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REVIEW ARTICLE

Role of Noise-Induced Cellular Variability in Saccharomyces cerevisiae During Metabolic Adaptation: Causes, Consequences and Ramifications

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Abstract | The concept of genetic determinism as illustrated by the metaphors such as 'genetic blueprint', or 'genetic program' had its beginning immediately after the rediscovery of growth lag observed when bacteria were exposed to a mixture of glucose and galactose or lactose. This concept got reinforced with the discovery of the mechanisms of how lactose activates the transcription of *lac* operon of *E. coli*. According to this doctrine, genetically identical cells exposed to the same environment respond in equal measure. However, studies carried out in the past two decades in organisms ranging from prokaryotes to eukaryotes, have clearly established that genetically identical cells need not necessarily respond in an identical fashion when exposed to a given environment. It has now become amply clear that organisms can stochastically switch from one physiological state to the other, thereby resulting in a phenotypically heterogeneous population. Such exhibition of heterogeneity by a population has been, in several contexts, shown to be beneficial in a temporally changing environment. In this review, we have discussed how individual cells of a genetically identical population of *Saccharomyces cerevisiae* remain fit by exploiting this fascinating phenomenon of stochastic switching from one metabolic state to the other when exposed to glucose and galactose, as a source of carbon and energy. We suggest that this inherent stochastic switching seems to have been exploited for an adaptive response in a fluctuating environment.

1 How Did It All Start

Following Pasteur's discovery that yeast proliferates by fermenting glucose to ethanol[1, 2], Dienert observed that yeast exposed to glucose and galactose simultaneously, consumes glucose first and only when all the glucose in the media is exhausted, switches over to galactose[3]. This phenomenon, rediscovered by Karstrome in bacteria[4], gave birth to the concept of enzyme adaptation, where presence of the substrate drives the pre-existing equilibrium between inactive and active forms towards the active form[5]. Monod, not being aware of the phenomenon of enzyme adaptation, observed that *E. coli* grown in presence of glucose and mannose showed a monophasic growth, with no adaptive phase, while in glucose and galactose or glucose and lactose showed a biphasic or diauxic growth with a lag between consumption of the two sugars[6]. Monod then demonstrated that 'enzyme adaptation' was abolished if oxidative phosphorylation was inhibited[7]. This observation ushered in a new era of looking at microbial growth and physiology from the perspective of enzyme adaptation,

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Stochasticity: Biological variation can arise because of genetic or environmental or a combination thereof. Genetic variation is heritable, while environment dependent variation is non-heritable but can be predicted from measurable variables. The non-heritable variation that cannot be predicted from measurable variables is stochastic.

which continues to dictate our thinking of microbial systems till date. Monod then went on to provide evidence that presence of lactose triggers synthesis of enzymes required to breakdown lactose. This breakdown takes place by activating gene transcription, and not by activation of pre-existing inactive enzyme molecules, as proposed by Yadkin. That is, lactose (we now know that it is allolactose) acts as a relay system to convey the signal of its presence to the genetic machinery by interacting with the repressor. This eventually causes de novo synthesis of the enzymes necessary for lactose breakdown (reviewed in[8]). In a landmark paper, Monod also coined the term "allostery"[9, 10] to convey the idea that the enzyme activity can be modulated by mechanisms that do not involve fresh synthesis of enzymes, but involve a conformational change. Allostery also explains how interaction of allolactose with the repressor transmits the information to downstream targets, resulting in the activation of gene transcription. The upshot of the above studies was that the biological response, a hallmark of living systems, was brought under strict control of genes.

The above observations reinforced the then prevailing concept of genetic determinism borne out of the rediscovery of Mendalian genetics and modern evolutionary synthesis[11–13]. The metaphors such as "genetic blueprint", "genetic program" and "genetic switch" became standard lexicon of molecular biology literature. Ironically, even when this 'gene centric' view was taking deep roots during mid-twentieth century, sporadic studies had hinted at the insufficiency of the above view to explain the complex relationship between genotype and phenotype. These studies indicated that even when grown in a homogeneous environment, clonal cells, meaning cells with identical genotype, exhibit more than one phenotype. The earliest example of this phenomenon was the demonstration of the existence of persister cells, which constitute a small fraction of a population which does not get killed upon treatment with penicillin. Once penicillin gets depleted from the medium, the persister cells start proliferating[14–16]. Similar observations were reported in enzyme inducible systems in bacteria[17, 18], which showed that the production of β-galactosidase, at a single-cell resolution, in a population in a homogeneous environment was variable. Variability was also observed in chemotactic behaviour between clonal members of a population of bacteria exposed to an attractant[19]. Initial attempts to explore the theoretical underpinning of this inter-cellular variability examined the role of *stochasticity in dictating this heterogeneity*[20]. As early as 1950s, Speigelman asked "Is it possible for two cells of identical genotype to exhibit heritable differences in enzyme forming capacity?"[21]. Although the answer was in the affirmative, the time was not ripe and therefore the concept of cellular heterogeneity in isogenic population faded away quickly. In fact, cell to cell variability that arises because of fluctuations in the biochemical reactions was considered 'noise', an unavoidable feature of the system itself, with either no biological consequence or a nuisance to the orderly behaviour of organisms.

Nevertheless, these studies questioned the long-held assumption that every member of a population of genetically identical cells under identical conditions needs to respond in equal measure. They also highlighted that the growth pattern of a population of cells in response to a stimulus, in all its attendant complexity, can hardly be represented by measuring an average gene expression output. Several studies carried out in different organisms in the past two decades, clearly indicate that the members of a population, or a tissue, function autonomously, but within the broad confines of the constraints imposed by the genetic circuit, and therefore exhibit a seemingly uniform behaviour expected of an isogenic population. If so, is cell to cell variation within anisogenic population of any biological relevance? Are there mechanisms which can amplify this variability or 'noise' to introduce an alternate phenotype which could be beneficial to the population as a whole? Can evolutionary forces feed on such noise to provide adaptive value for evolution to proceed? Understanding the molecular basis, role and the evolutionary significance of 'noise' (stochastic transitions) has now become one of the mainstays of modern biology. The focus of this review is to highlight how *Saccharomyces cerevisiae* exploits stochastic transitions as a means of tackling changes in the availability of glucose and galactose.

The second half of twentieth century saw rapid progress in our understanding of the genetic and biochemical basis of the observation of Dienert[3]. The 'GAL Genetic Switch' of *S. cerevisiae* became a favourite model to study how transcriptional machinery responds to the activating and repressing signals of galactose and glucose, respectively (Figs. 1 and 2), with the tacit assumption that members of a clonal population behave identically[22–26]. More recently, studies have revealed that individual yeast cells of anisogenic population take divergent decisions when exposed to identical conditions and yet behave in a

