MOLECULAR
METABOLISM

Metabolic transcriptional memory

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ABSTRACT

Background: Organisms can be primed by metabolic exposures to continue expressing response genes even once the metabolite is no longer available, and can affect the speed and magnitude of responsive gene expression during subsequent exposures. This "metabolic transcriptional memory" can have a profound impact on the survivability of organisms in fluctuating environments.

Scope of review: Here I present several examples of metabolic transcriptional memory in the microbial world and discuss what is known so far regarding the underlying mechanisms, which mainly focus on chromatin modifications, protein inheritance, and broad changes in metabolic network. From these lessons learned in microbes, some insights into the yet understudied human metabolic memory can be gained. I thus discuss the implications of metabolic memory in disease progression in humans — i.e., the memory of high blood sugar exposure and the resulting effects on diabetic complications.

Major conclusions: Carbon source shifts from glucose to other less preferred sugars such as lactose, galactose, and maltose for energy metabolism as well as starvation of a signal transduction precursor sugar inositol are well-studied examples of metabolic transcriptional memory in *Escherichia coli* and *Saccharomyces cerevisiae*. Although the specific factors guiding metabolic transcriptional memory are not necessarily conserved from microbes to humans, the same basic mechanisms are in play, as is observed in hyperglycemic memory. Exploration of new metabolic transcriptional memory systems as well as further detailed mechanistic analyses of known memory contexts in microbes is therefore central to understanding metabolic memory in humans, and may be of relevance for the successful treatment of the ever-growing epidemic of diabetes.

1. INTRODUCTION

Organisms that are very well-adapted to a specific niche environment have a fitness advantage when that environment is stable [1,2]. However, being well-adapted to one environment can come at a fitness cost when the environment is changing [2—6]. Therefore some organisms have evolved so-called "bet-hedging" strategies, where organisms maintain a sub-optimal fitness in their typical conditions in exchange for increased fitness in anomalous or stressful conditions. Heterogeneous response to a stimulus by an isogenic population is also a part of bet-hedging strategies in unicellular organisms, which ensures that at least some part of the population will have a growth advantage to survive when unpredictable environmental changes are encountered.

Another strategy that many organisms have developed is the capacity to deal with potentially recurring environmental fluctuations through adaptive memory. Adaptation to environmental cues occurs via a range of mechanisms, including transcriptional regulation. This "transcriptional memory" of a previous exposure to an environmental stimulus can be present as changes in gene expression that persist long after the inducing stimulus is no longer present or manifest as changes in the rate or strength of gene expression during a subsequent induction (Figure 1A,B). The former is similar to the process of differentiation,

where a transient inducer results in permanent changes of gene expression important for maintenance of cellular identity. Both types of transcriptional memory are referred to in the literature as history-dependent behavior or hysteresis, while the latter can also be referred to as reinduction memory. Transcriptional memory arises from various inducing conditions, including environmental cues, such as exposure to stress, chemicals, and temperature, and has been observed in all kingdoms of life [7—10]. In multicellular organisms, transcriptional memory is critical for many biological processes, including inflammatory response to repeated exposure of pathogens [11,12].

A prime example of transcriptional memory involves changes in metabolism to optimize fitness in fluctuating nutrient conditions. Therefore "metabolic transcriptional memory" relates to specific metabolic exposures that lead to transcriptional memory — i.e. altered gene expression outlasting the period of altered metabolism and/or the ability to adapt subsequent responses in metabolic pathways differently than during an initial exposure (Figure 1A,B). Some of the most well-studied cases of metabolic transcriptional memory involve the availability of specific carbon sources, especially glucose. Several examples in microbes show that in glucose-limiting conditions, there can be metabolic transcriptional memory exhibited by rapid reinduction kinetics of enzymes involved in the metabolism of other sugars. In
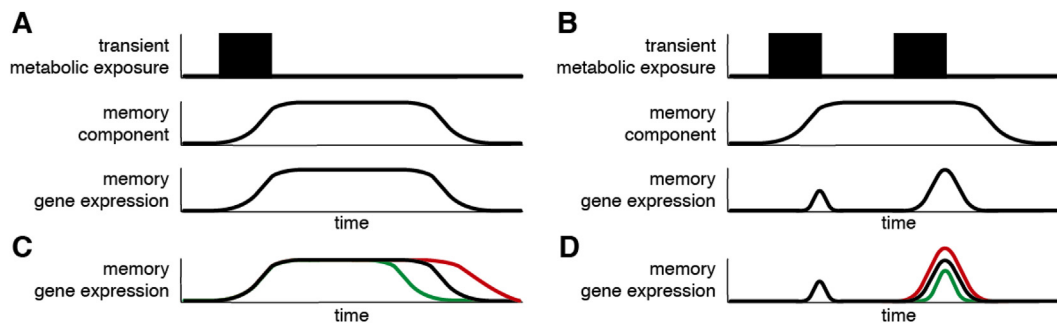
Figure 1: Schematic representation of metabolic exposures inducing metabolic transcriptional memory. (A) Environmental stimuli induce changes to gene expression. Memory of metabolic exposures via a variety of mechanisms can result in changes to gene expression that persist even after the initiating stimulus has been removed. Metabolic transcriptional memory is often associated with positive feedback, resulting in exponential gene expression until reaching a steady state plateau. Depicted here is a memory component with a limited lifetime (scale may be minutes to generations) resulting from, for example, dilution by cell division and degradation, unless actively maintained to result in permanent gene expression changes. (B) Metabolic transcriptional memory can also result in adaptation such that subsequent stimuli induce changes in gene expression with an altered rate or strength in comparison to the initial exposure. (C and D) Factors that cause loss of memory have been discovered in several metabolic transcriptional memory systems and have provided insight into the mechanisms of metabolic memory. Further large-scale screens are needed to identify more loss of memory factors (green) and to search for speculative gain of memory factors (red).

humans, exposure to high glucose (hyperglycemia) can lead to long-lasting effects, even after glucose levels are controlled, resulting in diabetes and disease progression. In this process, there is transcriptional memory for specific metabolic enzymes that promotes further effects in downstream biochemical pathways, all contributing to the disease process and collectively referred to more generally as "metabolic memory" of hyperglycemia in the literature [13—15]. I will discuss these examples in more detail below.

