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# A model for generating several adaptive phenotypes from a single genetic event

## *Saccharomyces cerevisiae GAP1* as a potential bet-hedging switch

Henrik D. Møller,* Kaj S. Andersen and Birgitte Regenberg
Molecular Integrative Physiology; University of Copenhagen; Copenhagen, Denmark

**Microbial populations adapt to environmental fluctuations through random switching of fitness-related traits in individual cells. This increases the likelihood that a subpopulation will be adaptive in a future milieu. However, populations are particularly challenged when several environment factors change simultaneously. We suggest that a population can rapidly adapt to multiple environmental changes if individual members stochastically flip a hub-switch that controls a set of adaptive phenotypes in a single event. This mechanism of coupling phenotypic outcomes via a hub-switch can protect a population against large fluctuations in size. Here we report that the general amino acid transporter Gap1 is a potential hub-switch. The *GAP1* gene is flanked by two direct repeats that can lead to *GAP1* deletions (Δ*gap1*) and a self-replicating *GAP1* circle. Thus, an isogenic *GAP1* population can differentiate into two variant, reversible genotypes, Δ*gap1* or *GAP1*$^{circle}$. These subpopulations have different phenotypic advantages. A Δ*gap1* population has a selective advantage on allantoin or ammonium as a nitrogen source and high stress tolerance. Advantages of the *GAP1* population include amino acid uptake, fast energy recruitment by trehalose mobilization, and in some cases, adherent biofilm growth. Our proposed model of a hub-switch locus enhances the bet-hedging model of population dynamics.**

**Keywords:** DNA circle, fitness, social, extrachromosomal element, hub-switch, Gap1, evolution, biological adaptation, population dynamics

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*Correspondence to: Henrik D. Møller; Email: hdmoller@bio.ku.dk

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In nature, microbial populations face fluctuations in environmental parameters such as temperature, osmotic pressure, pH and nutrient concentration. Environmental fluctuations challenge the population structure. A phenotypically uniform population with optimized fitness to a particular environment is likely to decline and could become extinct when the environment changes. A population that is phenotypically diverse has an increased chance of survival, if it contains subpopulations with fitnesses that are optimal for growth in different, randomly occurring conditions.[1]

One mechanism for population diversification is bet-hedging. Bet-hedging is the random switching of fitness-related traits among individuals within a population that increases the likelihood of diverse subpopulations will be adaptive in a future milieu.[1] The definition of bet-hedging has three criteria: First, phenotypic variation of a population occurs randomly through epigenetic switches. Second, the likelihood of a shift must be constant and independent of the environment. Finally, the different resulting phenotypes must have optimal fitness in at least one environmental condition, so that the mean population fitness in fluctuating environments is higher for a mixed population than for a phenotypically uniform population (defined in ref. 1–4). We propose that, in addition to epigenetic switches, reversible genetic mutations are also a mechanism for bet-hedging.

Bet-hedging is reported for several microorganisms including bacteria[1,5,6] and yeast.[7] A classic example of a stochastic genetic switch that creates phenotypic variation is antigenic variation in *Salmonella*. Here, the promoter that controls two