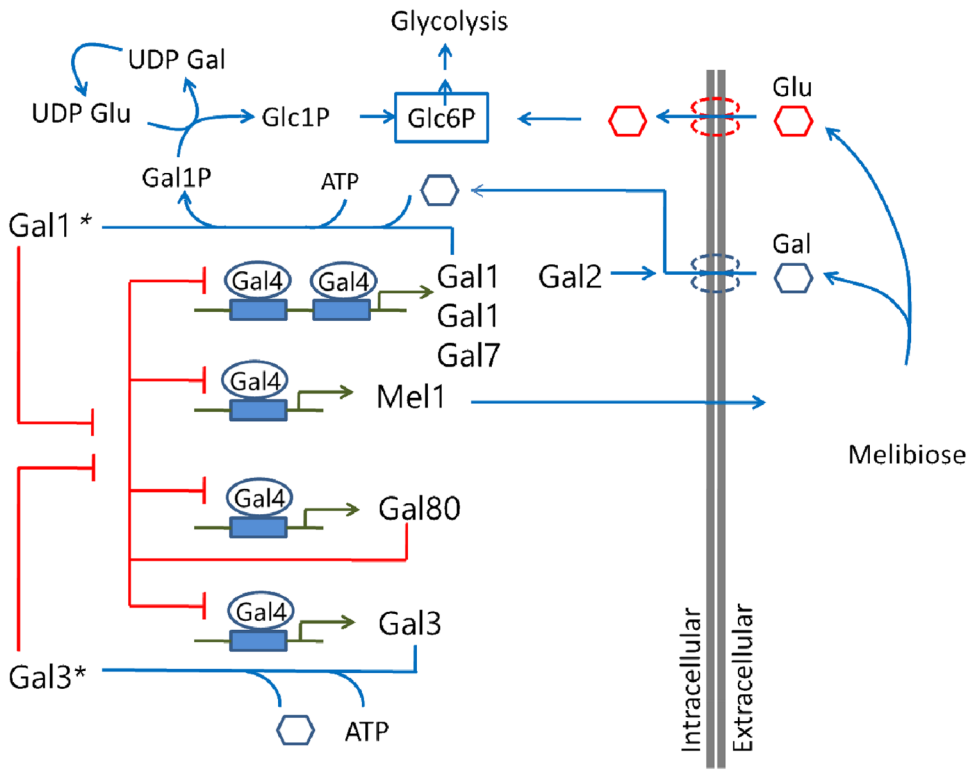

Figure 1: Schematic representation of genetic regulation of GAL/MEL regulon. Red hexagon represents glucose and blue represents galactose. Glucose and galactose can also be produced from melibiose a disaccharide consisting of glucose and galactose. Blue box represents the 17 base pair upstream activating sequence (UASG) present at the 5′ end of promoter of the galactose inducible genes to which Gal4 binds. Red line indicate negative feedback loop of Gal80which prevents Gal4 from activating transcription. Green lines indicate the positive feedback loop operational only when Gal1 and Gal3 get converted to their active conformation, upon recognising galactose and ATP as indicated by Gal1* and Gal3*. Gal2 dependent positive feedback loop is indicated by green arrow. When cells are grown in a neutral carbon source such as glycerol, the DNA binding transcriptional activator protein Gal4 remains bound to UASG but unable to activate the transcription because of the binding of Gal80, the repressor. Once galactose enters the cell, it allosterically activates Gal3, in to an active form. Active Gal3 physically interacts with Gal80 to free Gal4. Free Gal4 activates the transcription of the GAL genes. Thus, one can visualise that a population of cells induced with galactose will turn on the switch and as a function of time, the structural genes would be induced more than Gal3 and Gal80 at any point of time. This is because, promoters with single UASg, that is promoters of (GAL3, GAL80 and MEL1, have high basal but low fold induction. In contrast, promoters of GAL1, GAL7, GAL10 and GAL2 have two UASg. The genes with two UASG have low basal (in the absence of galactose) but have high fold induction (in presence of galactose). Within a few hours of induction, the cells reach a steady state expression of GAL genes. It is clear from the figure that a small change in galactose concentration is sufficient to take the cells from inactive to active state because of the positive feedback loops of Gal3and Gal2. This is counteracted by the negative feedback loop of Gal80 so that the cells tend to respond in a more coherent fashion (See Ref.[26] for more details).

concerted manner at the population level. In this review, we will first discuss the delay in growth phenotypes exhibited by certain mutants of *S. cerevisiae* to illustrate how *noise-induced heterogeneity arises during glucose and galactose utilisation. We will then focus on recent findings that highlight the role of noise induced heterogeneity during the transition from glucose to galactose, in both laboratory strains and wild type isolates. We then argue that the above findings have far-reaching ramifications beyond microbial ecology that would help us better understand the evolutionary aspects of metabolic basis of fundamental processes such as development, differentiation and diseases states.*

Noise-induced heterogeneity: Stochastic fluctuation induced phenotypic diversification is often considered as transient and non-heritable. However, noise-induced effects have the potentiality to introduce epigenetic changes which are heritable provided the regulatory circuit has certain features, such as positive feed back loops. In case phenotypic diversification caused due to stochastic fluctuation results in subsequent epigenetic changes that can be inherited, then it is generally referred as non-genetic heterogeneity, although the origin of such heterogeneity is stochastic. In this article, the term noise-induced heterogeneity is used to connote any heterogeneity caused solely due to stochastic fluctuation without involving subsequent epigenetic alterations.

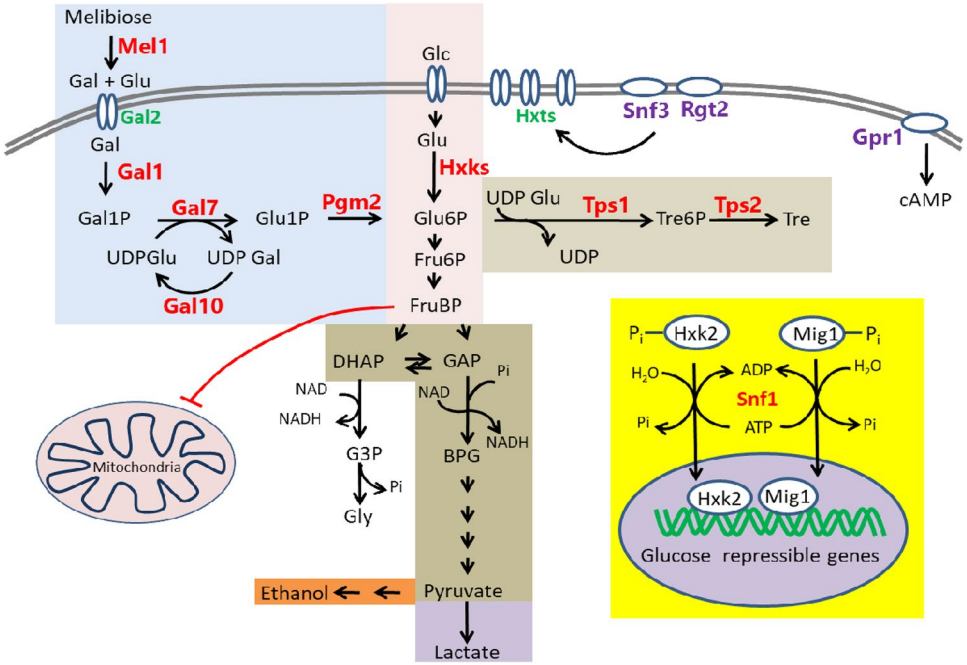

Figure 2: Schematic representation of glucose and galactosemetabolic pathways and glucose signalling. **a** SNF3/RGT2 sense extracellular glucose and activate the transcription (indicated by the arrow) of afamily of hexose transporters (HXTs) depending upon the glucose concentration. **b** *GPR1,* a GPCR, also sense extracellular glucose, and activates the transcription of genes through cAMP pathway, required to metabolise glucose to ethanol. Intracellular sensing of high glucose flux (high ATP/AMP ratio) is sensed by AMP protein kinase (Snf1). When the glycolytic flux is high, Snf1 remains inactive and is unable to phosphorylate transcriptional repressor protein Mig1 and Hexk2, which then enter the nucleus and prevent the transcription of genes coding for respiration and galactose utilisation. High fructose-1-6 bisphosphate suppresses the mitochondrial function at the biochemical level. The above independent mechanisms collectively contribute to glucose repression or Crabtree effect. Humans lack the pathway to ferment glucose to ethanol as well as to synthesise trehalose from glucose 6-phosphate. In humans, pyruvate is converted to lactate while in *S. cerevisiae* pyruvate is converted to ethanol. Abbreviations of Gene-enzyme nomenclature: *Mel1* α-galactosidase, *Gal2* galactose permease, *Gal1* galactokinase, *Gal7* galactose-1-phosphate uridyltransferase, *Gal10* UDP galactose 4-Epimerase, *Pgm2* phosphoglucomutase, *Hxk* Hexokinase, *Tps1* trehalose 6-phosphate synthase, *Tps2* trehalose-6-phosphate phosphatase, *Hxt* hexose transporters. Snf3 and Rtg*2* encode glucose sensors. Gpr1 encodes GPCR coupled glucose sensor. Abbreviations of metabolic intermediates. *Gal1P* galactose-1-phosphate, *Glu1P* glucose-1-phosphate, *UDPGal* uridinediphospho galactose, *UDPGlu* uridinediphosphoglucose, *Glc6P* glucose-6-phosphate, *Fru-6-P* fructose phosphate, *FruBP* fructose 1,6-bisphosphate, *DHAP* dihydroxyacetone phospate, *GAP* glyceraldehyde 3-phosphate, *BPG* 1,3-bishosphoglycerate, *Gly* glycerol, *Tre6P* trehalose-6-phosphate, *Tre* trehalose.

