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*Letter to the Editor*

# Mechanisms Implicated in the Response of System A to Hypertonic Stress and Amino Acid Deprivation Still Can Be Different

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Alfieri et al. (2004) analyzed the response of system A to hypertonic stress by determining changes in SNAT2 mRNA levels in a panel of cell lines, including the same cell type, CHO-K1, that we routinely used in the past for this kind of studies. It is evident that a discrepancy between their results and ours regarding the effect of hypertonicity on SAT2/SNAT2 mRNA levels exists. Our CHO-K1 clone was originally obtained from E. Englesberg (University of California, Santa Barbara, CA), who developed a panel of somatic mutant cell lines with altered system A activity and regulatory properties (Moffett and Englesberg, 1984, 1986; Englesberg and Moffett, 1986; Moffett et al., 1987; Qian et al., 1991; among others). In our experiments, the SAT2/SNAT2 cDNA probe used for Northern blot analysis was a fragment cloned from that particular cell line (this is the hamster SNAT2 orthologue, accession no. of the partial sequence AF363584). Although we do not believe these slight differences might explain this discrepancy, the study we published in the *Journal of General Physiology* (López-Fontanals et al., 2003) does not particularly rely upon this observation to conclude that the osmoregulatory and the amino acid–regulated responses of system A are mediated by different signal transduction pathways.

In that study, we combined inhibitors of the MAP kinase pathway, as well as negative dominant cells for selected kinases in this transduction pathway, and modulators of the cell cycle machinery, to demonstrate that, at least in CHO-K1 cells, both stimuli trigger independent responses. All these experiments were performed by looking at system A functional activity, which is presumably associated with SNAT2 expression in CHO-K1 cells. This is in agreement with our previous work (Ruiz-Montasell et al., 1994) in which we used a particular somatic cell CHO-K1 mutant (CHO-K1 ala$^r$4), generated at Ellis Englesberg's laboratory (Moffett and Englesberg, 1984, 1986), that lacked the ability to respond to amino acid starvation but, interestingly, still retained the hyperosmotic response. Although we agree that the existence of the system A activating protein is still an open question, as Alfieri et al. discuss, the conclusion that both pathways must converge at some point cannot be drawn from the mere observation that the two stimuli induce an increase in SAT2/SNAT2 mRNA levels. We respectfully believe that this is a simple interpretation of these data. Most genes can be transcriptionally activated by different agents/stimuli without bearing any common step in their transduction pathways, except that they converge, obviously, at the end somewhere on the gene promoter. Nevertheless, as the authors point out, even for the response of system A to amino acid starvation, traditionally assumed to be exclusively associated with gene transcription even before system A cloning, it has been now reported that protein recruitment from intracellular stores is also responsible for this response (Ling et al., 2001). Thus, even if SAT2/SNAT2 mRNA accumulates after hypertonicity in all cell lines they have tested, it does not rule out other mechanisms, which would explain for instance why cells lacking the amino acid–regulated response still show an increase in system A activity after hypertonic shock. In fact, the authors cite, as a demonstration of the hypertonic sensitivity of the system A gene, a recent paper by Trama et al. (2002), showing that SAT2/SNAT2 gene shows dependence on NFAT5, also known as TonEBP, a transcription factor implicated in osmotic responses. However Alfieri et al. do not comment on the fact that these authors themselves conclude that "system A amino acid transporter gene ATA2 exhibited NFAT5 dependence under hypertonic conditions but not in response to amino acid deprivation." This would argue against common pathways mediating both stimuli.

We do not believe that the Northern blot shown by Alfieri et al. provides unequivocal basis for a rebuttal of the key message of our contribution, which is that both stimuli trigger system A up-regulation, measured at the functional level (system A transport activity) by independent signal transduction pathways.

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