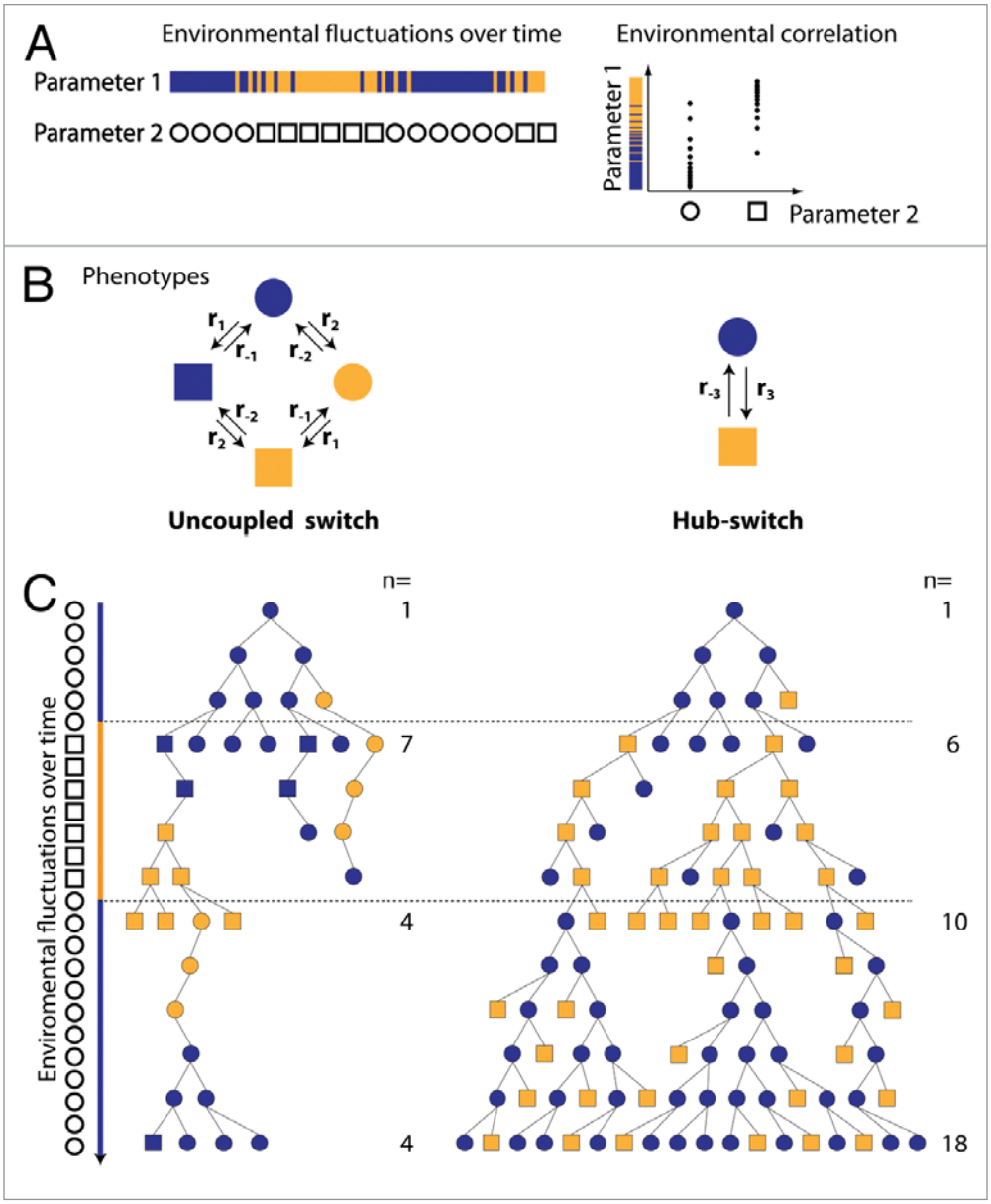


**Figure 1.** Model of uncoupled and coupled phenotypic switches with consequences of selection in an environment with two correlated conditions. (**A**) When the values of two environmental parameters (color and shape) correlate, the selection pressure has an additional layer, compared with each parameter acting alone, if the combination of parameters has an additive or distinctive selection. (**B**) Phenotypic outcomes of uncoupled or coupled switches. An uncoupled switch changes over two generations (e.g., from blue circles to yellow squares) at a rate of: $2r_1r_2+r_1r_2(1-r_{-1})+r_1r_2(1-r_{-2})$ while a hub-switch controls several adaptive phenotypes and switches at a rate of: $r_3(1-r_{-3})+r_3$. (**C**) Cellular lineages of two theoretical clones over a couple of fluctuations, showing that uncoupled switches (left) and hub-switches (right), have considerable differences in population sizes (n). The example is based on switching rates of 1/3 for $r_1$, $r_2$, $r_3$, $r_{-1}$, $r_{-2}$ and $r_{-3}$, with two offspring for optimal color and shape, one offspring when only color or shape is optimal and zero offspring when neither color nor shape is optimal.

flagella synthesis genes is flanked by inverted repeats.[8] Promoter inversions, facilitated by the inverted repeats, lead to changes in the expression of cell surface flagella antigens. This creates a heterogeneous population in which some variants can evade the host immune system.[9]

Genetic and epigenetic switches that enable a species to alternate between two phenotypic modes are selected evolutionarily through adaptation to an environment in which a parameter fluctuates between two conditions.[1] However, when several environmental parameters change simultaneously, selection of multiple phenotypic switches might be required in order to adapt. On that assumption, the environmental multidimensionality is a challenge for the bet-hedging theory, since under its criteria, populations might be vulnerable to simultaneous changes in several environmental parameters. Frequent occurrence of such conditions would result in large variations in population sizes over time, due to lower probability of a subset of individuals evolve an adaptive phenotype (**Fig. 1**, left).

