

# How to survive within a yeast colony?

## Change metabolism or cope with stress?

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Yeast colonies growing on solid medium begin at a particular point in their development to produce volatile ammonia and to alkalize their surroundings.<sup>1</sup> Ammonia serves as a long-range signal between neighboring colonies and was shown to influence various aspects of colony biology including metabolic reprogramming and differentiation. In a recent paper we presented the impact of deleting of key stress defense enzymes on ammonia signaling and colony development. New findings suggest that it is not stress defense, but rather proper development guided by ammonia signaling and related metabolic changes that are important factors in the long-term survival of a colony cell population.

Ammonia signaling was found to be connected with extensive expression changes<sup>2</sup> including metabolic reprogramming consisting of the repression of mitochondrial oxidative phosphorylation and the mitochondrial tricarboxylic acid cycle alongside the activation of amino acid and nucleotide metabolism and some poorly understood branches of carbon metabolism (e.g., genes *ICL2*, *CIT3*, *FDHI*) and transport (e.g., genes *ATO1*, *ATO2*, *ATO3*, *JEN1*). In parallel, the expression of stress-related genes, including those for oxidative stress defense enzymes, falls during colony development. This is rather surprising, since in liquid cultures aging results in stress factor accumulation, and the culture fitness is proportional to stress defense enzyme activities. What is then the importance of stress defense in colonies?

Studies of the differentiation between central and margin region cell populations of the colony revealed that cells with apoptotic-like features (e.g., reactive oxygen species (ROS) production, chromatin fragmentation, half-empty shrunken cells) are restricted to the colony centre, leaving colony margin cells healthy and fully able to propagate the colony towards an uncolonized area.<sup>3</sup> Similar centre-margin differences were found in the expression of various proteins and antioxidant enzyme activities.<sup>4</sup> Hence, there is clear evidence that cells located in distinct colony regions differentiate and that the starting point of this differentiation approximately correlates with the initiation of ammonia production. This raises the question as to whether differentiation is connected to ammonia signaling.

Additional results came from studies of colonies with a deleted *SOK2* gene, which encodes for a transcription regulator involved in various physiological processes. Colonies of this *sok2Δ* strain were unable to produce ammonia or activate variety of metabolic genes typical for ammonia-connected metabolic reprogramming.<sup>5</sup> Even more interesting was the fact that these colonies also have an impaired colony differentiation.<sup>3</sup> Unlike in the wild type, there was only a slight difference between the *sok2Δ* colony central and margin cells in the studied parameters. Is this lack of centre-margin differentiation of *sok2Δ* colonies connected to the reduction in ammonia signaling and inability to undergo metabolic changes?

To answer these questions, we performed a detailed analysis of colonies

**Key words:** yeast colonies, stress defense and metabolic adaptation, differentiation, aging and long-term survival, ammonia signaling

