 4F).

![Fig. 3. Short-lived memory is mediated by trehalose synthesis and metabolism.](http://stke.sciencemag.org/)

(A) The bar graph shows the memory effects in response to the high-amplitude transient priming input (15 min, 3 μM 1-NM-PP1) with a 10-min break time in wild-type (WT) and mutant strains. (B) The bar graph shows trehalose levels in WT and tps1Δ hxk2Δ, with and without PKA inhibition. Trehalose levels were measured after a 20-min treatment of 3 μM 1-NM-PP1. (C) Memory dynamics in tps1Δ hxk2Δ in response to the high-amplitude prolonged priming input (45 min, 3 μM 1-NM-PP1). Left, schematic illustrating the treatment procedure of the priming experiment. Right, memory dynamics in WT cells, which are defective in PB formation (Fig. 4D) (55). We observed that two processes, gene transcription and mRNA storage, are operated together to generate long-lived memory. (D) A diagram illustrating the network motif that gives rise to short-lived memory.
Priming input

A computational model can simulate and predict memory dynamics

To quantitatively understand the dynamic encoding of memory, we constructed a computational model. In the model, the network is composed of two memory-encoding motifs, one for short-lived memory and the other for long-lived memory (Fig. 4F). The short-lived memory motif comprises a fast process, in which PKA regulates the activities of Tps1 and Nth1 by phosphorylation (38, 39). In response to PKA inhibition, Tps1 is activated, boosting trehalose production; at the same time, Nth1 is inhibited, slowing down trehalose degradation. As a result, the trehalose level increases rapidly. Subsequently, when the PKA inhibition input is removed, Tps1 is inhibited, whereas Nth1 is activated, leading to a rapid decline in trehalose levels during the break time. This feedforward loop enables quick tuning of trehalose levels, accounting for the fast-changing component of memory effect. The long-lived memory motif consists of two processes that function together to regulate gene expression dynamics, a transcriptional response mediated by transcription factors Yap1 and Msn2/4 (60) and a mRNA storage process mediated by Pat1 (53). For the model output, we assumed that the memory effect linearly depended on the sum of the amounts of trehalose and stress resistance gene products in most of the kinetic regimes, unless the substance concentrations reached extremely high levels. We validated this assumption using a computational model developed by Muzzey et al. (31), which explicitly simulates the adaptation dynamics of the osmotic stress signaling pathway and has been well constrained by experimental data (fig. S13, A to C).

With computationally obtained best-fit parameters, our model reproduced all the experimental data (fig. S14). In particular, in our model, the high-amplitude transient input (Fig. 5A, left) specifically induces the trehalose production process (Fig. 5A, middle), generating the fast-decaying memory (Fig. 5A, right; compare with data in Fig. 2B). The low-amplitude prolonged input (Fig. 5B, left) only induces the transcriptional response but not the mRNP granule formation because the input was too weak (Fig. 5B, middle), resulting in a slow exponential decay of the memory effect after input removal (Fig. 5B, right; compare with data in Fig. 2E). The high-amplitude prolonged input (Fig. 5C, left), however, led to the coactivation of the fast trehalose production and the slow transcriptional response with mRNA storage by mRNP granules (Fig. 5C, middle). Once the transcriptional response was initiated, a portion of the newly synthesized mRNAs for stress resistance genes was stored in mRNP granules because of PKA regulation of Pat1. Upon input removal, these mRNAs were slowly released and translated, resulting in a long-lasting (up to ~90 min) memory plateau (Fig. 5C, right). The high-amplitude prolonged input induced the formation of mRNP granules, as indicated by DCS2 mRNA foci, whereas the high-amplitude prolonged input did not. The transcriptional response was inactive under the latter condition (Fig. 5C, middle).
transient input or low-amplitude prolonged input could not (Fig. 5, A to C, right insets). These live-cell mRNA results support our model in which the formation and function of mRNP granules depended on both the input amplitude and duration. In summary, our model suggested that the three PKA-regulated processes—trehalose metabolism, gene transcription, and mRNP granule formation—operated and coordinated in a specific temporal order to enable the biphasic memory dynamics observed experimentally.