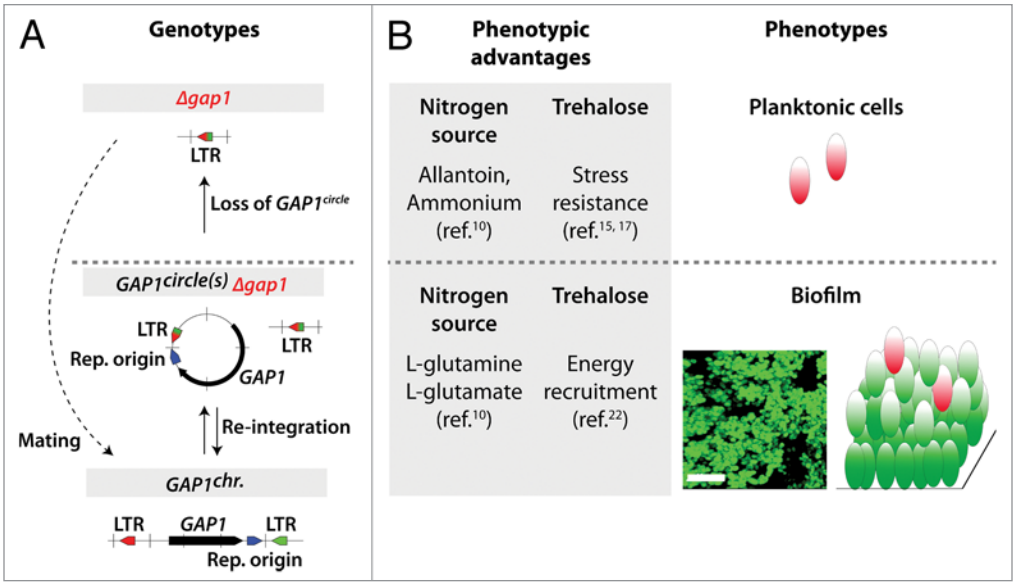


**Figure 2.** Bistability of *GAP1* and a putative biofilm model. (**A**) At least three different forms of the *GAP1* locus can exist in yeast: the chromosomal *GAP1* locus (*GAP1*$^{chr}$), the *GAP1* locus on an extrachromosomal circle (*GAP1*$^{circle}$) and Δ*gap1* from loss of *GAP1*$^{circle}$. A Δ*gap1* cell can become a *GAP1* cell after mating with a *GAP1*$^{chr}$ cell or by reintegration of the *GAP1*$^{circle}$ to the chromosome via the homologous LTR element. (**B**) Model of phenotypic consequences of *GAP1* locus variability in biofilm growth. Biofilm formers contain *GAP1* or *GAP1*$^{circle(s)}$ while planktonic cells arise from *GAP1* deletion. The Δ*gap1* cells have a selective advantage on allantoin and ammonium and have higher levels of trehalose, which confers stress resistance. *GAP1* cells have a selective advantage on glutamine and glutamate as nitrogen sources and fast recruitment of glucose from trehalose provide a selective advantage when nitrogen becomes available (see text for fitness advantages). A biofilm image of a *S. cerevisiae* mutants overexpressing *GAP1* (RB3)[20] after fluorophore-labeling and confocal laser scanning microscopy as described.[21] White bar = 30 μm.

We propose that adaptation to multiple fluctuating parameters can occur by stochastic switching of a regulatory component that couples several phenotypes—a hub-switch—that can alter an entire set of adaptive phenotypes in a single event. A hub-switch would be selectively advantageous in situations where changes in several physical parameters occur at once, such as seasonal changes in rain, temperature, nutrients and light intensity. The wiring or coupling of phenotypic outcomes via a hub-switch could protect a population against fluctuations in size and the ultimate risk of extinction. The selection of a hub-switch is likely to occur when occasional fluctuations occur in coordinated physical parameters (**Fig. 1**, right).

