Development and optimization of a new gene regulation system controlled by nutrition applicable for gene therapy

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INTRODUCTION

Our lab is specialized in nutrition research and mainly in characterizing the molecular mechanisms regulating the cellular adaptation to a nutritional stress. More specifically, eating a meal devoid of one Essential Amino Acid (EAA) causes a dramatic decrease of the limiting EAA in the blood, which in turn activates the GCN2/eIF2α/ATF4 signaling pathway (Figure 1). This leads to a translational up-regulation of the transcription factor ATF4 that binds to a particular DNA sequence: the AARE (Amino Acid Response Element) to initiate a transcription program allowing the stress adaptation.

In gene therapy, the control of transgene expression remains a major concern. Therefore, we have generated an innovative gene regulation system controlled by nutrition by transfering the AARE in an artificial patented promoter. This system is associated to an EAA-deficient inducing diet developed in our lab.

1 Generation of a gene regulation system controlled by amino acid availability: the AARE-Gene system.

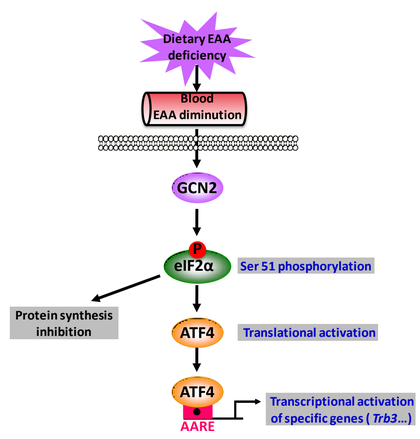


Figure 1 : Presentation of the GCN2/eIF2α/ATF4 pathway controlling the AARE-Gene system activity. GCN2 senses Essential Amino Acids (EAA) deficiency in the blood caused by the consumption of a diet lacking one EAA. Activation of this pathway leads to an increase in the translation of ATF4 transcription factor that functions as a master regulator for transcription of specific target genes playing a crucial role in nutrional stress adaptation.

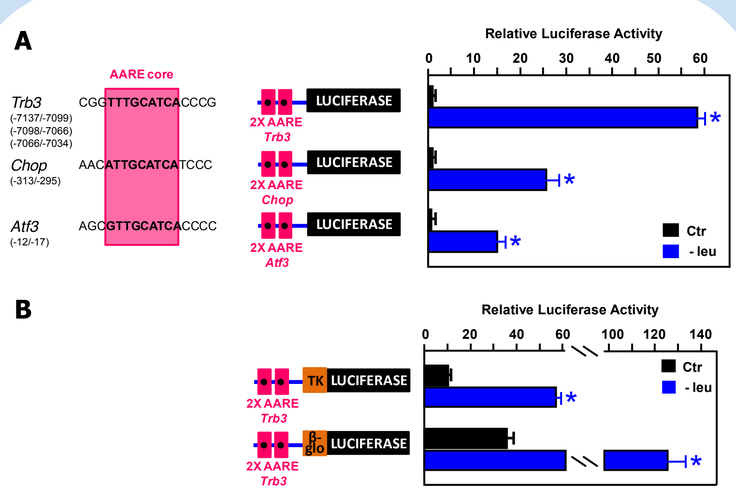


Figure 2 : The Gene regulation system containing 2 copies of *TRB3* AARE combined to TK minimal promoter provides the highest amino acid inducibility associated to low basal expression. (A) Functional comparison of 3 different AARE sequences. (B) Functional comparison of 2 different minimal promoters.