Submitted: 12/22/09

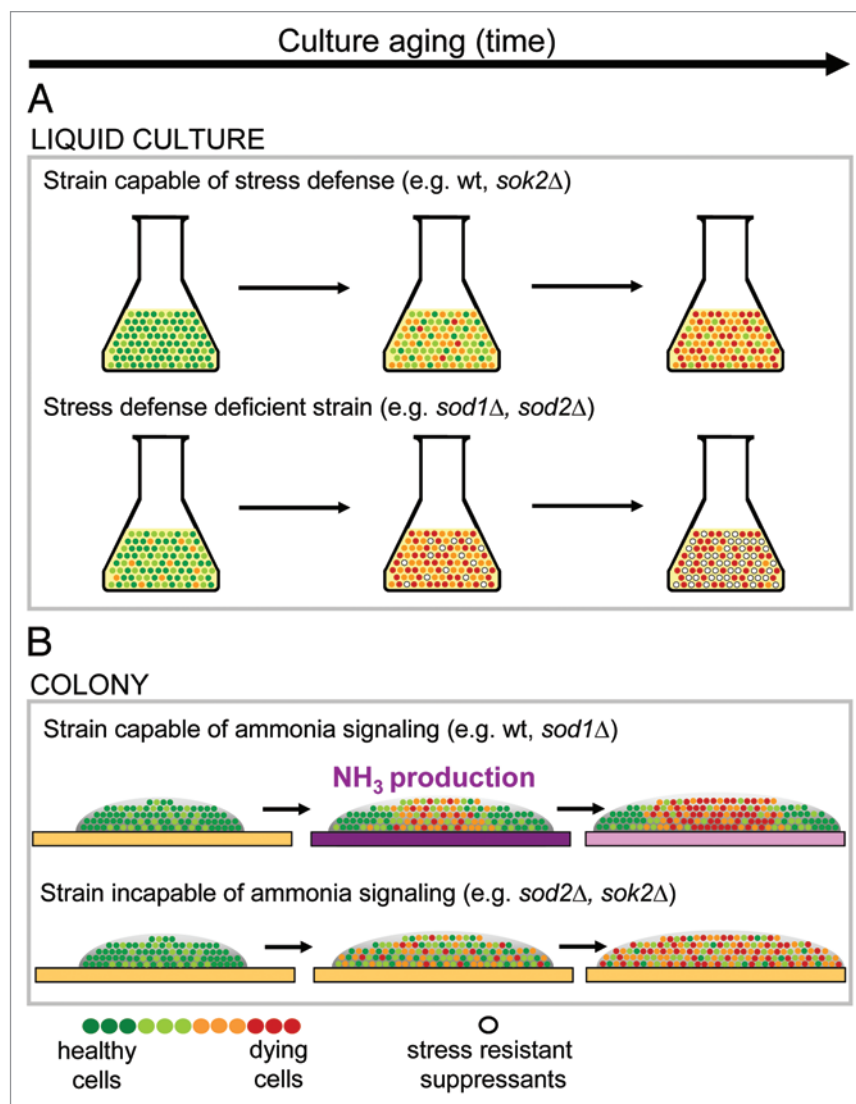
Accepted: 12/22/09

Previously published online:

[www.landesbioscience.com/journals/cib/article/11026](http://www.landesbioscience.com/journals/cib/article/11026)

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Addendum to: Čap M, Vachova L, Palkova Z. Yeast colony survival depends on metabolic adaptation and cell differentiation rather than on stress defense. *J Biol Chem* 2009; 284:32572–81; PMID: 19801643; DOI: 10.1074/jbc.M109.022871.



**Figure 1.** Different factors are important for survival in liquid cultures and in colonies. (A) Survival in liquid culture is dependent on stress defense. Stress-defense-deficient population dies rapidly unless stress-resistant mutants appear and overgrow the dying population. (B) Survival in a colony is dependent on metabolic adaptation of the population. Colony cell population switches metabolism as a result of ammonia signal which enables survival of cells at colony margin. Cells in colony centre die and provide nutrients for the benefit of cells at the colony margin. Development of a colony incapable of ammonia signaling is not orchestrated, cells are not able to activate their alternative metabolism and consequently cell death is spread throughout the whole colony. Color of the agar-medium indicates pH changes during colony development: yellow, pH below 6.5; violet, pH above 6.5.

formed by strains deleted in key enzymes of oxidative stress defense: i.e., *sod1Δ* deficient in cytosolic superoxide dismutase, *sod2Δ* lacking mitochondrial superoxide dismutase and *ctt1Δ* with the deletion of the gene for cytosolic catalase. As for their ability to produce ammonia and undergo ammonia related metabolic changes, *sod1Δ* was fully able to produce ammonia and activate genes typical for the ammonia-connected metabolic switch (e.g.,

*CIT3*, *ATO1*, *ATO3*, *JEN1*), while the *sod2Δ* strain was incapable of both ammonia production and sufficient induction of metabolic genes. Preliminary microarray data for the *sod2Δ* strain also revealed other similarities between *sod2Δ* and *sok2Δ* colonies at the transcriptional level, especially the lack of induction of amino acid, nucleotide and other metabolic changes typical for the ammonia-producing phase of wt colonies (unpublished data). Thus,