We have observed a potential hub-switch in the yeast *Saccharomyces cerevisiae,* in which the *GAP1* gene couples several phenotypes related to stress response and nutrient utilization. We found that the genetic structure around *GAP1*, which is flanked by two long-terminal repeat (LTR) elements, frequently leads to deletions of *GAP1*.[10] Gap1p is originally described as an amino acid transporter in the *S. cerevisiae* plasma membrane that is responsible for uptake of all common L-amino acids,[11,12] D-amino acids[13] and certain dipeptides.[14]

In competitive growth assays, isogenic Δ*gap1* cells have lower fitness than ancestral *GAP1* cells when grown with L-glutamate as the sole and limiting nitrogen source (fitness 0.93 gen$^{-1}$). In contrast, Δ*gap1* cells have significantly higher fitness than *GAP1* cells when grown in either ammonium or allantoin as the sole and limiting nitrogen source (fitness 1.09 and 1.64 gen$^{-1}$, respectively). Moreover, Δ*gap1* cells dominate mixed populations grown in urea.[10] The different fitness observed under different growth conditions, as well as the ability to differentiate from an isogenic population into Δ*gap1* and *GAP1* cells could be described as a bet-hedging mechanism. To fulfill the criteria for a bet-hedging mechanism, Δ*gap1* cells must be able to revert to *GAP1* cells, which is possible through mating between Δ*gap1* and *GAP1* cells.

The *GAP1* locus contains a replication origin and two solo LTR elements, YKRCδ11 and YKRCδ12, that can undergo homologous recombination, leading to DNA circularization of the locus[10] (**Fig. 2**). A screen of Δ*gap1* clones revealed that 90% of all Δ*gap1* cells were formed through recombination between the two LTRs. We found that the recombination product of this event is a self-replicating *GAP1* circle (*GAP1*$^{circle}$) that allows amplification of the *GAP1* copy number (*GAP1*$^{amp}$). This suggests that the structure of the *GAP1* locus with its LTR elements was selected to allow stochastic deletion of the locus from the chromosome. The *GAP1* circle is found in cells evolved on L-glutamine as the sole and limiting nitrogen source. Under these conditions, the relative fitness of clones with *GAP1* amplifications over *GAP1* clones is 1.14–1.31 gen$^{-1}$ (see ref. 10). Thus, cells carrying multiple copies of *GAP1*$^{circles}$ as *GAP1*$^{amp}$ might have higher fitness than cells with a single *GAP1* allele, presumably through increased amino acid uptake.

In addition to its function as amino acid transporter, Gap1p is also a regulator of the protein kinase A pathway, which has the stress-protecting sugar trehalose as one of its targets.[15,16] Accumulation of trehalose in *S. cerevisiae* cells increases their survival rate when exposed to stresses such

as ethanol, hydrogen peroxide,[17] heat,[18] or osmotic changes.[19] Exposure of Gap1p to amino acids triggers trehalose degradation via trehalase, which allows *GAP1* cells to mobilize a carbon and energy source[22] when nitrogen becomes available. The Δ*gap1* cells, which cannot elicit this signal, have higher levels of trehalose.[15] Diversification in trehalose content in yeast was recently shown by Levy et al., which reported a bet-hedging mechanism through trehalose-mediated heat resistance of slow-growing cells while fast-growing cells were heat-sensitive with a low trehalose content.[7] Hence, while still under investigation, Δ*gap1* cells are presumably more resistant than *GAP1* cells to certain types of stress due to their higher trehalose content. Opposite, *GAP1* cells might have a fitness advantage over Δ*gap1* cells because of faster energy recruitment from trehalose when external nitrogen becomes available (**Fig. 2** and ref. 15).

An additional phenotype linked to Gap1p that underscores the likelihood of *GAP1* as a hub-switch, is the ability to activate a sessile form. We found that cells expressing high levels of *GAP1* form a dense biofilm and express cell-surface proteins in the Flo family that are involved in cell-cell and cell-surface adhesion (**Fig. 2B** and ref. 20). Deletion of *GAP1* in these mutants leads to loss of *FLO11* expression and a planktonic form, revealing that Gap1p can regulate biofilm formation via *FLO11*.[20]

In summary, the architecture of the *GAP1* locus enables frequent deletions and amplifications. We propose that the coupling of amino acid uptake, trehalose degradation and biofilm formation through Gap1 allows mixed populations of *GAP1*, *GAP1*$^{circle}$ and Δ*gap1* to occur, providing a mechanism for adaptation to fluctuating stress and nitrogen levels. The *GAP1* hub-switch connects adaptation to stress and lack of amino acids in the environment. The switch also couples adaptation to certain amino acids in the environment and the ability to mobilize glucose from trehalose, and in some cases a biofilm lifestyle.

Hence, loci that act as hub-switches, controlling a set of phenotypes through a single genetic or epigenetic event, could increase mean fitness of a population when changes in several physical parameters occur simultaneously. Hub-switches could thus enhance the bet-hedging theory by adding a new mechanism for phenotypic variation in populations.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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