2 Molecular Basis of Noise-Induced Heterogeneity During Growth on Glucose

Screening for mutants to understand the regulation of metabolism was a routine practice in the 1960s. Efforts aimed at understanding the regulatory features of glycolysis led to the identification of three independent loci that caused growth defect when cells were grown on glucose but not when grown on galactose[27–29]. Later on, these three genes were found to be alleles of trehalose-6-phosphate synthase (*TPS1*)[30]. Trehalose, a disaccharide of glucose, normally accumulates when yeast cells are exposed to stress conditions, such as high temperature, nutrient depletion and high osmolarity. Note that, *TPS1*is not a member of the glycolytic pathway (Fig. 2) and is therefore not expected to cause a defect in glucose utilisation. Therefore, it remained a paradox as to why a*tps1Δ* strain suffers from growth defects only when glucose is used as a carbon source. Early attempts to explain this conundrum were based mainly on genetic and biochemical observations. They hinged on the premise that when excess glucose enters the cell, availability of inorganic phosphate (Pi) is depleted, because two molecules of

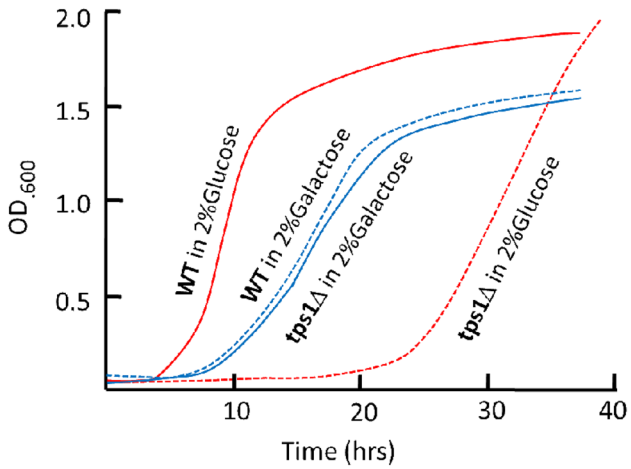

Figure 3: Schematic representation of growth pattern of wild type and *tps1*Δ strain in glucose (red) and galactose (blue). (Adapted from Ref.[33]).

adenosine triphosphate (ATP) are required to convert glucose to fructose 1,6-bisphosphate, during the first phase of glycolysis. This causes a block in the reaction of the second phase of glycolysis, catalysed by glyceraldehyde-3-phopshate dehydrogenase which requires Pi as one of the substrates (Fig. 2).That is, entry of excess glucose outpaces the energy generating reactions located in the lower phase of the pathway, causing the overall glycolytic pathway to come to a halt. If so, the intermediates of the upper part of glycolysis are expected to accumulate in *tps1Δ* strain in presence of excess glucose[31]. Consistent with this hypothesis, it was observed that fructose 1,6-bisphosphate (FBP)accumulates in *tps1Δ* mutants[32]. Mutations that otherwise decrease the flux through the upper part of glycolysis, were shown to overcome the growth defect associated with the *tps1Δ*[33]. In addition, over-expression of genes that encode for enzymes necessary for glycerol biosynthesis overcame the defect by enhancing the availability of Pi (Fig. 2) required to catalyse the glyceraldehyde 3phopshate dehydrogenase reaction[34]. Observations that trehalose 6 phosphate (T6P) is a competitive inhibitor of hexokinase (Hxk)[35] suggested that T6P probably reduces the flux of glucose into glycolysis and ensures the upper part of glycolytic pathway does not outpace the downstream reactions. Consistent with this, even deletion of hexokinase (yeast has additional isozymes of hexokinase) could overcome the growth defect of *tps1Δ*strain[36], suggesting that the excess glucose entry is the cause of the defect. This idea was also supported by an independent observation that the defective growth on glucose of a strain having a weak *TPS1* allele can be overcome by deleting *TPS2*. This was interpreted to mean that T6P accumulated to a level sufficient to inhibit the Hxk. However, cells bearing T6P insensitive mutant of Hxk do grow on glucose arguing against the above possibility[37, 38]. The above explanations provide circumstantial evidences for the observed growth defect of a *tps1Δ* strain, but fail to explain the long lag observed by these mutants when glucose is used as a carbon source (Fig. 3).

More recently, the above problem was addressed by setting up a kinetic model ofglycolysis ina *tps1Δ* strain[39]. The model indicated that the *tps1Δ* strain can exist intwo distinct metabolic stable states, one state similar to the *tps1Δ* mutant and the other similar to wild type. This observation led the authors to look for experimental evidence in support of this possibility. They predicted that the members of population of *tps1Δ* cells that attain the wild type steady state in the model are likely to exhibit normal growth on glucose. To test this, a known number of *tps1Δ* cells pre-grown in glycerol, were plated on galactoseto obtain the total cell count. When the same number of cells were plated on glucose, only 1 out of 10^3–10^4 cells gave rise to distinct colonies while the rest did not. This result was further supported by studying growth kinetics of wild type and the mutant in glucose (non-permissive medium) and galactose (permissive medium). Here, (Fig. 3) wild type and the*tps1Δ* strain were separately inoculated into a medium with glucose as the sole carbon source. As expected, the *tps1Δ* strain showed a long lag while the wild type did not (Fig. 3). In control experiments with galactose, both the

strains showed an identical lag. These results were interpreted to mean that the population of *tps1Δ* strain has a small fraction of cells, of the order of 1 in 10^3 to 1 in 10^4, which grow on glucose like a wild type strain and eventually populates the culture. In the past, such glucose positive *tps1Δ* cells were discarded on the premise that they are genetic suppressors. In fact, this small fraction of glucose positive *tps1Δ* cells are not genetic revertantsbutrather have transiently acquired a metabolic state that can overcome the defect that otherwise exists in *tps1Δ* strain is demonstrated by the following experiment. When this small fraction of *tps1Δ* strain population that can grow on glucose are cultivated in galactose (permissive carbon source) and retested for their ability to grow on glucose subsequently, again only 1 in 10^3 cells is able to grow.

Thus, the ability of a small fraction of the population of *tps1Δ* strain to grow on glucose is not because of a genetic suppression. The authors then investigated whether members of the wild type population can also exist in two physiological states: one state similar to *tps1Δ* strain and the other state similar to the wild type. It turned out that when wild type cells were tested for the above possibility, nearly 7% of the cells were unable to grow on glucose, similar to the phenotype exhibited by the *tps1Δ* strain.

This indicates that even wild type strain, when grown in glucose, is not robust enough to overcome the growth defect due to cellular metabolic heterogeneity.

3 Molecular Basis of Noise-Induced Heterogeneity During Growth on Galactose

As early as 1940s, it was observed that a recessive mutation in *GAL3*confers a delay in the induction of *GAL* genes in response to galactose[40]. This unusual long-term adaptation phenotype became a powerful paradigm to analyse the phenomenon of enzyme adaptation. Given the design of the *GAL* switch (Fig. 1), how does one explain the long-term adaptation phenotype of *gal3Δ* strain? In early 1990s, it was demonstrated that*GAL1* encoded galactokinase (Gal1), the first enzyme of the galactose metabolic pathway, is a bifunctional protein having Gal3-like signalling activity as well as galactokinase activity[41]. Thus, *GAL3* and *GAL1* are paralogous. The mechanism of Gal1-mediated induction involves the same mechanism as Gal3, in that in response to galactose, Gal1 also interacts with Gal80 and prevents Gal80-dependent sequestration of Gal4. Further, if Gal3 function is abolished after the GAL switch is fully induced, Gal1 substitutes as a signal transducer and the induced state continues unchanged[42, 43]. In contrast, in a *gal3Δ*strain, the kinetics of transition of the population from a Gal OFF to Gal ON state is quite distinct from the wild type. Specifically, the *gal3Δ*strain exhibits a slower transition to switch on the Gal switch. That means that Gal3p is required only for the initiation of induction and not for the maintenance of the induced state, as long as Gal1 function is intact. As expected, if both *GAL1* and *GAL3* are deleted, that is, *gal3 gal1Δ*the Gal switch cannot be induced (Fig. 4).