To date, the molecular mechanisms and factors involved in transcriptional memory are not well understood. Local changes in chromatin structure and protein inheritance have been proposed as the two main mechanisms for general transcriptional memory as well as specifically in metabolic transcriptional memory. In addition, broad changes in metabolic networks have also been implicated in metabolic transcriptional memory (Figure 2). As transcriptional responses result from transcription factor binding accompanied by changes in chromatin structure and organization, transcriptional memory may result from, for example, transcription factors that are not removed from the induced gene even after the inducing signal is no longer present, or could be the result of changes in DNA methylation, non-coding RNAs, histone variants and/or modifications, nuclear localization, chromatin remodelers, and chromatin architecture [16—20]. These mechanisms have the potential not only to ensure that a particular cell retains

transcriptional memory, but also to be "epigenetic" in nature, allowing for the inheritance of transcriptional memory through cellular divisions. Long-term maintenance of epigenetic transcriptional memory, however, would require bookmarking or positive feedback (cooperativity between chromatin modifier and modification), such that these modifications are actively maintained through cell divisions [21].

With the onset of a new inducible network in response to an inducer, proteins that are newly expressed may persist longer than the inducing signal itself. Transcription factors that are expressed as a result of the inducing signal, or proteins involved in positive feedback loops of the inducible gene network, can both contribute to transcriptional memory [22—24]. These proteins may even be stable enough to linger through cell division and also result in "epigenetic" transcriptional memory. However protein-based transcriptional memory is limited by protein half-lives and dilution during cell growth and division.

More recently, it has been proposed that locus-specific mechanisms involving chromatin and the expression of specific proteins is only a small part of much broader changes in metabolic and transcriptional networks, where transcriptional memory involves the kinetics of overall changes in respiratory state rather than the specific manner in which the respiratory state was changed [25]. Metabolic network effects can be particularly appreciated in transcriptional memory regimes involving glucose, as changes in this nutrient lead to broad changes in cellular growth, TCA cycle, gluconeogenesis, fatty acid synthesis, and so on [26,27].

Although the underlying metabolic transcriptional memory mechanisms are distinctly described above, by no means are they mutually exclusive from one other. In fact, as I will discuss below, in some systems, all of these mechanisms may be at play. Furthermore, the relative contribution of the various mechanisms is still unclear. There are only a few well-described examples of metabolic transcriptional memory, and likely many more memory systems to be discovered. In line with this, further large-scale screens are necessary to identify factors that affect both kinds of transcriptional memory. By identifying factors that result in loss and even gain of memory (Figure 1C,D) and memory of gene induction as well as repression, we can begin to tease apart the exact mechanisms of metabolic transcriptional memory.

While the two "types" of transcriptional memory, persistently altered expression and reinduction memory, might seem unrelated, they are both manifestations of the same gene induction process. Depending within which biochemical processes transcriptional memory is encoded and the nature of the metabolic fluctuations determines hysteresis
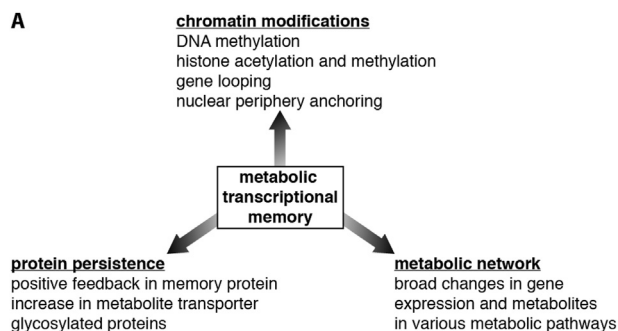