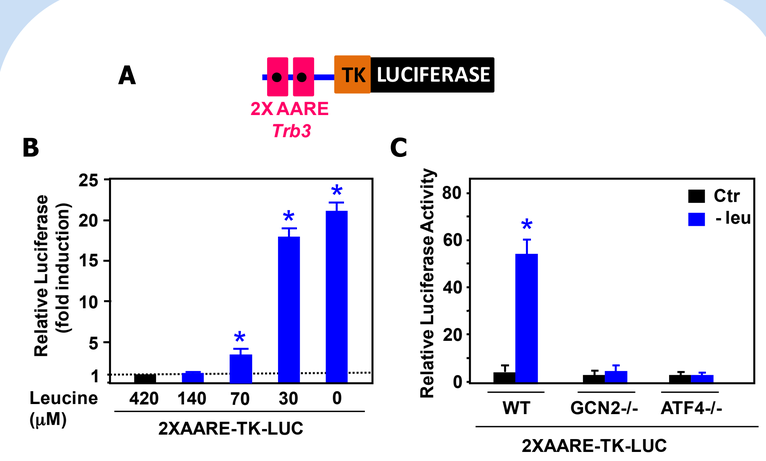


Figure 3 : The AARE-Gene system can be activated by physiological EAA concentrations and requires GCN2 and ATF4 expression. (A) The plasmid construct contains two copies of the *TRB3* AARE fused to the minimal promoter thymidine kinase (TK) and the Luciferase (LUC) coding sequence. This transgene is inducible (B) by different concentration leucine (C) via GCN2 (amino acid starvation) and the transcription factor ATF4.

2 Identification of target tissues and definition of a nutritional protocol dedicated to long term-usage of the AARE-Gene system.

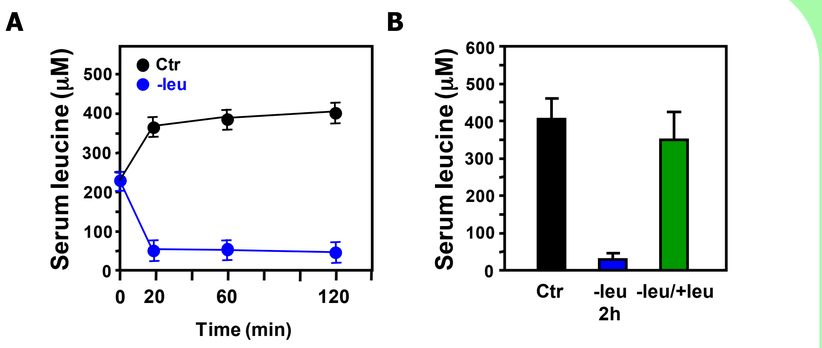


Figure 4 : Rapid change in the plasmatic amino acid concentration following the consumption of a complete and an EAA-deficient diet . (A) Mice were fed either with a control (Ctr) or a leucine-deficient diet (-leu) for 20 to 120 min (B) or refed for 2h on the control diet after a 2h-consumption of the leucine-deficient diet (-leu/+leu).