Moreover, our model largely reproduced the memory dynamics observed in the mutants of key regulatory factors. In the absence of Tps1, the priming input (fig. S15A, left) could not induce the production of trehalose (fig. S15A, middle), resulting in a loss of the fast-decaying component of memory, whereas the long-lived memory remained intact (fig. S15A, right; compare with data in Fig. 3C). Upon deletion of the transcription factors Msn2/4 and Yap1, the priming input (fig. S15B, left panel) could no longer induce the expression of stress resistance genes, resulting in a loss of long-lived memory. Meanwhile, the absence of these transcription factors led to the loss of Tps1 expression required for the short-lived memory (fig. S15B, middle). As a result, both short- and long-lived memories were abolished (fig. S15B, right; compare with data in Fig. 4A). When Pat1 was deleted, the newly synthesized mRNAs, induced by the priming input (fig. S15C, left), could no longer be stored in mRNP granules (fig. S15C, middle) but instead underwent immediate translation, resulting in a higher initial level of stress resistance than that of WT cells (during break time 0 to 30 min). This stress resistance, however, decayed more quickly because the gene products are being directly and continuously degraded, and cells can no longer maintain a plateau of long-lived memory (fig. S15C, right; compare with data in Fig. 4C).

To test our model, we first used it to predict how the memory effect changes as a function of priming input duration while keeping...
the break time constant (Fig. 6A). We chose a 60-min break time to focus on the long-lived memory, which depended on input duration (Figs. 2B and 5A). Our model predicted that the memory effect would increase sharply between 15- and 30-min input duration, the time window needed to sufficiently activate gene expression and mRNP granules, and would gradually reach saturation with increasing input duration beyond 30 min (Fig. 6A, “prediction”). The data from our experiments agreed with the model prediction, with a curve shape similar to that of the simulated one (Fig. 6A, “experiment”).

Furthermore, we used the model to predict the memory dynamics in response to a combined pattern of priming input. Neither low-amplitude prolonged input nor high-amplitude transient input could induce the plateau phase of memory (Fig. 2, B and E), yet our model predicted that these two inputs, when applied sequentially (Fig. 6B), should be capable of generating a memory plateau phase that is Pat1 dependent (Fig. 6C, prediction). In this scenario, the low-amplitude prolonged input would first produce a high amount of mRNAs, and the subsequent high-amplitude transient input would induce mRNP granules to store newly synthesized mRNAs, enabling the plateau phase. This prediction was intriguing because it illustrated that the memory effect to the combined input was not simply the sum of the effects to the two single inputs (Fig. 6C, prediction; compare the purple and light pink curves) due to the mRNP-dependent storage mechanism. Therefore, because the memory-encoding processes were largely independent, the memory effects would become additive when the mRNA storage process was removed in the pat1Δ mutant (Fig. 6D, prediction). In agreement with the model, our experiments showed a plateau phase of memory in response to the combined input (Fig. 6C, experiment). Moreover, in the absence of Pat1, the plateau phase was abolished, and the memory dynamics largely resembled the sum of memory effects to the two single inputs (Fig. 6D, experiment), consistent with the model prediction.

These results validated our model and demonstrated its predictive power. Given that the memory-encoding processes were kinetically separated, the model-guided analyses suggest the possibility of rationally designing patterns of priming input and reprogramming the temporal order of regulatory processes to generate the desired forms of memory dynamics.

**DISCUSSION**

Cellular memory allows cells to adjust their responses to environmental cues based on their prior experience. In this study, we used GSR in yeast *S. cerevisiae* as a model system and demonstrated that the memory effect on stress adaptation was biphasic, composed of a fast decaying component (short-lived memory), which was mediated by posttranslational regulation of the trehalose metabolism pathway, and a long-lasting component (long-lived memory), which was mediated by transcription factors and mRNP granules. These memory-encoding processes were mediated by PKA, an evolutionarily conserved kinase with a central role in many molecular and cellular processes that is also associated with diverse diseases (61, 62). Because of the functional pleiotropy of PKA, it has been difficult to target pharmacologically to achieve therapeutic specificity. Here, we found that different PKA signaling dynamics, depending on the input amplitude and duration, selectively induced specific downstream pathways or processes, leading to distinct memory dynamics. These results raise the possibility of perturbing the dynamics of signaling hubs for specific therapeutic outcomes (63, 64).