Figure 2: Various mechanisms underlie metabolic transcriptional memory. (A) Chromatin modifications, proteins, and metabolic network have all been described as contributors to metabolic transcriptional memory, and can work in concert to widen memory parameters and potential.
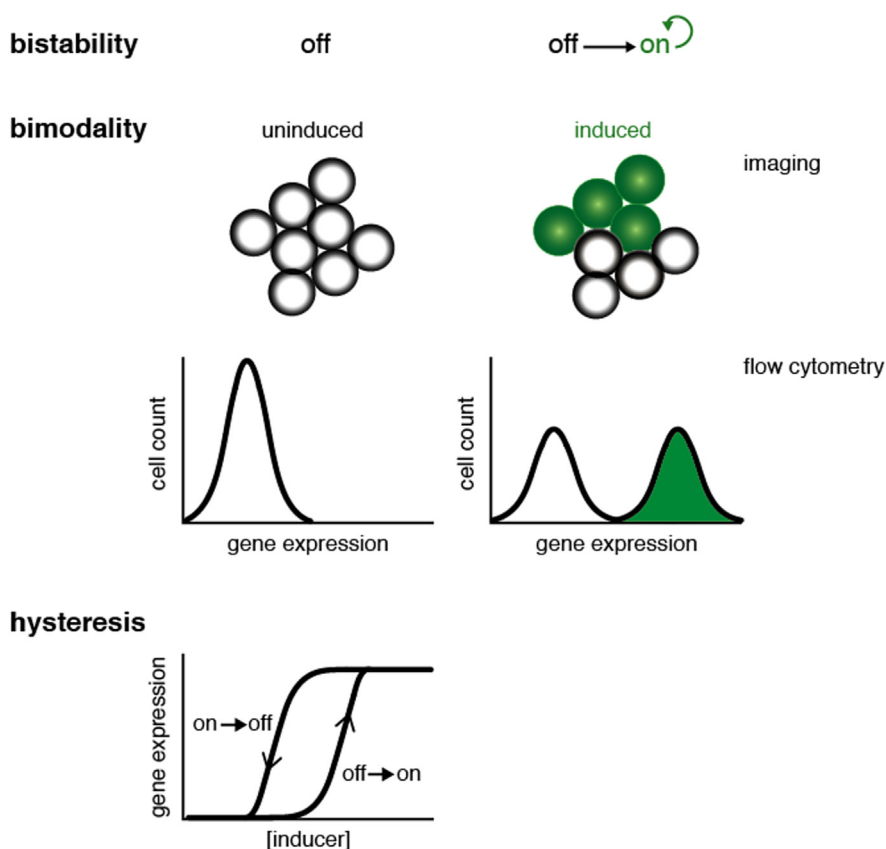
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Figure 3: Hysteresis often emerges from bistability and bimodality. (A) Gene activation processes with various kinds of feedback (shown here is an example of positive feedback) are associated with bistability, such that at certain inducer concentrations and gene induction times, stochastic mechanisms cause some cells in the population to turn on and rapidly produce an expression product (green). This results in a bimodal distribution of gene expression, with induced cells having hundreds-to thousands-fold of product in comparison to uninduced cells. To observe bimodal gene expression, single-cell measurements are necessary, such as by time-resolved imaging or flow cytometry analysis. Bistable gene expression is often associated with hysteresis, where the threshold concentration of a pathway component to turn a gene on is higher than to turn it off due to, for example, a positive-feedback process in the gene activation pathway.

and whether it results in maintaining gene expression in lower concentration/absence of inducer and/or whether rates of gene expression during reinduction might be affected. During the process of gene induction, memory components (for example, molecules, covalent modifications, or structures that differ after the metabolic stimulus) are produced to encode transcriptional memory. Importantly, the concentrations of the memory components and time of inductions and repressions are critical — they determine the window of memory in these systems. When the memory components are able to self-perpetuate gene induction, this can result in persistently altered expression. However, when the memory components are not sufficient to self-perpetuate gene induction in the absence of the stimulus (e.g. due to falling below a critical threshold concentration), but remain long enough until the next stimulus, they can affect the rate-limiting steps of gene induction to result in reinduction memory. Therefore a metabolic stimulus that can lead to persistently altered transcriptional memory is likely to also have the ability to display reinduction memory.

Bistability in these processes also plays an important role, by widening the memory window [28]. Layers of control and feedback loops in the biochemical process of gene induction amplify the effects of stochasticity to generate bistability, where there are two possible stable states, but not something between the two. Bistability is often associated with bimodal gene expression and hysteresis, where thresholds to transition from uninduced to induced may not be the same as in the reverse transition, induced to uninduced (Figure 3) [28]. This highlights the importance of single-cell analyses, as bimodality in gene expression is obscured in population-averaged measurements. These concepts and their role in metabolic transcriptional memory will be further expanded in the specific examples below.