Despite the above knowledge, the molecular basis of the induction delay of *gal3Δ*strain was not clearly understood. Two mutually exclusive hypotheses, postulated almost 25 years apart, were in vogue till recently. The first hypothesis posits that in a*gal3Δ*strain, Gal1 accumulates slowly in every cell over a period of time and reaches a threshold. Once this threshold is reached, the Gal switch is turned ON[44]. In this hypothesis, all cells in a population behave identically. The second hypothesis posits that from among the cells in the entire population, only in a few cells doesGal1 expression reach above a threshold required to induce the GAL switch[45]. It is these few cells that keep proliferating in galactose, which eventually populate the culture, giving rise to the long-term adaptation phenotype. Implied in this hypothesis is that there exists a variation between members of *gal3Δ* population in responding to galactose.

Experimental evidence in support of the latter hypothesis came about a decade later. It turned out that when galactose is provided, approximately only 3 out of 1000 *gal3Δ* cells pre-grown in glycerol(neutral carbon source) have sufficient Gal1 to activate the switch. That is, 3 out of 1000 cells are rapid inducers by virtue of having sufficient Gal1, while in the wild type strain, all cells are rapid inducers by virtue of basal expression of Gal3. This implied that the rest of the population had Gal1 expression below this threshold. This observation indicates that a population of *gal3Δ* cells grown in glycerol are not identical with respect to the basal expression of Gal1p. What is the origin for this difference? It has been suggested that a small fraction of *gal3Δ* cells growing in glycerol, express Gal1stochastically[46]. That is, Gal80 is not robust enough to suppress this stochastic expression of Gal1. It was later demonstrated that a non-coding RNA whose

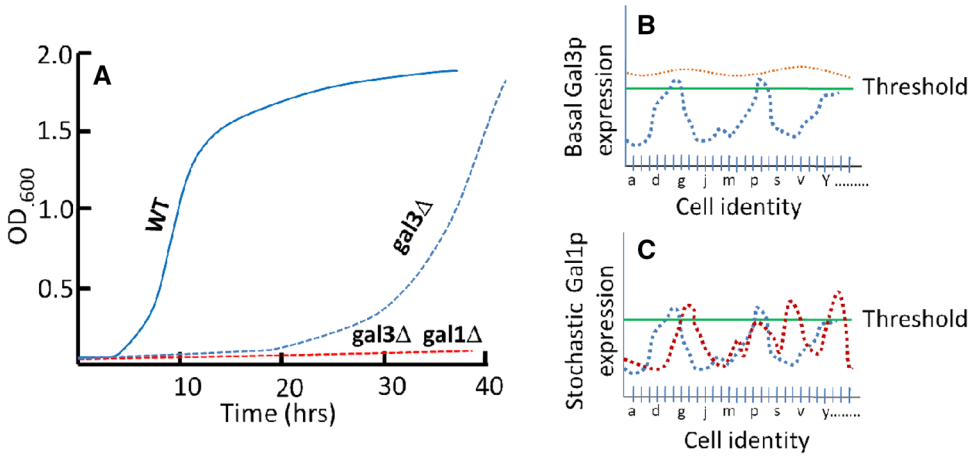

Figure 4: **a** Schematic representation of growth pattern of wild type (blue solid line), *gal3*Δ (blue dotted line) and *gal1*Δ *gal3*Δ (red dashed line) strains in 2% galactose. **b** The expression of basal Gal3when wild type cells are grown in glycerol. Note that Gal3 in every cell is above the threshold and therefore every cell would respond to galactose. **c** The stochastic expression of Gal1in *gal3*Δ strain lacking the noncoding RNA(red dotted line) and *gal3*Δ strain with the intact non-coding RNA (blue dotted line) grown in glycerol. Each dot represents the level of Gal3 or Gal1 in that cell at the time of galactose exposure. Note that only few cells have Gal1 expression above the threshold and only these cells would sense galactose and turn ON the GAL switch and would grow on galactose as the sole carbon source.

transcription runs over the *GAL1* promoter plays a significant role in ensuring that the expression of Gal1is at its minimum during growth on glycerol. If the expression of this non-coding RNA is abolished, the rapid inducer number jumps from 3 to 35 out of thousand and accordingly the lag exhibited by the *gal3Δ* strain is also reduced[47].

Thus, in a wild type strain, of the two signal transducers, Gal3 has a basal expression while the basal expression of Gal1 is below the threshold concentration required to recognise galactose (Fig. 4b). When galactose is present, Gal3 acts as a primary transducer turning on all the GAL genes, including Gal1 (alternative signal transducer). However, a *gal3Δ* strain can still activate the system, but with a long delay due to the presence of few cells that express Gal1stochastically above a threshold (Fig. 4c). This expression of Gal1 is likely due to the inherent stochasticity in transcription, thus resulting in noise-induced heterogeneity in the population.

4 Noise-Induced Heterogeneity Under Changing Conditions

The noise-induced heterogeneity observed in the above example should be viewed as aberrations due to deletion of specific genes. That is, *TPS1* and *GAL3*ensure that all the members of the population respond uniformly to glucose and galactose, respectively. This implies that wild type cells have evolved mechanisms to reduce the cell to cell variation (see the previous section, where, even the wild type cells exhibit heterogeneity) and confer a deterministic behaviour. Alternatively, noise-induced heterogeneity exists in wild type cells as well, but identifying growth conditions where noise-induced heterogeneity exists, is not trivial. This is because, it is difficult to predict a priori, the conditions under which an isogenic population would exhibit phenotypic diversification. Second, if at all phenotypic heterogeneity is exhibited, the fraction of cells in a population that would deviate from the majority is expected to be small making it difficult to establish existence of noise-induced heterogeneity. Thirdly, even if the noise-induced heterogeneity can be demonstrated under a specific experimental condition, assigning a biological role to such a phenomenon is all the more challenging. Despite the above constraints, independent studies carried out in the recent past demonstrate that when *S. cerevisiae* cells are exposed to an environmental perturbation, members of an isogenic population exhibit cell to cell variation due to intrinsic noise (see below). Moreover, there is compelling evidence to suggest that this noise induced heterogeneity is not an aberration in the design per se, but rather an advantageous trait in changing environmental conditions. We will focus on recent studies that demonstrate that *S. cerevisiae* cells exhibit noise-induced heterogeneity mainly

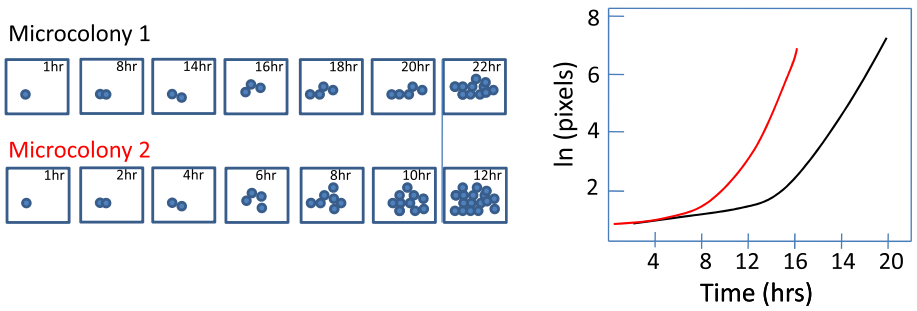

Figure 5: Microcolony growth assay. Top panel represents the growth of a cell into a micro colony as a function time. Lower panel, the above data is transformed into a growth curve (adapted from Ref.[52]).

at the metabolic level when exposed to a mixture of glucose and galactose.