we had an experimental model where the deletion of a single enzymatic activity located in different compartments (cytosol vs. mitochondria) led to differing effects on ammonia signaling and the connected alternative metabolism activation. We then examined in detail the differentiation in several stress-related physiological parameters (ROS production, cell death occurrence, thermoresistance, activity of oxidative stress defense enzymes) between central and margin regions in colonies formed by oxidative-stress-impaired mutants. It turned out that center-margin differentiation is dependent on the ability of the particular strain to undergo the ammonia-mediated metabolic switch. Hence, in *sod2Δ* colonies we observed a similar consequence of the absence of ammonia signaling as in *sok2Δ* colonies: an inhibition of the activation of the metabolic changes and consequently an inhibition of *sod2Δ* colony differentiation. This complex colony phenotype was thus found in strains lacking two functionally unrelated proteins, the pleiotropic transcription regulator Sok2p and ROS scavenging enzyme Sod2p, indicating the key role of ammonia-guided metabolic and possibly other changes. This finding is also supported by observations of *ctt1Δ* colonies, which produce a reduced amount of ammonia and, correspondingly, exhibit a level of activation of metabolic changes as well as differentiation that is in-between the wt and *sod2Δ*. Interestingly, the overall level of ROS (superoxide or hydrogen peroxide) was not elevated in aging colonies of any of the stress-defense mutants compared to the wt colonies.

The above results raise the possibility that Sod2p and/or cellular ROS homeostasis are somehow involved in signaling pathways leading to ammonia production and the subsequent metabolic switch. This hypothesis predicts that a higher mitochondrial superoxide level in the early stages of colony development could somehow impair the onset of ammonia signaling. And indeed, in contrast to aging colonies, superoxide production is elevated in the mitochondria of very young *sod2Δ* colonies (1- and 3-day old colonies, unpublished data). One could imagine several mechanisms for the ROS imbalance effect, one of them being that

mitochondrial ROS could block or cross-talk with pathways leading to ammonia signaling. Several mitochondrial redox-sensing pathways were proposed in higher organisms that regulate either mitochondrial physiology or nuclear gene expression<sup>6-8</sup> but none in yeast so far. Alternatively, mitochondrial ROS may act directly by damaging particular enzyme(s) necessary for ammonia generation. A number of amino acid metabolic genes are concentrated in mitochondria, many of which are induced during the ammonia-production phase. Since it has been shown that amino acid metabolism is source of ammonia,<sup>9</sup> oxidative damage to some enzymes could affect ammonia production at the enzymatic level. Finally, *sod2Δ* could have another role in cellular signaling besides its enzymatic activity. Mitochondrial superoxide dismutase was recently suggested to have a regulatory role in DNA repair machinery and chromatin remodeling in yeast.<sup>10</sup>

Stress-defense-deficient mutants, especially *sod1Δ* and *sod2Δ*, are severely damaged in liquid cultivations, as documented by their slow growth, high ROS level and rapid cell dying.<sup>11-13</sup> In colonies, however, the situation is different. *sod2Δ* and *ctt1Δ* colonies (incapable of ammonia signaling) lack differentiation, which results in a large proportion of stressed and dead cells at the colony margin. In contrast, *sod1Δ*, the strain most damaged in liquid cultivations, is as prosperous as the wt under colonial conditions. Since the *sod1Δ* cell population is known to efficiently suppress defects evoked by oxidative stress by accumulating stress-resistant suppressor mutations, it was a surprise that no such

mutants were detected in *sod1Δ* colonies. *sod1Δ* cells taken from colonies, however, acquired some new heritably stable physiological features, such as different growth properties and survival rate in liquid cultures, showing that this strain, although developing in a colonial environment, is prone to changes (mutations or epigenetic switches). The lack of accumulation of stress-resistant suppressor mutants thus indicates that in colonies there is no selection pressure in favor of stress-resistant mutations. This demonstrates that completely different factors are important for survival in homogeneous, aerated, fast growing liquid shaken cultivations and in the differentiated multicellular population of a colony (Fig. 1). A colony represents a highly complex and heterogeneous population developing in an environment where gradients of various compounds, nutrients or gases form many different niches, where each cell occupies its particular space and cell-to-cell interactions are preserved. In this respect, the colonial model resembles to some extent the tissues of real multicellular organisms and could contribute to uncovering and understanding new aging and stress-related processes in slowly dividing or non-dividing differentiated cell populations.

#### Acknowledgements

This work was supported by Grant Agency of the Czech Republic (204/08/0718), by Ministry of Education (LC531, MSM0021620858 and AV0Z50200510) and by the Howard Hughes Medical Institute (International Research Award 55005623 to Z.P.).

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