I begin this review by exploring metabolic transcriptional memory in bacteria and yeast exposed to different sugars. Expanding the lessons learned from microbes, I end with the phenomenon of metabolic memory in a disease context, which occurs in some humans with diabetes.
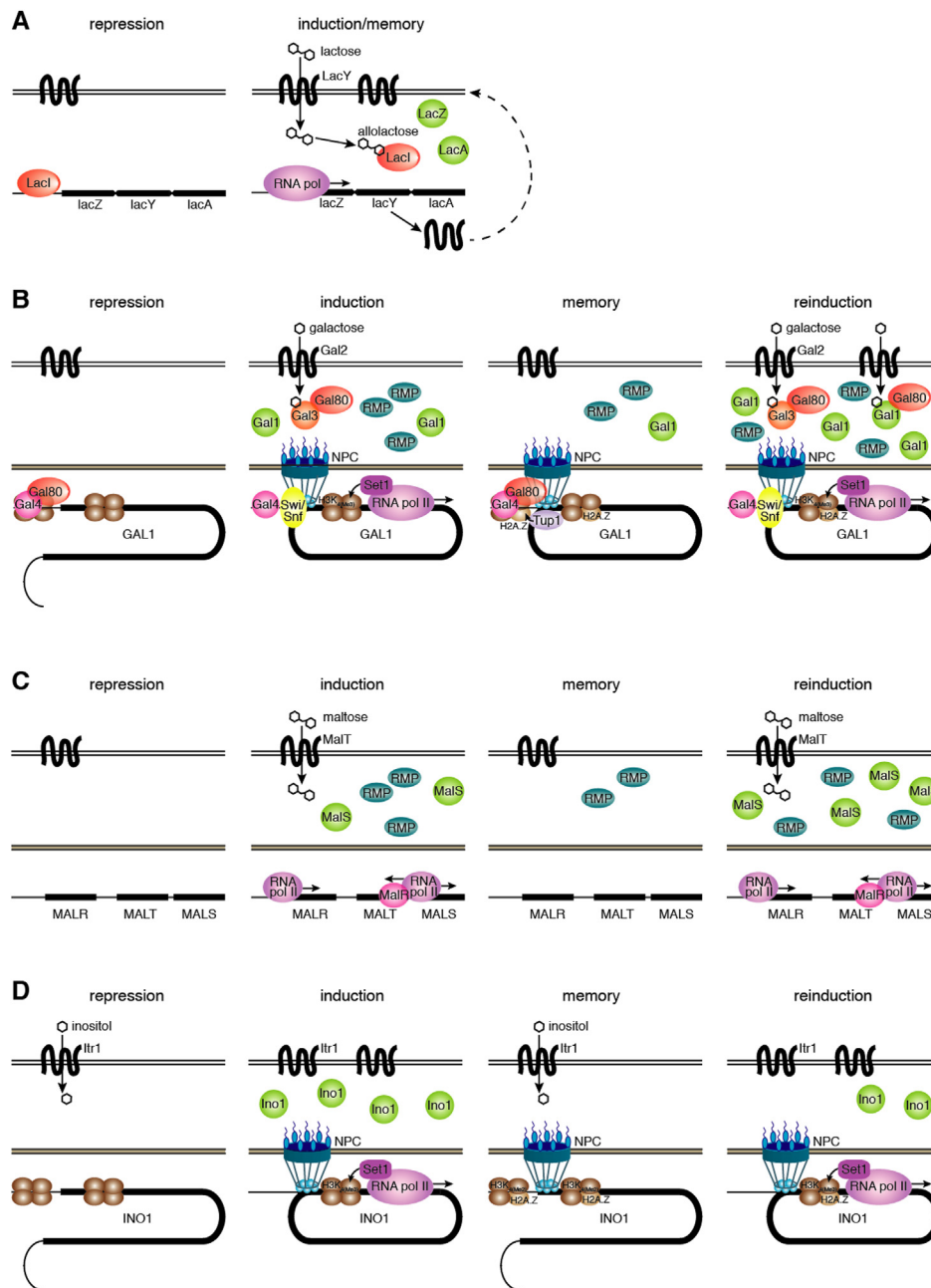
Figure 4: Microbial metabolic transcriptional memory mechanisms. (A) Lactose metabolism and lac operon hysteresis in *E. coli*. The lac operon consists of lacZ, lacY, and lacA, which are responsible for the uptake and metabolism of lactose. The LacI repressor maintains the operon in an inactive state when glucose available. Under inducing conditions, lactose uptake is followed by conversion to allolactose, which binds to and causes release of the repressor, allowing for the lac operon to be expressed. This results in a positive feedback of the LacY permease and metabolizing enzymes LacZ and LacA, even when inducer concentrations fall below the initial threshold for induction, resulting in hysteresis. (B) Galactose metabolism and GAL reinduction memory in *S. cerevisiae*. Under repression, Gal4 activator bound at GAL promoters (GAL1, GAL2, GAL7, and GAL10) is repressed by Gal80. In the presence of galactose, intracellular galactose binds to Gal3, which then binds and removes the Gal80 repressor, allowing the GAL genes to be expressed. GAL1 anchors to the nuclear periphery by the nuclear pore complex (NPC) and forms an intragene loop. GAL reinduction memory is accomplished by a combination of chromatin modifications and protein inheritance, including expression of respiratory metabolism proteins (RMPs) and Gal1 during initial induction, which remain during the memory state. RMPs accelerate the transition back to respiration and Gal1 can function like Gal3 to relieve Gal80 repression during reinduction. The GAL1 intragene loop can be maintained during memory to also facilitate reinduction. Tup1-dependent H2A.Z incorporation during memory possibly also promotes reinduction, by forming a less stable nucleosome. Chromatin remodeling by the SWI/SNF remodeling complex and Set1-mediated histone H3 lysine 4 trimethylation (H3K4me3) are important factors involved in GAL gene expression, and have also been implicated in GAL metabolic transcriptional memory. (C) Maltose metabolism and MAL gene reinduction memory. Maltose metabolism involves a transporter MalT, a maltase MalS, and a transcriptional activator MalR that induces MalT and MalS that are expressed in the presence of maltose. Growth in maltose results in a change towards respiration, resulting in expression of respiratory metabolism proteins (RMPs) that precedes MAL gene expression and metabolism. These RMPs can persist during the memory state, and facilitate MAL reinduction memory by promoting the shift back to the respiratory state. (D) Inositol starvation and INO1 reinduction memory. During inositol starvation, Ino1 is expressed to synthesize inositol. Reinduction of INO1 is actually *lower* than the initial induction; however localization to the nuclear pore complex (NPC) during the initial induction is maintained during memory, and promotes reinduction. Thus although INO1 metabolic transcriptional memory results in lower Ino1 expression, nuclear localization in combination with chromatin modifications including incorporation of H2A.Z and H3K4me2 during the memory state cooperate to permit Ino1 expression during reinduction.

1 *E. coli* — lactose metabolism

Metabolic transcriptional memory in unicellular organisms, such as bacteria and yeast, can induce rapid response to cycling environmental conditions, which can be an advantageous survival strategy. Because expression of unnecessary metabolic proteins is energetically costly, microbes use repression mechanisms to limit the expression of these proteins only when it is advantageous to the organism [29—31]. This is clearly observed when microbes are changed from one carbon source to another — there is a lag phase in growth and expression while the repression mechanisms are removed and transcription is adapted to the new environment [32]. While this provides flexibility for different nutrient sources, the growth disadvantage during such a lag phase can be debilitating to the survival of the population. Thus microbes employ a variety of other "bet-hedging" responses, including heterogeneous expression, sometimes amplified by bistability in gene expression. In addition, some microbes have evolved anticipatory transcriptional responses, where potential future environmental changes are less costly due to the memory of a previous response.