Saccharomyces cerevisiae has evolved mechanisms to respond to a range as wide as ten-fold variation in glucose concentration[48]. At very high (around 2%) glucose concentration, *S. cerevisaie* exhibits high growth rate by fermenting glucose to ethanol. Under these conditions, glucose is fermented (and not processed through the mitochondrial oxidative pathway) despite the presence of oxygen in the medium. In fact, at high concentration, glucose suppresses mitochondrial function as well as the enzymes required for the utilisation of alternative carbon sources (Fig. 2) through a phenomenon known as glucose repression or *Crabtree effect*[49–53]. At concentrations below 0.1%, glucose-repression gets disabled and the yeast switches over to the oxidative metabolism of glucose using oxygen as an electron acceptor. Thus, *S. cerevisaie* is considered as a facultative anaerobe. Using a high throughput microscopic microcolony growth assay, an increase in growth rate as a function of glucose concentration was observed[54]. Second, the growth rate at any particular concentration of glucose varied from strain to strain. These results revealed a continuum of growth strategies across strains obtained from different ecological niches. Surprisingly, the lag of individual microcolonies was vastly different when individual cells of a given strain pre-grown in glucose to stationary phase to ensure cell cycle arrest, and then shifted to a fresh medium containing glucose (Fig. 5). While some cells start growing without lag, others took as much as ten hours to initiate growth. This cell to cell difference in the lag gets accentuated at low glucose concentrations[54]. Thus, not only genetic but even noise-induced mechanisms seem to have evolved during their ecological diversification.

It has been reported that when *S. cerevisiae* cells transit from glucose to an alternate carbon source, expression of more than 40% of its genesis altered[55]. Till recently, it was thought that yeast species, as a rule, exhibit *diauxy lag during glucose to galactose transition. A recent study however, revealed that closely related species belonging to sensu strict* group exhibit a wide variation in the diauxy lag. While *S. cerevisiae* shows a prominent diauxy lag, its sister species *S. bayanus*, *S.castelie* and *S. miketi* do not show any discernible diauxy lag[56]. Let alone different species, even natural isolates of *S. cerevisiae*, obtained from a range of geographical locations, showed a wide variation in the time needed to transition from using glucose to galactose, indicating that diauxy lag is likely subject to evolutionary pressures[57–59]. What was even more surprising is that the variation in diauxy lag between strains could be genetically mapped to *GAL3*. Further, it was demonstrated that, polymorphism of the GAL3 ORF alone was sufficient to confer varying diauxy lag[57]. That is, *GAL3* alleles obtained from the natural isolates conferred the characteristic diauxy lag of their parent strain when transplanted into a laboratory strain of known genetic background. Thus, it appears that existing regulatory networks have sufficient flexibility to accommodate avenues for distinct adaptive trajectories, should adaptation to a new environment becomes a necessity.

In the studies discussed above, diauxy lag was monitored at the population level. This collective behaviour could belie the contribution of cellular decision making at a single-cell resolution to the overall performance of the population. Thus, it is possible that variation in diauxy lag observed in different strains of *S. cerevisiae* could arise because individual members of a population of a given strain respond differently to the glucose–galactose transition. Many interesting questions emerge if indeed a correlation, negative or positive, exists between cell-to-cellvariation and the

Crabtree effect: It refers to the phenomenon of glucose suppression of mitochondrial function. Glucose repression or carbon catabolite repression is the alternative designation of the Crabtree effect, used only in the context of microbial growth.

Diauxy: It literally means double growth. Normally it refers to the growth pattern obtained when microbes such as *Saccharomyces cerevisiaeis* grown in a mixture of glucose and a less preferred carbon such as galatcose. Initially, cells grow in glucose and after it is exhausted, the growth temporarily ceases before the cells switch over to the second carbon source. The lag in between the two growth phases is commonly referred as diauxy lag.

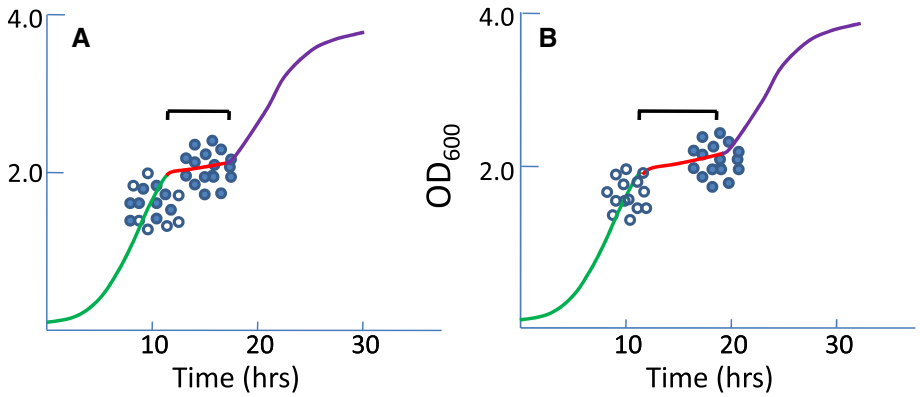

Figure 6: Growth pattern of *Saccharomyces cerevisiae* growing in an equimolar mixture of glucose and galactose. Glucose growth phase, diauxy lag and galactose growth phase are indicated in green, red and violet colours, respectively. Coloured circles indicate cells that express GAL genes while open circles indicate cells that do not express GAL genes. **a**, **b** Different strains showing difference in the diauxy lag. Note that in strain **a**, even before the diauxy lag begins, that is during growth on glucose, a fraction of the population has induced GAL genes while in strain **b**, GAL genes are induced only after glucose is completely depleted. The black bar indicates the difference in the dauxy lag between two strains.

diauxy lag exhibited by a population. For example, would the phenotypic diversification lead to a trade-off between the ability to utilise alternate carbon source and fitness in the preferred carbon source. Do these populations respond to the rate of change of glucose depletion or the absolute level of glucose concentration or the ratio of glucose to galactose? And how does this influence responses at a single-cell resolution? Is bet-hedging at play during this transition? In the last decade, many detailed studies have been carried out that touch upon the above issues using different experimental regimes.

When grown in a medium containing glucose and galactose, it was observed that a fraction of cells of an isogenic population activate transcription from the *GAL1*promoter even before glucose is completely depleted from the medium (Fig. 6a). The rest of the population expresses from the *GAL1* promoter only after glucose is exhausted from the media[58]. Thus, not all the members of the population turn on the *GAL* genes in unison, as previously thought. The diauxy lag among a large battery of strains isolated from different geographical locations ranged from 0 to 9 h. Strains that turn on the *GAL* genes even before glucose exhaustion, have a short diauxy lag while strains that exhibit long diauxy lag express *GAL* genes only after glucose is completely depleted from the medium[58]. There is no difference in the final biomass yield between strains that have short-lag and long-lag. However, the strains that have a short diauxy lag took less time to reach saturation as compared to the strains that had longer lag[58]. The above observations clearly indicate that growth lag, diauxy or otherwise, which occurs normally during transition from preferred carbon source to the alternate is a regulatory strategy and appears to be an evolvable trait.

For example, the pre-induction of *GAL* genes appears to be a response to the impending glucose depletion, coupled with the fact that galactose is present in the media, to prepare themselves for galactose utilisation (Fig. 6).This pre-emptive decision taken by a fraction of the population reduces the ability of the overall population to quickly adapt to glucose, should glucose return to the environment. For example, a strain that has a short-lag has a doubling time of 0.62/h and 0.51/h in glucose and glucose plus galactose, respectively. On the other hand, a strain that has a longer-lag has a doubling time of 0.67/h in glucose as well as glucose plus galactose. Thus, there is a clear trade-off between longer diauxy lag and the ability to maintain a better growth rate during the first phase of the growth. Mechanistically, mutants that alleviate glucose repression have a shorter lag as compared to their wild type counterparts[59] suggesting that glucose repression is a determinant of the diauxy lag and glucose repression is an evolvable trait. But how reduced glucose repression, in effect, brings about larger intercellular variability is not clearly understood (Fig. 7).

In one study, cells were exposed to combinations of concentrations of glucose and galactose ranging from 0 to 2% of each sugar. At low glucose concentrations, the population showed a

unimodal ON state of GAL genes while at high glucose concentrations, the population exhibited a unimodal OFF state. In contrast, when the two sugars are present in an equimolar amount, the population showed a transient bimodal distribution with respect to *GAL* gene induction. Further experiments suggested that this behaviour is an outcome of a response to the sugars and not due to stochastic transition from OFF to ON state[60]. Surprisingly, despite the presence of subpopulation of GAL ON cells, galactose was not consumed, an observation quite opposite to what was observed in an independent study (see Ref.[58]). In contrast, if the GAL genes are turned ON in a galactose independent fashion before glucose is depleted, glucose consumption rate decreased. Thus, sacrificing the overall growth rate on glucose is the price to be paid for activating *GAL* genes in a subpopulation before glucose is completely depleted. On the other hand, if a strain is not prepared to activate *GAL* genes, the growth rate in glucose will not be sacrificed but a longer delay in transiting to galactose consumption would ensue. Given an environment of glucose and galactose, the strains that can consume both sugars simultaneously are considered as generalists while those strains that consume the two sugars hierarchically are considered specialists. Thus, a given strain seem to have an option to choose from the spectrum between these two extreme choices[58, 61].

