

# Amino acid-dependent signal transduction for control of transport and metabolism

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**Abstract.** Amino acids modulate fundamental processes in mammalian cells, including transcription via the Amino Acid Response pathway. Target genes, such as asparagine synthetase and SNAT2 amino acid transporter, contain genomic elements that mediate increased transcription following amino acid limitation. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** SNAT2, System A, asparagine synthetase, starvation

## Amino acids as signal molecules

Amino acids are known to modulate a number of fundamental processes in mammalian cells, including transcription, via a signal transduction process referred to as the Amino Acid Response (AAR) pathway (1). In this context, amino acids serve as signal transduction messengers to transmit the nutritional status of the entire organism to individual cells. Detection of a limiting amount of any single amino acid has been linked to the GCN2 kinase that is activated by uncharged tRNAs, phosphorylates the eukaryotic initiation factor eIF-2 $\alpha$  and, as a consequence, suppresses global translation initiation (2). However, certain mRNAs, that contain short upstream open reading frames, such as that for the transcription factor ATF4, exhibit increased translation under these conditions. ATF4 mediates the increased transcription of AAR pathway target genes, including asparagine synthetase (ASNS) (3) and the sodium-dependent neutral amino acid transporter 2 (SNAT2) (4).

## Asparagine synthetase

*In vivo* footprinting identified two amino acid-responsive protein binding sites, originally labeled si-

tes V and VI (5), and later renamed Nutrient Sensing Response Elements (NSRE-1, -2). Mutagenesis confirmed the sequence and location of NSRE-1 (5'-TGATGAAAC-3', nt -68 to -60) and NSRE-2 (5'-GTTACA-3', nt -48 to -43) and demonstrated that both were required for increased transcription from the ASNS gene following activation of either the AAR pathway or Unfolded Protein Response pathway (5). *In vitro* and *in vivo* DNA binding analysis as well as over-expression studies revealed that the transcription factors, ATF4, ATF3, and C/EBP $\beta$  bind to the NSRE-1 sequence and modulate ASNS expression following amino acid deprivation (3).

## SNAT2 amino acid transporter

System A transport activity mediates the sodium-dependent transport of selected neutral amino acids in mammalian cells, and is increased by amino acid limitation (6). Three genes (SNAT1,2,4) encode System A activity, and of these, the SNAT2 mRNA content is increased the most following amino acid deprivation (7). Palii et al. (4, 8) have established that the SNAT2 gene contains an amino acid response element (AARE) within the first intron that functions as an enhancer (Fig. 1). The sequence of the SNAT2 AARE dif-

		<b>CAAT box</b>				
Human	+676	<b>AGACGAGTTGGG</b>	--	<b>AACATTTGACAATCGA</b>	----	<b>CGATCG</b>
Mouse	+481	<b>AGGCGAACCGCGCGAGGGCTTGACAATCAATCGCCCTCG</b>				
Rat	+506	<b>AGGCGAGCCGAG</b>	--	<b>AGGGCTTGACAATCGC</b>	----	<b>CCCTCG</b>
		<b>AARE</b>		<b>PuR box</b>		
Human	+710	<b>ATATTGCATCAGTT</b>	<u>TTCTTTCCGGACATAGGAGGGGGCTGG</u>			
Mouse	+521	<b>GTATTGCATCAGTT</b>	<u>C-CTCCCGGGGTAGAGGAGGGGC</u>	-GG		
Rat	+540	<b>GTATTGCATCAGTT</b>	<u>C-CTCCCGGGGCAGAGGAGGGGC</u>	-CG		

Figure 1. The SNAT2 intronic sequence surrounding the AARE (underlined)

fers by 2 nt from the ASNS NSRE-1, but analysis suggests that the two genes are regulated by similar mechanisms.

### Amino acid regulated transcription

The chromatin immunoprecipitation (ChIP) data from the ASNS and SNAT2 genes led to a working model that proposes two distinct phases (Fig. 2). Phase I encompasses the first 4 h after amino acid withdrawal and Phase II covers the time from 4-24 h. Within 30 min of amino acid depletion, translational control of ATF4 mRNA results in increased *de novo*

synthesis and subsequent binding of ATF4 to the AARE. Acetylation of histones H3 and H4 is increased and subsequently, general transcription factors, as well as RNA polymerase II, are recruited to the promoter. During Phase I, C/EBP $\beta$  is constitutively bound to the AARE, whereas in Phase II, C/EBP $\beta$  *de novo* synthesis and subsequent binding increases at a time when transcription has peaked and is beginning to decline. Likewise, the synthesis and action of ATF3 also increases during this period (3). It is proposed that during Phase II ATF3 and C/EBP $\beta$  act in concert to suppress, but not to completely reverse, the increased transcription.

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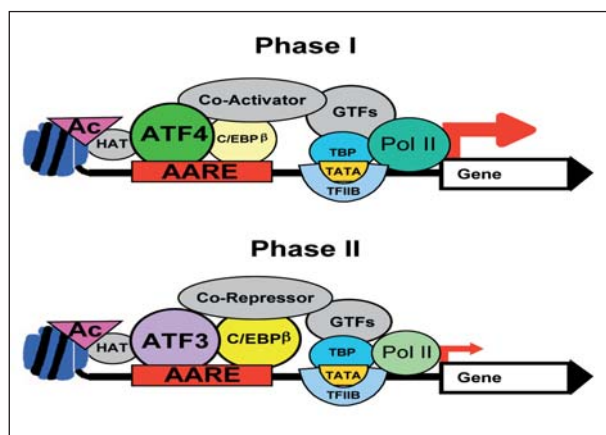


Figure 1. A working model for control of the asparagine synthetase (ASNS) and SNAT2 genes by amino acid limitation

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