Lactose metabolism and hysteresis in *Escherichia coli* has been extensively studied. In the presence of glucose (the most abundant monosaccharide) and lactose (a disaccharide), *E. coli* prefer glucose as a carbon source. However, when glucose is no longer available and lactose is present, *E. coli* express components of the lactose metabolism pathway from the lac operon. The lac operon consists of three polycistronic genes, lacZ, lacY, and lacA, which are responsible for the uptake and metabolism of lactose and related sugars (Figure 4A). The lacI gene expresses a repressor that binds the lac promoter and maintains the operon in an inactive state until glucose is no longer available and lactose is present. After uptake, lactose is converted to the inducer allolactose, which binds to and causes release of the repressor, allowing for components of the lactose metabolism pathway to be expressed [1,33]. This results in a positive feedback of permease and metabolizing enzyme expression, where lactose is both the inducer and a carbon source for the cells (Figure 4A).

In *E. coli*, the lac operon achieves a hysteretic effect by maintaining the ability to metabolize lactose even once lactose is present at a lower concentration than would be required for initial induction. As a result of the positive feedback loops, the lactose metabolism system is bistable, resulting in bimodal expression of lac operon genes, and also in hysteresis [34,35]. This history-dependent behavior is observed within a range of inducer concentrations — in populations of cells that are uninduced, a high concentration of lactose is necessary for the cells to induce. However, in cells that are induced, a low concentration of lactose is sufficient to maintain expression (up to 10-fold below the concentration necessary for initial induction), as there is sufficient positive feedback of the permease at these lower concentrations [35,36]. Within this range, there is metabolic transcriptional memory, and strikingly, it is maintained in subsequent generations as a sufficient number of permease molecules is inherited by each daughter cell to maintain lac operon expression, even at low inducer concentration and further perpetuate the positive feedback [36]. Elegant experiments have shown that disrupting the positive feedback loop by titrating the ratio of repressor to operon results in loss of bistability, producing a graded response of lac gene expression and a loss of the history-dependent behavior [34,35]. When lactose is completely removed and the system is repressed, this hysteretic effect enhances adaptation and lactose-metabolizing gene expression during lactose reinductions with intermittent repression on short time-scales (<1 generation) [24].

With longer repressions in glucose (∼1—10 generations), reinduction memory is rather based on residual lac proteins from an initial induction.

2 *S. cerevisiae* — galactose metabolism

The budding yeast *Saccharomyces cerevisiae* offers one of the most well-studied paradigms for metabolic transcriptional memory. Several metabolic pathways in this organism have been shown to display hysteresis. An as yet controversial example of metabolic transcriptional memory is that of the galactose-metabolism pathway proteins, including the Galactokinase 1 (GAL1), GAL2, GAL7, and GAL10 genes in *S. cerevisiae*. Galactose is a non-preferred carbon substrate for growth; however, in the event that galactose is available and the preferred sugar glucose is not available, galactose-metabolizing enzymes are expressed. Under non-inducing conditions, the Gal4 activator may bind to the GAL promoters, but is maintained in a repressed state by the repressor Gal80. When galactose is available, Gal3 binds and removes the Gal80 repressor, allowing the GAL genes to be expressed (Figure 4B). There are multiple positive and negative feedback loops in the GAL network that contribute to GAL expression rates and profiles. Whereas galactose-naïve cells have a considerable lag period before expression, cells that have been previously exposed to galactose re-induce the galactose-metabolizing enzymes much faster [20]. This lag period is also characterized by growth arrest and recovery [25].

Though Gal metabolic transcriptional memory itself is not disputed, the mechanisms by which this gene retains memory are somewhat incongruent and elusive. Understanding Gal metabolic transcriptional memory is further complicated by the fact that there are two memory states, where the prevailing memory mechanism is based on length of repression between subsequent inductions. As Gal1 is a paralog of Gal3, it also has the ability to remove the Gal80 repressor. Though it is substantially less effective at relieving repression, high numbers of Gal1 can be expressed during induction and have a significant inducing effect. In long-term metabolic transcriptional memory (∼4—12 h of glucose repression), Gal80 repression presents the most significant challenge to expression, and therefore undegraded Gal1 confers memory [23,37]. In this scenario, Gal1 protein relieves Gal80 repression, bypassing the need for Gal3 to result in faster reinduction. In short-term metabolic transcriptional memory regimes (up to 4 h of glucose repression, corresponding to 2—3 cell divisions), relief from Gal80-mediated repression is not the rate-limiting step to reactivation, and rather changes in chromatin organization leading to nucleosome loss and transcription factor recruitment play a larger role. Chromatin remodeling by the SWI/SNF remodeling complex and an intragene loop between the 5′ and 3′ ends of Gal1 have been postulated to be required for Gal1 metabolic transcriptional memory [19,20,38]. The intragene loop can even be maintained through at least one cell division (Figure 4B). Tup1-dependent H2A.Z (histone H2A variant) incorporation during repression and Set1-mediated histone H3 lysine 4 trimethylation (H3K4me3) have also been implicated in Gal1 metabolic transcriptional memory; however, their specific effects only during reinduction appear to be limited to certain strains and media change protocols [37,39—42].