Among the PKA-controlled pathways, our results highlighted the contributions of two major biological processes to acquired stress resistance. The first one was the metabolism of trehalose, which is critical for mediating yeast stress responses. For instance, under nonoptimal temperatures, Tsl1, a regulatory factor of the trehalose synthase Tps1, mediates the heterogeneous cellular phenotypes in growth, survival (65), and bimodal gene expression (66). Because Tsl1 is also encoded by a GSR gene (67), it would be interesting to expand our approach to evaluate the priming effects of PKA input on the single-cell responses to temperature stress. This analysis could shed light on how priming inputs influence cell to cell variability and fate decision in stress response.

The second process that we revealed was the mRNP granule-regulated gene expression. Cytoplasmic mRNP granules, such as PBs and SGs, play important roles in controlling the translation, degradation, and storage of mRNAs upon environmental changes (53, 68). Although the biochemical and biophysical characteristics of mRNP granules have been elucidated (69), their functional roles in cell physiology remain largely unclear. mRNP granules have been related to cellular memory formation. For example, transient over-expression of proteins with intrinsically disordered domains (highly enriched in RNA binding proteins) can induce inheritance of biological traits in yeast cells over many generations (70). Another report showed that the aggregation of Whi3, a yeast RNA binding protein, can encode the memory of previous unsuccessful mating attempt and modulate cells' mating capacity (71). Moreover, a synthetic biology study revealed that phase-separated protein droplets can memorize transient spatial stimuli in mammalian cells (72). In accord with these findings, our results provide additional independent evidence that the PKA-regulated mRNP granules contribute to a long-lasting memory of previous environmental challenges and facilitate the adaptation to future stresses. Further analyses will systematically determine the identities of these stored mRNAs that mediate the memory effect, the detailed mechanisms that direct these mRNAs to mRNP granules, and the prevalence of this mRNP-dependent memory effect in regulating other cellular responses, such as the hormetic effect on aging (73).

In addition, through our modeling analysis, we obtained a quantitative understanding about the dynamics of cellular memory and the regulatory network that controls these dynamics. Previous systems biology studies focused on cellular memory originated from bistability, in which different initial conditions lead to distinct steady states, resulting in history-dependent cellular behaviors or outcomes (74, 75). These systems often contain positive feedback loops that give rise to bistability underlying differentiation or fate decision processes (76–78). In contrast, in this study, we investigated the cellular memory in adaptive stress response, which arose from regulated storage and decay of gene products. The memory dynamics are governed by a network composed of multiple parallel pathways with highly diversified inherent kinetics. This network architecture conferred signal processing capability and plasticity in shaping memory dynamics, enabling cells to determine their memory patterns in response to the rapidly changing environments. In particular, this system coupled two low-pass filters with different thresholds to effectively separate the short-term responses to transient signals from the chronic ones to prolonged signals. Moreover, the system also featured a PKA-regulated mRNP granule formation process, which represented a new network motif for the biological information storage. The initiation of the storage depended on input amplitude, whereas how long the storage could last depended on the amount of newly synthesized mRNAs being
localized in the granules and, hence, depended on both the amplitude and the duration of priming inputs. In this way, mRNP granules enabled cells to integrate the information about input amplitude and duration and tune the dynamics of their memory. Guided by the dynamic regulatory schemes revealed by modeling, we further designed priming input patterns to reprogram the temporal order of fast- and slow-acting processes in the network and reshape the memory dynamics. For future studies, advanced fluorescent reporters and
imaging technologies (68, 79) could be used to track on a single-cell level, the explicit spatiotemporal dynamics of key species in the model, such as mRNAs and mRNP granules. These data would enable us to constrain and improve our model and, ultimately, enhance the predictive power of the model. We anticipate that a quantitative and predictive understanding of memory control would produce opportunities for a broader and more effective use of priming treatments as a low-cost nongenetic approach for stress management in agriculture, biotechnology, and clinical intervention.