Different experimental protocols were used by independent groups to look at the mechanism of glucose sensing when cells are exposed to a mixture of glucose and galactose. One study looked at the response of single cells to a varying ratio of glucose to galactose concentration over 1000-fold. This study indicated that cells respond to ratio of glucose and galactose and not the absolute concentration. This behaviour is invariant of starting cell density and is exhibited not only by domesticated strains but also by wild strains. More importantly, this ratio sensing occurs upstream of the canonical GAL signalling pathway, most likely at the level of transport[62]. An independent study looked at the mechanisms of glucose sensing as it gets depleted during diauxy growth. As the glucose levels drop to 0.0625% from a starting concentration of 0.25%, strains that show shorter lag start express *GAL1* just before the cells enter the diauxy state. It was observed that cells start expressing *GAL1*, when transferred to media containing an initial concentration of 0.0625% glucose and 0.25% galactose. These results suggest that cells do not respond to the rate of glucose depletion, rather they respond to the absolute

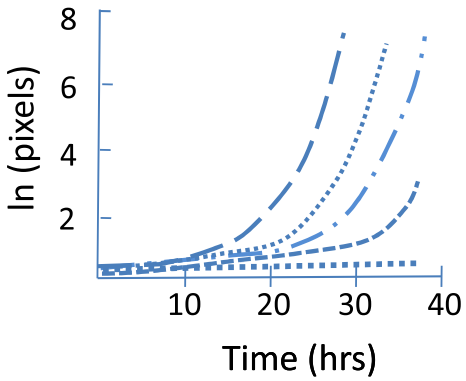

Figure 7: Single cell lineages of cells pre-grown in glucose and then shifted to galactose medium. Each trace represents the growth pattern starting from a single cell as determined by the micro-colony growth assay.

levels of glucose present in the medium[58]. An independent study came to the opposite conclusion. Using a microfluidic device, cells exposed to a constant level of galactose were subjected to varying rates of glucose depletion. It turned out that the GAL1 accumulation occurs more quickly if glucose is depleted slowly. This response is thought to be due to the wastage of energy when glucose is depleted fast[63].

How would cells exposed to constant levels of galactose respond to transient pulses of glucose? It has been shown that cells exposed to constant galactose exhibit multiple dynamic regulatory patterns if challenged with transient pulses of glucose[64]. Another group used a microfluidic devise to investigate how cells growing in a constant concentration of galactose would respond, if exposed to sinusoidal waves of glucose of varying concentration and frequency. Only at low frequency of exposure of glucose, cells responded to the glucose repression while at a high frequency, cells continued to induce the GAL system, suggesting that the response of the cell is quite robust to dynamically changing environment[65].

The above studies illustrate how cells respond toa setting where the total amount of glucose and galactose are made available at the start of the experiment. What if, the glucose and galactose concentrations available to the cell were to be dictated by the cells themselves? Melibiose, a disaccharide consisting of glucose and galactose, is hydrolysed by *MEL1* encoded α-galactosidase extra-cellularly to free glucose and galactose. The expression of *MEL1* is under the control of *GAL* switch. That is, α-galactosidase is induced by galactose and repressed by glucose. How would

cellsrespond to melibiose, as galactose and glucose concentration would depend not only on their rate of consumption but also on the dynamics of their production. Preliminary analysis indicated that cells growing on melibiose exhibit a monophasic growth. We observed that *S. cerevisiae* pre-grown inglycerol (glycerol is a neutral carbon source while glucose is a repressive carbon source with respect to GAL gene induction) and then inoculated into melibiose exhibits an unusually long lag during its growth on melibiose as compared to the lag observed during growth on glucose and galactose separately as well as in a mixture of glucose and galactose. In such a context, where glucose utilization represses, and galactose utilization triggers hydrolysis of melibiose, it is not clear the metabolic strategy that would be employed,at a single-cell resolution, by the yeast growing on melibiose. Several possibilities exist in this context. One, each cell behaves identically where it utilizes glucose (which represses further glucose release via melibiose hydrolysis) and then switches to galactose (which triggers release of α-galactosidase). But this strategy means continuous switching between glucose and galactose—a strategy that hardly seems optimal. Second, the population diversifies into two groups where one uses glucose and the other galactose. Alternatively, any combination of the above two strategies could also be used by the population (Kavatalkar and Bhat, unpublished observations). This "confusion" regarding metabolic fate of the population suggests that, in principle, there is an avenue for evolutionary forces to modulate the phenotypic diversity within a population of isogenic individuals so as to evolve as per the ecological demand.

5 Bioenergetic Bottleneck Drives Noise-Induced Heterogeneity in *S. cerevisiae* Cells

A recent study tracked the expression of *GAL3* and *GAL1* in single cells in response to galactose after pre-growing the cells in glycerol, galactose or glucoseseparately[66]. Recall, that unlike glucose, in glycerol, the expression of enzymes required for the alternate carbon sources is not repressed. When cells are transferred from glycerol to galactose, cells express *GAL1* and *GAL3* in a unimodal fashion. On the other hand, cells pre-grown in glucose, not only exhibit a lag but also exhibit a bimodal expression of GAL3p and GAL1p[66]. Only those cells where a rare stochastic molecular event turns on *GAL3* or *GAL1*above a threshold are capable of activating the switch and can thereafter grow on galactose. This is because, both *GAL3* and *GAL1*are severely repressed during glucose pre-growth condition[66]. Moreover, pre-growth in glucose also causes mitochondrial repression and only few cells can come out of this bio-energetic bottleneck. Thus, the cells need to make either Gal3 or Gal1in sufficient amount to kick-start the *GAL* switch, which can then, because of the positive feedback loops, sustain itself. Based on these results, the authors point out that cells that have come out of the above bioenergetic bottleneck eventually populate the culture and those that cannot lose out. The authors also observed that the reduction in the number of un-induced cells as a function of time, is not because un-induced cells induce over a period of time. Instead, the fraction of cells that stochastically induce, demographically replace the cells that were unable to induce. In fact, the cells unable to induce *GAL1* or *GAL3*, eventually die as a function of time. It is clear that the metabolic state imposed by glucose repression is so stable that only few cells seem to be capable of escaping from this through a stochastic event to enter into the induced state.

Curiously enough, if glucose repression is so severe, how do we explain growth history dependent cellular memory? Previous studies have demonstrated that cells pre-grown in glucose for 12 h show a detectable lag in growth kinetics when switched to galactose. However, this lag is reduced if cells are grown on galactose before growing the cells in glucose for 12 h. Based on this growth history dependent behaviour, it was inferred that pre-growth in galactose imprints a memory that remains even after many cells divisions during growth on glucose for 12 h. This galactose induced memory is due to either epigenetic changes ingrained in nucloesome structure or could be due to theresidualGal1 protein that lingers on or a combination of both, which does not get erased even after 12 h of growth on glucose[62].

To dissect out the molecular basis of growth history dependent cellular memory, the authors initially exposed the cells to maltose followed by 12 h of growth on glucose and monitored the lag for the induction of *GAL* genes in single cells. What is expected is that prior growth on maltose, should not confer galactose memory. It turned out that the induction lag of GAL genes was similar regardless of whether cells were exposed to maltose or galactose prior to exposing the cells to glucose for 12 h[67–69]. This observation argues against the growth history dependent cellular memory. Following up on this lead, further

studies demonstrated that the rate-limiting step that determines the lag during the transition from glucose to galactose is the de-repression of proteins required for respiratory function. Using a Bar-Seq technology for screening mutants, two independent studies identified mitochondrial genes crucial for this transition. For example, loss of cytC, complexes III, IV and coenzyme Q increase the lag at least two times as compared to their wild type counterparts. Strains deleted for the above genes fail to adapt to galactose. Consistent with this observation, the authors observe that during the glucose to galactose transition, the recovery of oxidative metabolism precedes the activation of the pathway for the secondary carbon source. Thus, the mitochondrial function seems inevitable for the induction of the genes required for the metabolism of galactose. Only cells that come out of this bottleneck respond to galactose[70]. Thus, we have come a full circle on growth lag experienced by microbial cells during their transition from glucose to alternate carbon source. Recall, that Monod had observed that when respiratory activity was abolished, bacterial cells were unable to adapt.