Interestingly, the observation of reinduction memory requires glucose repression between inductions [23,42]. When cells are first grown in glucose and then induced in galactose, there is bimodal expression of Gal1. This is not the case during reinduction, or when cells are grown

from a non-repressing sugar like raffinose or glycerol to galactose [22,23,43]. Once again, hysteresis in this system is linked to bistability. Intriguingly, an initial growth in maltose (which does not induce GAL genes) also results in some Gal reinduction memory, providing evidence for global changes in the respiratory state of the cell as a mechanism for memory [25]. In line with broader mechanisms underlying Gal metabolic transcriptional memory, several proteins with no direct role in galactose metabolism (TKL2, STL1, HXT5) have also been shown to have reinduction memory in response to galactose [44,45]. In addition to gene activation memory in response to galactose, there are hundreds of genes that are repressed by galactose exposure and which have metabolic transcriptional repression memory [45]. It has been proposed that the active histone mark H3K4me3 plays a repressive role via recruitment of the Rpd3L histone deacetylase complex to genes that were expressed during glucose repression. Deacetylation at promoters of these genes leads to loss of RNA pol II and faster and stronger repression during subsequent galactose exposures.

3 S. cerevisiae — maltose metabolism

Metabolic transcriptional memory also occurs in yeast when cells are grown in a medium containing maltose, another sugar and carbon source [25,32]. Maltose metabolism is carried out by a simple pathway, similar to galactose metabolism, involving a transporter MalT, a maltase MalS, and a transcriptional activator MalR that induces MalT and MalS, all three of which are encoded in five independent MAL loci in S. cerevisiae (Figure 4C). Reinduction memory in maltose metabolism is apparent by reduced lag phases during a growth in maltose, which temporally coincides with the expression of MAL genes. Key to this memory appears to be cycling between glucose and maltose, where glucose-mediated repression of respiratory pathways plays a large role. S. cerevisiae are facultative-fermentative — depending on the type and concentration of sugar(s) and availability of oxygen, they can either undergo fermentation or respiration; in addition, they are a Crabtree-positive yeast, such that growth in glucose represses respiration in favor of fermentation, even in the presence of oxygen. The length of the lag phase for growth and Mal metabolizing protein expression in maltose medium, which induces respiratory growth, is proportional to the length of glucose repression [25].

Interestingly, an initial induction in galactose, another sugar that triggers changes in respiration, also leads to maltose metabolic transcriptional memory, and overexpression of a respiration regulator abolishes memory by reducing the lag phase during an initial induction [25,46]. Therefore maltose reinduction memory appears to be not the result of a few specific chromatin-related factors or maltose metabolizing proteins, but coincides with massive changes in the metabolic network, including broad changes in gene expression, metabolite production, and protein persistence that occur during shifts between fermentation and respiration. The persistence of respiratory metabolism proteins (RMPs) expressed from initial maltose induction through intermittent glucose repression allows for faster reinduction of MAL genes by accelerating the transition back to a respiratory state that is important for MAL gene expression (Figure 4C). Galactose reinduction memory is also influenced by changes in metabolic network and expression of respiratory metabolism proteins (RMPs, Figure 4B), even when an initial induction was not in galactose but in maltose [25]. It will be interesting to further explore the importance of this mechanism in other metabolic transcriptional memory contexts.

4 S. cerevisiae — inositol starvation

Another well-characterized yeast metabolic transcriptional memory occurs during inositol starvation, a precursor sugar involved in cell signaling and gene expression. Inositol deprivation results in the expression of Ino1, an enzyme that synthesizes inositol. After a short repression, reinduction of INO1 is *lower* than the initial induction, offering an interesting example of reinduction memory unlike the previous examples above [17,19].

An additional layer of memory further regulates the expression of INO1. Upon activation, INO1 forms an intragene loop and relocates to the nuclear pore complex (NPC), which promotes transcription and may be involved in mRNA processing and export (Figure 4D). Intriguingly, INO1 remains anchored at the nuclear periphery even after inositol is provided and INO1 is repressed for several generations, though the intragene loop is not maintained [17,19]. Anchoring by the NPC facilitates reinduction — in the absence of this localization, reinduction is even lower [17]. This is unlike GAL1, which also translocates to the nuclear periphery after induction; however, for GAL1, this localization is not important for its faster reinduction [42].

Importantly chromatin modifications that occur once localized to the nuclear periphery during repression, including incorporation of H2A.Z and H3K4me2, are required to facilitate the positive effect of NPC-anchoring on INO1 reinduction [17,47,48]. Thus although INO1 metabolic transcriptional memory results in lower Ino1 expression, nuclear localization in combination with chromatin modifications help to regulate expression to ensure that some reinduction is possible (Figure 4D).

5 Humans — hyperglycemia

The microbial model organisms *E. coli* and *S. cerevisiae*, where metabolic transcriptional memory is well-established, have provided crucial insights into the molecular mechanisms underlying hysteresis. These mechanisms have been further explored in transcriptional memory schemes in various multicellular organisms, including in metabolic contexts but also in other types of memory regimes including development, environment, and stress. Though the exact mechanisms are not necessarily conserved, the lessons learned from the unicellular world have certainly built the bases for these studies. Here, I continue to discuss metabolic memory and the implications in humans in the context of disease — diabetes.

Prolonged or even transient elevated blood glucose (hyperglycemia) can lead to metabolic memory that has detrimental long-lasting effects that are cumulative and largely irreversible, driving obesity, diabetes, and even aging [15,49—51]. In fact, achieving tight glycemic control for periods as long as 3—5 years after hyperglycemic exposure does not prevent the manifestation of all diabetes-related effects. This is referred to as "hyperglycemic memory" or the "legacy effect" [52,53]. In this section, I will use the terms "metabolic memory" and "hyperglycemic memory" to include not only genes with transcriptional memory in response to high glucose, but also the downstream effects in biochemical pathways that collectively contribute to the disease process.