Last, we want to highlight the biological relevance of our findings. We revealed that, because the molecular processes governing the two memory components have distinct kinetic properties, short- and long-lived memories could be selectively induced by different dynamics of priming inputs. Whereas a high-amplitude transient input induced fast-decaying memory that enabled short-term stress resistance, a prolonged input elicited long-lasting memory conferring long-term stress resistance. This regulatory scheme is analogous to the fast-responding innate immune response and the long-lasting adaptive immune response in mammals. Moreover, mRNP granules were responsive to the amplitude and duration of inputs and could function to further tune the duration of the long-term memory component based on the input dynamics. Together, this integrated regulatory network enables cells to process the information of a previous stress encounter and determine the length of their memories. We speculate that this type of regulation may represent a strategy for cells to optimize resource allocation for future challenge preparation and may be widely applicable to organisms living in rapidly changing environments. Furthermore, given that this regulation is readily tunable, cells could evolve their memory dynamics through natural selection to match the environmental fluctuations in their habitats.

MATERIALS AND METHODS

Strain construction

Standard methods for the growth, maintenance, and transformation of yeast and for manipulation of DNA were used throughout. All S. cerevisiae strains used in this study are derived from the W303 background (MATa leu2-3,112 trp1-1 can1-100 ura3-1) and from the W303 background (MATa leu2-3,112 trp1-1 can1-100 ura3-1). The strains used in this study are listed in table S1.

Microfluidics

The previously reported Y-shaped microfluidic device (18, 28) with two inlets has been modified to accommodate three inlets on a single device and used in this study. The device fabrication and the setup of microfluidic experiments were performed as described previously (18, 20, 24, 27, 28).

Time-lapse microscopy

All time-lapse microscopy experiments were performed using a Nikon Ti-E inverted fluorescence microscope with an Andor iXon X3 DU897 electron multiplying charge-coupled device camera and a Spectra X light-emitting diode light source. A CFI Plan Apochromat Lambda DM 60x oil immersion objective [numerical aperture, 1.40; working distance (WD), 0.13 mm] was used for all experiments. Three positions were chosen for each microfluidics channel. For each position, phase contrast, YFP, mCherry, and infrared red fluorescent protein (iRFP) images were taken sequentially every 2 min. When the acquisition of the image series started, cells loaded in the microfluidic device were maintained in synthetic complete (SC) medium (2% dextrose) for the first 5 min before 1-NM-PP1 was introduced. Media input was switched manually between SC medium, SC medium with 1-NM-PP1, and SC medium with KCl at the indicated time points. The exposure and intensity settings for each fluorescence channel were set the same as that used in our earlier work (28).

For priming experiments, cells were inoculated from a yeast extract, peptone, and dextrose plate into 2 ml of SC liquid media 2 days before the experiment. On the second day, saturated cells were diluted by 1:20,000 into fresh SC media and grown overnight to reach optical density (OD) = 0.6. These exponentially growing cells were diluted by 1:2 and grown for another 2 hours before being loaded into the microfluidic devices.

Image analysis

The images were processed using custom MATLAB code for single-cell tracking and fluorescence quantification. The whole cell was segmented using the phase contrast images, and the nucleus was segmented using the iRFP images. The cytoplasm was the region of the cell that was not the nucleus. The nuclear to cytoplasmic ratios of Hog1-YFP were calculated using the mean fluorescence intensities of Hog1-YFP in the nucleus and in the cytoplasm. The ratios were subtracted by baseline that is the ratio right before KCl was introduced (close to 1) and then plotted against the time. The duration of Hog1 translocation for each condition was quantified using the full width at half maximum (FWHM) and used to calculate the memory effect for each break time (fig. S2). We determined the sample size of our single-cell data based on similar studies published previously (18, 27–29).