We now recognise that the diauxy transition occurs only after the cells have shifted to respiratory mode of energy generation. In past, disparate observations had hinted at the role of respiratory energy in the activation of galactose utilisations pathway in *S. cerevisiae*. It was known for long that that a*gal3* strain lacking mitochondria was unable to grow on galactose as the sole carbon source but would grow on glucose[70]. Much later, it was demonstrated that in *gal3Δ* strain, the *GAL1* dependent induction is dependent on mitochondrial function[41, 71]. Based on the phosphorylation state of S699 of Gal4p, it was proposed that the GAL system needs to receive the signal from galactose as well as a signal originating from the energy status of the cell[72]. The latter signal was shown to be mediated by phosphorylation of S699 by *CDC10* encoded kinase[73]. Recall that, both galactose and ATP are required to convert Gal3p and Gal1p to an active form. It appears that unless the cell is fully prepared for respiratory metabolism[74]; it may not venture into activating *GAL* gene induction, an energy-intensive pathway. It was demonstrated early on that in a strain lacking mitochondria the generation time when grown on galactoseis twice as compared to its wild type counterpart, whereas the generation time remains unchanged when grown glucose regardless of the mitochondrial contribution[75].

6 Glucose as the Primaeval Source of Carbon and Energy

Glucose, the most abundant monosaccharide in nature, is also the most preferred source of carbon and energy for a vast majority of life forms. Immediately following the evolution of photosynthesis, there presumably would have been a fierce competition for glucose as a source of energy and carbon. Therefore, it is not surprising to see that organisms have optimised their growth strategies for metabolising glucose through different pathways, fermentation being one of them[76]. Evolutionary considerations dictate that organisms with reduced lag during transition from glucose to the less-preferred carbon sources would outcompete organisms that have extended lag. If so, what selective forces moulded *S. cerevisiae* to be an avid glucose fermentor and exhibit extended lag during transition from glucose to galatcose? For example, *S. cerevisiae* activates the expression of glucose transporters and glycolytic enzymes after sensing the concentration of extra-cellular glucose while intracellular glycolytic flux (Fig. 2) and energy status sends signals to repress the expression of unwanted proteins such as proteins required for respiration and other carbon utilisation pathways. This design seems to have contributed immensely for *S. cerevisiae* to be a prolific glucose fermentor[77–79]. The consequent tradeoff of this commitment to glucose being that it is unable to rapidly switch over to mitochondrial respiration. When glucose sensing is decoupled from the transport activity the growth rate of the organisms decreases[80], indicating a tight coupling between the glucose uptake and glucose sensing, as a necessary mechanism for maintaining high growth rate. Because of its signalling role, glucose is considered as a hormone and not just a nutrient[53]. Availability of whole genome sequence of closely related yeast species allows us to peep into an otherwise closely guarded secret of the organism's evolutionary history.

The genomes of the members of the hemiascomycete lineage consisting of the genus *Klueveromyces* and *Saccharomyces,* (these two genus come under *Saccharomyces* complex, see Fig. 8) have been sequenced. *Saccharomyces* and *Klueveromyces* split from a common ancestor 100MYA[81, 82]. After the separation, *Saccharomyces* underwent a whole genome duplication (WGD) event following which the genome got reorganised leaving 551 duplicate pairs among a total of 5800 genes[83]. In contrast, the *Klueveromyces* genome did not go through a WGDevent and thus serves as a reference to track the evolutionary trajectory of *Saccharomyces*, which has five

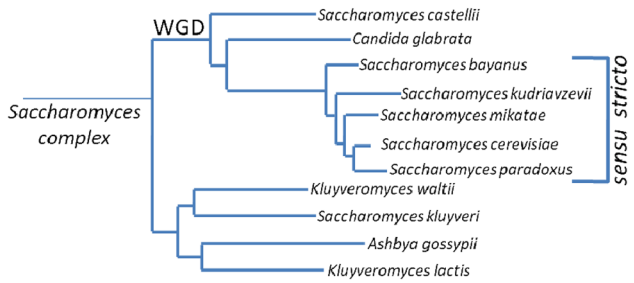

Figure 8: Phylogentic relationship among the members of Saccharomyces complex (adapted from Ref.[82]). *WGD* whole genome duplication.

distinct species. As mentioned before, among the members of the sensu stricto group, *S. cerevisiae* has a prolonged diauxy lag (Fig. 8). The question is whether WGD provided any selective advantage to *S. cerevisiae* in optimising rapid growth phenotype in glucose. An interesting idea that has received considerable attention in the recent past is that the appearance of fruit bearing angiosperms provided a niche wherein *S. cerevisiae* evolved sophisticated mechanisms to compete for the large excess of glucose available in nature[77–79].

Saccharomyces cerevisiae has a family of hexose transporters consisting of 18 members while *K. lactis* has only two members in this family. Of the 18, some got neo-functionalized into glucose sensors as mentioned before, while others got sub-functionalized by changing the expression pattern and acquiring different affinities for glucose and related hexoses[84, 85]. Thus, the presence of a variety of glucose transporters with different properties confer a unique advantage to *S. cerevisiae* in that it can continuously monitor the extracellular glucose concentration and accordingly modulate its growth rate. For example, when the extracellular glucose concentration depletes, high affinity glucose transporters are expressed to pick up even a miniscule amount of glucose and channelize into the oxidative pathway. At high glucose concentration, low affinity glucose transporters are expressed and glucose is fermented even in the presence of oxygen. Further, it is also suggested that the presence of duplicate glycolytic genes increase the flux through the pathway, while growing in glucose-rich environment[86]. This ability to ferment glucose even in presence of oxygen is known as *Warburg effect or aerobic glycolysis (see next section for details), which evolved following WGD. Warburg effect in yeast gets implemented because glucose represses mitochondrial function through multiple mechanisms. This phenomenon is generally referred as glucose repression and is also known by the term Crabtree effect (see next section). Thus, S cerevisiae* is a Crabtree-positive yeast while in *K. lactis* glucose does not suppress mitochondrial function and gets oxidised through mitochondrial respiratory pathway and therefore it is Crabtree negative[77]. The sequence motif AATTTT required for rapid proliferation is found in the promoters of genes that code for both mitochondrial and cytoplasmic ribosomal protein in species such as *K. lactis* (Crabtree negative)[77–79]. In contrast, this sequence is lost from the promoters of the genes that code for mitochondrial ribosomes but present in the genes that code for cytoplasmic ribosome in *S. cerevisiae* indicating that during growth on glucose, only the cytoplasmic ribosome synthesis is promoted. Further analysis indicated that this loss is observed in species that have gone through WGD[87].

Interestingly, the ability to ferment glucose to ethanol evolved independently 200MYA, in another branch of yeast.This example recapitulates a situation similar to *Saccharomyces* and *Klueveromyces*. Here, *Dekkera Bruxellensis* behaves identical to *S. cerevisiae* in its life style while *Brettanomyces naardenensis* behaves more like *K. lactis*[88]. Moreover, *Dekkera* genome lacked AATTTT in the ribosomal genes of mitochondria while *Brettanomyces* had retained it in both mitochondrial ribosomal genes. Overall, these observations suggest that fermentation in response to high extracellular glucose linked to rapid growth is an ancient metabolic regulatory scheme that has stood the test of time. It has a direct bearing on our understanding of how glucose fermentation is tightly coupled to cellular proliferation even in humans, a topic that has attracted considerable attention in recent past.