Metabolic memory was first observed in the context of diabetes during clinical trials where patients with Type 1 diabetes were treated daily with either standard (1—2 insulin injections) or intensive treatments (3—4 insulin injections) to control their blood glucose levels. The progression of diabetic complications was so profoundly reduced in patients undergoing intensive therapy that after 6.5 years, all of the patients receiving the standard therapy were also switched over to intensive therapy. However, the patients that initially received standard therapy and were later
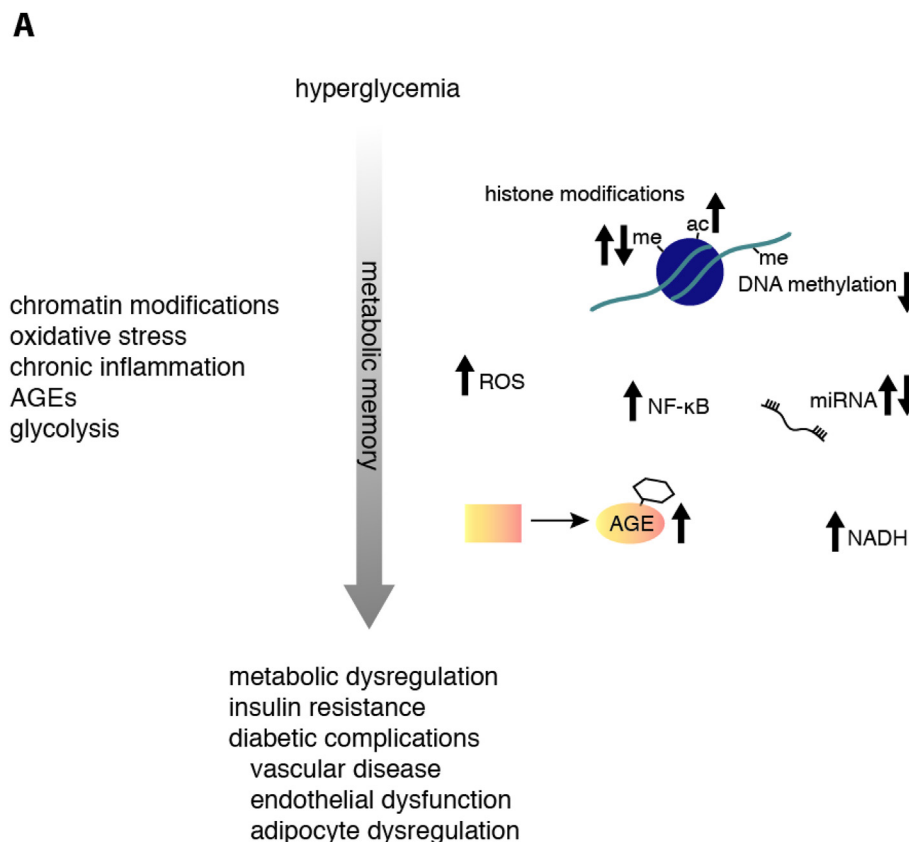
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Figure 5: Metabolic memory of hyperglycemia is accomplished via a combination of mechanisms. (A) Chromatin modifications such as decreased DNA methylation, increased histone acetylation, and changes in histone methylation as well as microRNAs (miRNAs) contribute to hyperglycemic memory. In addition, an increase in reactive oxygen species (ROS) leads to oxidative stress, and an increase in NF-κB leads to pro-inflammatory signaling and chronic inflammation. Overexposure to sugar leads to formation of advanced glycation end products (AGEs) that affect protein structure and function. Glycolytic genes are induced along with reduced binding of PPAR-γ to chromatin of genes inhibiting glycolysis, resulting in persistently upregulated NADH. These various factors together lead to metabolic dysregulation and insulin resistance, leading to diabetic complications such as vascular disease, endothelial dysfunction, and adipocyte dysregulation.

switched to intensive therapy had a much higher coincidence of diabetic complications in comparison to those patients that had initially received intensive treatments [54,55]. These and other findings not only highlight hyperglycemic memory, but also led to the hypothesis that early and intensive treatments leading to tight control of glycemic levels results in long-lasting improvements, even when glucose levels are not as tightly controlled later [49,56—58]. This was further confirmed in animal models of diabetes, such as in experiments done in rats where glycemic control initiated immediately after the onset of diabetes was sufficient to stave off diabetic complications, but when glycemic treatment was delayed by 6 months, the animals continued to develop effects of the disease [59,60]. Studies of glucose metabolism in microorganisms have well-established that changes in the concentrations of glucose and other sugars results in hysteresis of sugar metabolizing enzymes, which led to the hypotheses that hyperglycemic memory in humans results from glucose-induced hysteresis. As in the examples discussed above, hysteresis in this context first affects the genes directly involved in sugar metabolism itself; second, there are wider effects through downstream biochemical cascades in metabolic pathways including glycolysis, the pentose phosphate pathway, β-oxidation, and the TCA cycle [61]. These changes in metabolic profile mirror those occurring during aging, whereas glucose restriction in simple organisms has been shown to increase lifespan, leading to the hypothesis that cumulative glucose exposure over the lifetime plays a role in the aging

process [62]. Several elegant studies have observed the specific effects of glucose hysteresis in different cell types. In pancreatic cells, exposure to high glucose levels induces glycolytic genes, which induces a persistent increase in insulin secretion, and can ultimately lead to obesity and insulin resistance, and therefore diabetes [63]. Transient hyperglycemia has also been shown to induce fibronectin and collagen as well as a subunit of NF-κB in endothelial cells, both of which persist longer than cell division and can therefore have epigenetic effects [64,65]. Studies in Schwann cells suggested that metabolic memory of glucose metabolizing genes is perpetuated by the metabolite NADH and reduced binding of PPAR-γ at genes inhibiting glycolysis [61]. Intriguingly, oscillating high glucose was found to have more of a negative impact on endothelial function and oxidative stress in cells and in patients than constant high glucose [66,67]. This suggests that, like in the examples in microorganisms above, high glucose hysteresis might also be associated with reinduction memory. Furthermore, glucose homeostasis has been modeled with bistability, suggesting that hyperglycemia promotes hysteresis of glucose-induced genes [28].