Trehalose assay

Trehalose level was measured using the Trehalose Assay Kit (Megazyme). Using this assay, trehalose was converted into glucurate-6-phosphate, generating the reduced form of nicotinamide dinucleotide phosphate (NADPH) in a two-step reaction; the NADPH concentration was determined by measuring the absorbance at 340 nm. To determine trehalose concentrations, 13 ml of yeast culture at OD ≈ 0.5 was harvested and put on ice for 5 min before centrifuged for 5 min at 4°C. Cells were washed with 0.1 M phosphate buffer (pH 5.9) to remove glucose in media and resuspended in 1 ml of 0.25 M Na2CO3 solution, and OD was measured. Additional Na2CO3 solution was added to make the cell densities (OD) the same for 0 and 20 min samples. Samples (~1 ml) were boiled for 20 min to release intracellular trehalose. After cooling, the samples were centrifuged at 12,000g for 3 min to remove cell debris. Two aliquots (300 µl) of supernatant were transferred to two new tubes, which were separately used to determine total glucose level and preexisting glucose level. The following regents were then added to the cell lysates sequentially: 150 µl of acetic acid (1 M), 650 µl of distilled water, 100 µl of imidazole buffer [2 M imidazole, 100 mM magnesium chloride, and 0.02% (w/v) sodium azide (pH 7.0)], 50 µl of NADP 1/adenosine 5′-triphosphate (ATP) [NADP+ (12.5 mg/ml) and ATP (36.7 mg/ml)], 10-µl suspension of HK/G6PDH [hexokinase (425 U/ml) and glucose-6-phosphate dehydrogenase (212 U/ml)], and 10 µl of trehalase (490 U/ml). The mixtures were incubated at room temperature for 5 min for the reactions. Absorbance at 340 nm was recorded to determine the trehalose concentration in the solution first. To estimate the intracellular concentration, we assumed that cell density at OD = 1 is 1 × 107 cells/ml and that the yeast cell volume is 42 fl. Preexisting glucose was determined in a control reaction without added trehalase.
Live-cell mRNA visualization
The MS2-CP strains for mRNA visualization were constructed as described previously (54). The promoter and the coding region of genes of interest were amplified by polymerase chain reaction and then inserted into a template vector, which contains 12× MS2 loop sequences in the integration vector pRS305. The plasmid was linearized by Eco RV and integrated into the W303 background yeast strain, which has PKA analog-sensitive mutations (NH084) at the LEU2 locus. A plasmid that constitutively expresses MS2-CPs fused with GFP driven by MYO2 promoter (54) was also integrated into the same strain at the HIS3 locus. To visualize the colocalization of mRNAs and PBs, a pRS304 plasmid that expresses DCP2-mCherry under the native DCP2 promoter was integrated into the same strain at the TRP1 locus (NH0857).

To perform live-cell mRNA visualization, cells were cultured to OD 0.6 and then loaded into microfluidic devices for time-lapse microscopy. For each position, phase contrast, mCherry, GFP, and iRFP images were taken sequentially every 2 min. After the start of image acquisition, cells were maintained in SC media for the first 5 min to obtain a baseline for each fluorescence channel before the introduction of 3 μM 1-NM-PP1 treatment. The exposure and intensity settings for each channel were set as follows: GFP, 200 ms at 9% lamp intensity; mCherry, 1 s at 10% lamp intensity; and iRFP, 300 ms at 15% lamp intensity.

Computational modeling
Our model focused on the PKA-dependent memory-encoding network composed of the experimentally identified processes that regulate the levels of metabolites or proteins needed for enhancing stress adaptation, including trehalose and stress resistance gene products. The input of the model is the PKA inhibition signal (priming input). For the model output, we assumed that the memory effect linearly depends on the sum of the amounts of trehalose and stress resistance gene products in most of the kinetic regimes, unless the substance concentrations reached extremely high levels. This assumption left out the explicit inclusion of the downstream Hog1 pathway in our model and simplified our analysis.

To validate this simplification, we tested it using a computational model developed by Muzzey et al. (31), which explicitly simulates the adaptation dynamics of the osmotic stress signaling pathway and has been well constrained by experimental data. In this model, there are four subsystems. H represents the reactions that link an osmotic with Hog1 nuclear translocation. D and I are the Hog1-dependent and Hog1-independent subsystems, respectively. G represents the metabolic reactions involved in glycerol synthesis. The ordinary differential equation (ODE) model has three species and five parameters. Where \( s_1, s_2, \) and \( s_3 \) are the respective outputs of the \( H, D, \) and \( G \). The Hog1-independent subsystem, I, is a parameter in the ODEs, which is \( \alpha_i \). \( u(t) \) is the input, which takes the Heaviside step form in our simulation. This model produces a perfect adaptation of the \( s_1 \) species