7 An Aside on Warburg Effect, Crabtree Effect and Cancer

Historically, glucose metabolism was studied mainly in yeast and mammalian tissues. While yeast ferments glucose to ethanol even in presence of oxygen, aerobic organisms such as humans normally oxidise glucose to CO_2 through mitochondrial respiration. Conversion of glucose

Warburg effect: It is a phenomenon where in cells ferment glucose even when sufficient oxygen is available. Normal quiescent cells do not ferment glucose to lactate, instead oxidise it to CO_2. Normal cells destined to proliferate rapidly because of stimulation by growth factors or cancer cells which inherently proliferate rapidly exhibit Warburg effect. *S. cerevisaie* cells too exhibit Warburg effect under conditions of excess glucose in the medium and where glucose is fermented to ethanol. Warburg effect is often referred as aerobic glycolysis.

to lactate in muscle, a textbook example of fermentation in aerobic organisms, provides only a restricted perspective of its otherwise pervasive role even in aerobic organisms. Although it was known that the fermentation is not just limited to muscle tissue alone, interest in glucose fermentation to lactate got rekindled only somewhat recently. Glucose fermentation to lactate even in presence of sufficient oxygen was first observed in Flexner-Jobbling rat carcinoma cells by Warburg[89]. Based on this, he proposed that malignant cells derive energy by fermenting large excess of glucose to lactate because of a defect in mitochondrial respiration. It is now clear that malignant cells respire and mitochondria remain functional[90]. Thus, one of the hallmarks of cancer cells is excess glucose uptake with concomitant fermentation to lactate even in the presence of sufficient oxygen, a phenomenon known as Warburg effect or aerobic glycolysis. Immediately following Warburg's observation, Crabtree reported that glucose causes a decrease in oxygen consumption in Ehrlich ascities cells[91], a phenomenon later came to be known as Crabtree effect. In fact, the inspiration to provide an alternative designation to glucose repression and classify yeast into Crabtree positive and Crabtree negative[77]came from the observation made by Crabtree[91], although significant mechanistic differences exist between glucose repression and Crabtree effect. One of the mechanisms of Crabtree effect appears to be mediated through 1,6,bisphosphate fructose which suppresses mitochondrial function in *S. cerevisiae* as well as in humans (see Fig. 2)[92, 93]. Regardless of the above, the correlation between Warburg effect and high growth rate is conserved from yeast to humans, suggesting that a common metabolic regulatory strategy is at work. As mentioned before, *S. cerevisiae* proliferates rapidly in response to high extracellular glucose concentration. On the other hand, human cells proliferate rapidly only when glucose uptake is activated, as happens in normal cells exposed to growth factors or in malignant cells because of mutations. That is, normal human cells respond to growth factors and exhibit Warburg effect, meaning that they proliferate rapidly by increasing glucose uptake with lactate excretion even when sufficient oxygen is available. Else, normal cells follow the oxidative mode of glucose metabolism[94]. In contrast, malignant cells constitutively exhibit Warburg effect, meaning that because of genetic alterations, they proliferate rapidly by increasing glucose uptake rates, independent of growth stimulation.

Notwithstanding our current knowledge of glucose metabolism in all its ramifications, it has remained a vexing problem to understand why fermentative mode of glucose metabolism confers higher growth rate despite the efficiency of ATP production is severely compromised. Recall, that fermentation generates 2ATPs/molecule of glucose, an energy inefficient process as compared to oxidative phosphorylation which yields 36ATP/ molecule of glucose. One of the theories suggests that there is a trade-off between rate and yield (yield-rate-trade off-YRT) and which pathway would be followed depends on many alternative mechanisms[95, 96]. The make-accumulate-consume (MAC) theory posits that the accumulated ethanol during fermentation is utilised subsequently[79]. That is, glucose is consumed rapidly, thus making it not readily available to its competitors with the consequent accumulation of ethanol, a toxic end product that may be detrimental to the competing organisms. More recently, an alternative possibility has been considered which is based on an imbalance between the upper part of glycolysis, (energy consuming reactions) and lower part of glycolysis (energy producing reactions). This imbalance is thought to occur under glucose excess condition[97]. According to this proposal, fermentation and mitochondrial oxidation is a mechanism that overcomes this clash.

It was recently demonstrated that competition between *Lachancea kluyveri,* a weak Crabtree positive strain with bacteria, resulted in an evolved *Lachancea kluyveri* with features commonly present in a Crabtree positive yeast. Authors suggest that this result is difficult to explain based on the trade-off between rate versus efficiency. Rather, the acquired mechanisms during evolution to rapidly consume glucose and eventually utilise ethanol would have provided a distinct advantage to *Lachancea kluyveri*[98]. Further support that Warburg effect is evolutionarily conserved comes from a recent observation that as compared to genes that are unique to metazoans, the genes that are over-expressed in cancer cells required for Warburg effect are the ones that have orthologues in microbes[99]. Ifisogenic yeast cells can exist in different metabolic states under identical conditions as discussed herein, can similar mechanisms be operative in human cells as well?

The genesis of malignancy and metastasis has been viewed in terms of Darwinian evolutionary process. It is tacitly considered that Warburg effect, a universal feature of cancer cells, arises as a result of genetic alterations. That is, the acquired morphological and metabolic

phenotype normally associated with cancer, is thought to be preceded by heritable genetic and/or epigenetic alteration. Recently, however, non-heritable cellular variation due to stochastic transition is thought to play a role in such diverse cellular processes as multidrug resistance, behavioural individuality and even in the development of cancer[100–106]. In fact, Warburg did consider the possibility of metabolic heterogeneity as a cause of cancer. He stated that "Nothing hinders us from supposing an irregular distribution of glycolytic action and imagining that for example, resting epithelium is a mosaic in which a few cells strongly glycolyse while the majority do not at all. If a lack of oxygen acts on a mixture of cells of this type… the cells lacking glycolytic action must perish, however, the cells that are glycolytically active can survive"[89]. It is now clear, that stochastic transitions causing phenotypic diversification pervades across life forms and has finally caught the imagination of biologists and is reviewed periodically[107–111]. Yet, it is not easy to embrace the idea that stochastic transitions can have selective value for evolution to work its way through.

8 Conclusion

The fundamental tenet of evolutionary biology is that selection acts on phenotypic variation caused by heritable genetic variation for evolution to progress. According to this, the change in the genetic composition of the population drives the phenotypic variation. Analysis of growth lag during sugar utilisation, a phenotype commonly observed in microbes, laid the foundation for our understanding, at the molecular level, of how a genotype determines the phenotype. As discussed here, variation in diauxy lag is a heritable genetic trait and therefore snugly fits in to the classical concept of the evolutionary theory. We now know that stochastic transition causes variation in diauxy lag within the isogenic population. How would this concept fit in to the classical framework of evolutionary biology? The question is whether noise-induced heterogeneity is selected during evolutionary process. Experimental evidence points out that stochastic switching might be one of the earliest evolutionary strategies for facing the challenges of environmental fluctuation[112]. The role of *TPS1* or *GAL3*in the wild type cells only highlights the otherwise hidden potentiality for stochastic transition. A similar observation has been made in *C. elegans*, where mutations in developmental network genes lead to phenotypic variations otherwise absent in the wild type[113]. Therefore, it is possible that novel regulatory circuitry can arise that can stabilise metabolic steady states, and if such states happen to provide an advantage to the population as a whole, it will be selected. Existence of co-operation between cell population with differences in metabolic states driven by stochastic transitions can be more stable than between different genotypes. This is because genetic differences impose constraints to transit from one metabolic state to the other. Next, the timescale of stochastic transition is much shorter and therefore more relevant in a dynamically fluctuating environment. It is possible that genetically identical cells existing in multiple steady states can co-operate to build a complex community structure providing an opportunity for mutations to stabilise the same. In fact, two recent studies suggest that phenotypic diversification caused by stochastic transition can promote adaptive evolution[114, 115]. It appears that the inherent stochasticity associated with molecular interactions is exploited by evolutionary forces to shape myriad life forms spanning biological organisation from single to multicellular forms. Probably, stochastic transition leading to phenotypic diversification promotes division of labour and co-operation. More recently, it has been demonstrated that yeast colony of clonal population can self-organise into distinct interdependent metabolic subpopulation[116]. Thus, it is not too far-fetched to imagine that this would have served as the precursor for the evolution of metazoans, where we see a clear division of labour between different tissues which have to co-operate for the proper functioning of the organism as a whole.

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