A multitude of factors has been shown to contribute to hyperglycemic memory, engaging all three of the broad mechanisms found to play a role in microbial metabolic transcriptional memory mentioned above (Figure 5). Initially, oxidative stress and nonenzymatic glycosylation of proteins were found to partially explain metabolic memory [59,60,68—

70]. An increase in oxidative stress due to hyperglycemia was shown to persist even after achieving proper glycemic control in diabetic rats and humans. Similarly, excess advanced glycation end products (AGEs) that are produced nonenzymatically simply through exposure to excess sugar can affect protein structure and function and result in a variety of pathological effects in diabetic patients.

Recently, chromatin modifications have been implicated in hyperglycemic memory [14,53,71—76]. Hyperglycemia can induce a variety of chromatin modifications that are persistent even after normalization of glucose levels. In particular, histone modification activity, including changes in H3 methylation and acetylation that result in persistent gene activation, and DNA methylation can be permanently altered in response to high glucose levels [14,65,73]. For example, hyperglycemia results in downregulation of the lysine deacetylase SIRT1 and upregulation of the Set7/9 methyltransferase, leading to increased active histone marks acetylation and methylation (H3K4), respectively, and constitutive expression of many genes, including the active pro-inflammatory NF-κB subunit [77].

In addition, chronic inflammation could mediate metabolic memory. The conditions that lead to diabetes, and hyperglycemia itself, both stimulate inflammatory response, which in turn further promotes insulin resistance [71,78,79]. This has been well-documented via the activation of NF-κB in endothelial cells and monocyte-to-macrophage differentiation [14]. These inflammatory responses are accompanied by changes in chromatin, including histone methylation and acetylation and DNA methylation, by affecting the expression of the enzymes responsible for these modifications [14]. This suggests that the mechanisms of metabolic memory not only occur in primary glucose metabolism, but also via secondary pathways such as inflammatory response, and may reinforce one another. Thus the effects of hyperglycemia and the pleiotropic downstream effects employ all mechanisms of metabolic transcriptional memory to contribute to diabetic complications. Finally, as metabolic memory of hyperglycemia does not develop into debilitating diseases in all patients, the genetic background has been proposed to contribute to the legacy effect.

Hyperglycemia and diabetic complications also accelerate certain aspects of aging, suggesting that there are common mechanisms driving these pathologies. The progressive nature of aging is hypothesized to result not from increasing amounts of glucose intake over a lifetime, but rather from the cumulative effects of glucose exposure in combination with glucose-mediated hysteresis [80].

While glucose hysteresis is reversible, it is not so easily accomplished as by simply reducing blood glucose levels. Rather, early and aggressive treatment (for example, 3—4 insulin injections/day compared to a conventional 1—2 injections/day) to reduce blood glucose levels is vital to the success of therapy [54,55]. Other therapeutic approaches include curtailing AGE formation, decreasing inflammation, reducing oxidative stress generation, delivering micro-RNA mimics and antagonists, and targeting chromatin modifiers [81—86]. Therapies targeted towards specific pathways that might be involved in metabolic memory have only been mildly successful in slowing down the progression of diabetic complications [87]. This is likely because there are multiple mechanisms (oxidative stress, AGEs, chromatin modifications, chronic inflammation) supporting metabolic memory of hyperglycemia, and thus a combination of these therapies will likely be the most successful. Metabolic memory of hyperglycemia may also be explained by persistent bistable expression of glucose-induced genes. Critical to the success of reversing these pathologies is to understand the bistable behavior of hyperglycemic hysteresis.

While the advantages of metabolic memory are apparent in single-cell organisms, especially in terms of fitness and outcompeting other microbes, it is hard to imagine how hyperglycemic memory in humans could be beneficial and why it has evolved. One study has shown that acute hyperglycemia in human endothelial cells confers a proliferative advantage over persistent high or even normal glucose conditions [64]. Further investigations into the potential benefits of hyperglycemia in humans might be worthwhile.

6 CONCLUDING REMARKS

As is apparent from the examples in prokaryotes, lower eukaryotes, and humans reviewed here, the most well-studied cases of metabolic transcriptional memory involve changes in nutrient status involving carbon sources, and especially glucose. As we delve further into other carbon source shifts, it seems likely that we will discover more cases of hysteresis, including both inducing and repressive memory. Indeed, in-depth mechanistic analyses of other types of metabolic transcriptional memory beyond carbon sources, for example, due to nitrogen sources or amino acid fluctuations, should be further explored in microbes, as metabolic memory of e.g. protein intake is known to occur in humans [88,89].

Further, as metabolic transcriptional memory clearly plays a role in disease contexts such as diabetes, it will be interesting to find out what other diseases and therapies might be affected by hysteresis. First observed in microbes, dietary restriction has been shown to have a protective effect against aging in a number of organisms, accomplished through a variety of mechanisms including reduced glucose metabolism [90]. These findings have been extrapolated to mammals, where counteracting glucose-induced transcriptional hysteresis also increases lifespan [15,80]. Understanding metabolic transcriptional memory and, in particular, the molecular mechanisms of this process are important avenues of research that will contribute to our understanding of disease tolerance and may have important implications for therapies of these diseases.

CONFLICT OF INTEREST

None declared.

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