\[
\begin{align*}
\frac{d}{dt} \begin{bmatrix} s_1 \\ s_2 \\ s_3 \end{bmatrix} &= \begin{bmatrix} -\gamma_h & 0 & -k_h \\ \alpha_d & 0 & 0 \\ 0 & 1 & -(\alpha_i + \gamma_d) \end{bmatrix} \begin{bmatrix} s_1 \\ s_2 \\ s_3 \end{bmatrix} + \begin{bmatrix} k_h \\ 0 \\ \alpha_i \end{bmatrix} u(t)
\end{align*}
\]

In our simplification, we assumed that our priming input changes the initial condition of this system by producing substances (trehalose and stress resistance gene products) before stress and, hence, reduces the adaptation time. To study the dependence of the adaptation time (FWHM of the peak) on the preproduced substances, we either varied the initial condition of \( s_2 \), which is the Hog1-dependent substances, or the value of \( \alpha_i \), which is the Hog1-independent substances. All other parameters and initial values were kept the same as those described in (31). In both cases, we observed a near-linear relationship (fig. S13, A to C). These analyzes validated the simplification in our model.

In our model, the network consisted of three molecular processes, trehalose metabolism, stress resistance gene transcription, and mRNP granule formation, all of which are PKA regulated. For trehalose metabolism, PKA regulates both trehalose production and degradation by phosphorylating Tps1 and Nth1, respectively. More specifically, PKA-mediated phosphorylation inhibits Tps1 activity and enhances Nth1 activity based on the previous reports. Because the regulation is primarily through phosphorylation, we assumed that it was a fast process. For stress resistance gene expression, PKA regulated mRNA transcription through transcription factors and mRNP granules. More specifically, the PKA inhibition input activated transcription factors and mRNP granules. Once the mRNP granules were activated, a portion of the newly synthesized mRNAs was stored in mRNP granules where their translation and degradation were paused. Upon input removal, these mRNAs were slowly released, resuming their translation and degradation. Because gene transcription was a multi-step process, we assumed that it is a relatively slow process; in contrast, because PKA regulated mRNP granules through phosphorylation, we assumed that it was a relatively fast process. Moreover, the level of Tps1 also depended on the PKA-regulated gene expression, resulting in a connection between the trehalose metabolism pathway and the gene expression process. On the basis of the experimental observations, these three processes had different dependence on input amplitude and duration. Trehalose metabolism and mRNP granules could be activated only in response to high-amplitude inputs; by contrast, mRNA transcription could be induced by low-amplitude input, but a prolonged duration was needed.

Computational modeling and all the simulations were done using MATLAB. The model contained 13 variables and 27 independent parameters. The effect of growth-dependent dilution was incorporated in the decay rates in our model. The function “lsqnonlin” was used for data fitting. The data of three dynamic inputs and three mutants (tps1Δ, pat1Δ, and msn2/4Δ yap1Δ) were used for data fitting (fig. S14). To highlight the role of mRNP granules, time points for long-lived memory plateau (Fig. 1F) were weighted by 20-fold for data fitting. Fitting starting with completely random guesses failed. Because our model is an abstract model in which each equation or parameter represents a combination of a series of biochemical reactions, the initial guesses for most of the parameters are not available from the literature. Instead, we estimated the initial guesses based on the experimental data. In particular, the memory dynamics data from different priming conditions and various mutants allowed us to estimate the initial guesses of parameters, step by step, for each small subnetwork in the system. Here, we used the parameters for the trehalose pathway as an example to illustrate how we obtained the initial guesses. Our data showed that high-amplitude transient input activated the short-lived memory that depended solely on Tps1 (Figs. 2, A and B, and 3A). We further showed that the trehalose degradation enzyme Nth1 was involved in regulating the decay rate of trehalose and thereby the decay rate of memory effect (fig. S10, A and B). The short-lived memory decayed at ~15 min (Fig. 2B), which...
allowed us to estimate the limits for trehalose decay rate \( (k_{18}) \) and Nth1 activation rate \( (k_i) \). In addition, the memory effect remained at a plateau for ~10 min after the removal of the priming input (Fig. 2B), suggesting a delay between the inactivation of Nth1 and the decay of trehalose. This delay could arise from a thresholding effect in the enzymatic reactions of trehalose degradation, governed by the trehalose decay equilibrium constant \( (K_{M3}) \) and the Hill coefficient \( (n) \) and hence could be used for initial guesses of these parameters. Furthermore, the trehalose production rate \( (k_{19}) \) could be estimated by dividing the memory level (~0.08 from Fig. 2B) by the duration of priming input (15 min) and the Tps1 level.

Among the Hill coefficients in the model, \( n_3 \) governed the thresholding effects in Nth1-mediated trehalose degradation and was important for reproducing the ~10 min delay between Nth1 inactivation and trehalose decay (Fig. 2B) and \( n_4 \) governed the thresholding effect on mRNP formation, both of which were important for reproducing the response to the low-amplitude prolonged input (Fig. 2E). We chose the value of four for all these Hill coefficients because it generated enough nonlinearity required for the thresholding behaviors while not exceeding the biologically relevant range. We did not include these Hill coefficients in model fitting to reduce the number of parameters for fitting.

We tested 10 random sets of initial guesses that are randomly chosen within eightfold (1/8 to 8) of our first set of guesses and compared the squared norm of the residuals of the final fitting results and then selected the best-fit set of parameter values. We further expanded the range of parameter scan to 16-fold of the first initial guesses and tested additional 10 random sets of initial guesses. We did not find parameter sets that outperformed the chosen best-fit parameter set. Because of the large number of parameters in the model, the chosen best-fit parameter set might not be the global minimum mathematically. However, it did not prevent us from using this model to illustrate our hypothesized mechanism and to make testable predictions (Figs. 5, A to C, and 6, A to D).

Under our experimental conditions, the stress adaptation responses were relatively uniform among cells, and priming inputs did not alter cell to cell variability (fig. S3, A and B). Therefore, we did not consider stochasticity in our model.

The initial conditions are provided in table S2. Reactions and rate constants are provided in table S3.

Model equations

\[
\frac{d[T_{F_a}]}{dt} = k_{11} \cdot [TF_i] - \frac{k_{12} \cdot PKA^{n_1}}{K_{M3}^{n_1} + PKA^{n_1}} \cdot [TF_a]
\]

\[
\frac{d[TF_i]}{dt} = -k_{11} \cdot [TF_i] + \frac{k_{12} \cdot PKA^{n_1}}{K_{M3}^{n_1} + PKA^{n_1}} \cdot [TF_a]
\]

\[
\frac{d[Tps1RNA]}{dt} = \frac{k_{14} \cdot [TF_a]^{n_2}}{K_{M3}^{n_2} + [TF_a]^{n_2}} + k_{15} - k_{16} \cdot [Tps1RNA]
\]

\[
\frac{d[Tps1protein]}{dt} = k_{17} \cdot [Tps1RNA] - k_{13} \cdot [Tps1protein]
\]

\[
\frac{d[Nth1]}{dt} = \frac{k_1 \cdot PKA \cdot [Nth1]}{K_{M4} + [Nth1]} - \frac{k_2 \cdot [Nth1]}{K_{M5} + [Nth1_a]}
\]

\[
\frac{d[Nth1_a]}{dt} = -\frac{k_1 \cdot PKA \cdot [Nth1]}{K_{M4} + [Nth1]} + \frac{k_2 \cdot [Nth1_a]}{K_{M5} + [Nth1_a]}
\]

\[
\frac{d[Trehalose]}{dt} = \frac{k_{10} \cdot KM_6^{n_3}}{KM_6^{n_3} + PKA^{n_3}} \cdot [Trehalose]
\]

\[
\frac{d[Tre6P]}{dt} = -\frac{k_{19} \cdot KM_6^{n_3}}{KM_6^{n_3} + PKA^{n_3}} \cdot [Trehalose]
\]

\[
\frac{d[Pat1]}{dt} = k_4 \cdot [Pat1_a] + \frac{k_3 \cdot KM_6^{n_4}}{KM_6^{n_4} + PKA^{n_4}} \cdot [Pat1_a] - (1 - PKA) \cdot k_5 \cdot [mRNPs] \cdot \frac{k_5 \cdot [mRNPs]}{KM_2 + [mRNPs]}
\]

\[
\frac{d[Pat1_a]}{dt} = k_4 \cdot [Pat1_a] - \frac{k_3 \cdot KM_6^{n_4}}{KM_6^{n_4} + PKA^{n_4}} \cdot [Pat1_a]
\]

\[
\frac{d[mRNPs]}{dt} = (1 - PKA) \cdot k_6 \cdot [mRNAs] \cdot [Pat1_a] - k_5 \cdot [mRNPs] \cdot \frac{k_5 \cdot [mRNPs]}{KM_2 + [mRNPs]}
\]

\[
\frac{d[mRNAs]}{dt} = k_{10} \cdot [TF_a] - k_9 \cdot [mRNAs] - (1 - PKA) \cdot k_6 \cdot [mRNAs] - \frac{k_5 \cdot [mRNPs]}{KM_2 + [mRNPs]}
\]

\[
\frac{d[protein]}{dt} = k_8 \cdot [mRNAs] - k_7 \cdot [protein]
\]

\[
output = \frac{a \cdot [protein]^2}{b + [protein]^2}
\]

SUPPLEMENTARY MATERIALS

stke.sciencemag.org/cgi/content/full/13/632/eaay3585/DC1

Fig. S1. Time traces of Hog1 nuclear translocation with different break times.

Fig. S2. Data processing workflow for quantification of memory effects as a function of the break time.

Fig. S3. The effect of priming input on stress adaptation in single cells.

Fig. S4. A priming input with 3 μM 1-NM-PP1 cannot induce the memory effect in cells with WT PKA.

Fig. S5. Memory dynamics in response to 45 min 0.5 M KCl priming input.

Fig. S6. Time traces of Hog1 nuclear translocation with different break times when 0.5 M KCl is used as the priming input.

Fig. S7. Biphasic memory effects on the adaptation to glucose limitation.

Fig. S8. Time traces of Msn2 nuclear translocation with different break times when glucose limitation is the second stress.

Fig. S9. The effect of 15 min 3 μM 1-NM-PP1 on stress adaptation time is statistically significant.

Fig. S10. Short-lived memory is regulated by the trehalose-degrading enzyme Nth1.

Fig. S11. Identification of the transcription factors Msn2/4 and Yap1 in mediating long-lived memory.

Fig. S12. Colocalization of DCS2 or SIR18 mRNAs with Pbs in response to 3 μM 1-NM-PP1.

Fig. S13. Modeling the relationship between the level of priming products and Hog1 adaptation time.

Fig. S14. Model fitting with all the experimental data.

Fig. S15. Computational modeling reproduces the modulation of memory dynamics in the absence of key regulatory factors.

Table S1. Strains constructed in this study.

Table S2. Initial conditions of all species in the model.

Table S3. Best-fit parameter values used in the model.

View/request a protocol for this paper from Bio-protocol.


Submitted 11 June 2019
Resubmitted 03 February 2020
Accepted 27 April 2020
Published 19 May 2020
10.1126/scisignal.aay3585

A protein kinase A–regulated network encodes short- and long-lived cellular memories
Yanfei Jiang, Zohreh AkhavanAghdam, Yutian Li, Brian M. Zid and Nan Hao

Sci. Signal. 13 (632), eaay3585.
DOI: 10.1126/scisignal.aay3585

Remembering stress with PKA
A prior stress can prime organisms to successfully survive subsequent stresses. In yeast, the kinase PKA is inhibited by stress, resulting in changes in gene expression that mediate the general stress response. Using PKA inhibition as a mimic for stress, Jiang et al. examined how yeast encoded the memories of previous stresses. Short-term memory required metabolism of the sugar trehalose and was more sensitive to the amplitude of the stress than its duration. In contrast, long-term memory was sensitive to stress duration and required stress-induced transcription factors and the storage of messenger ribonucleoproteins in granules. These results show how yeast use PKA to "remember" previous exposures to stress in the short term or over a longer period of time.