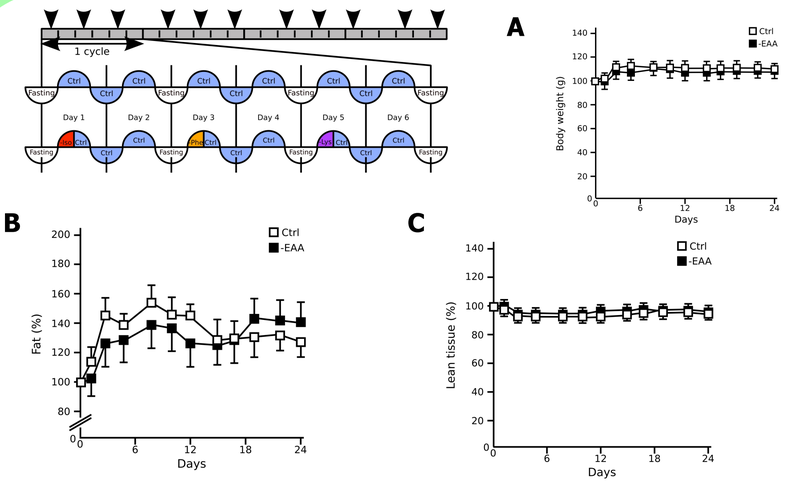
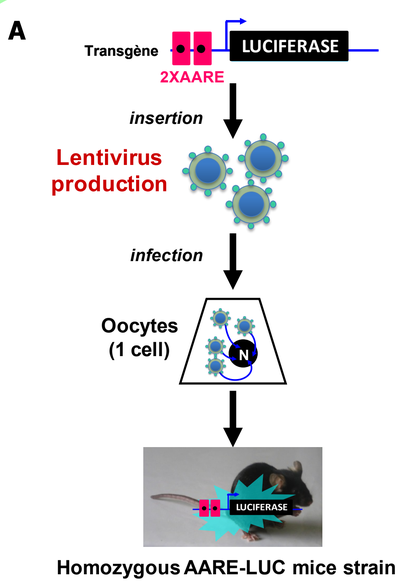
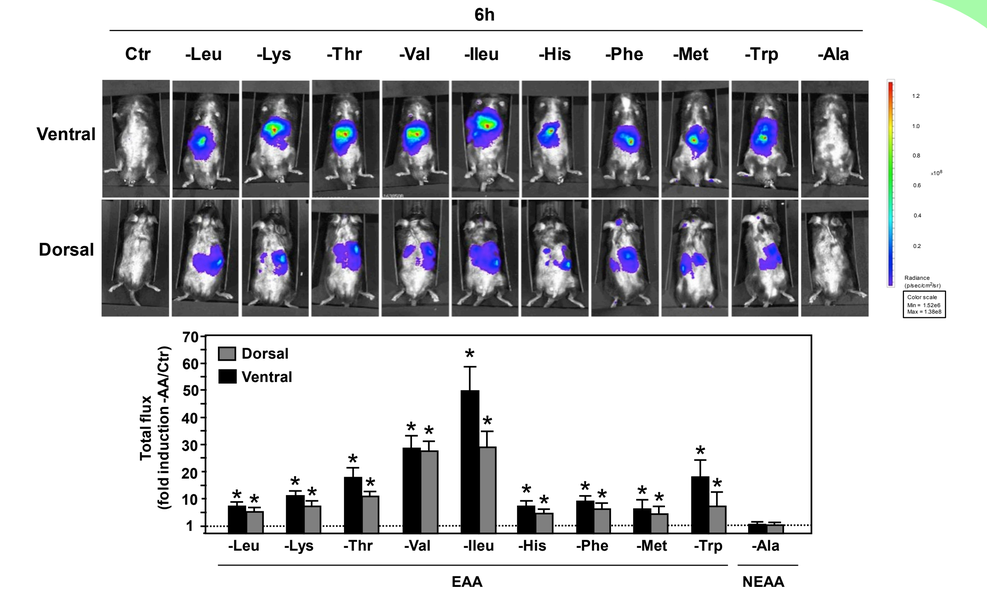
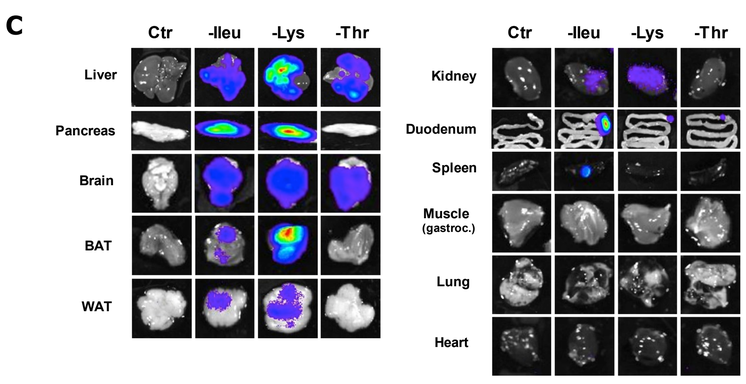


Figure 6 : Rotation of several diets lacking one EAA (Ileu, Phe, Lys) doesn't affect neither (A) body weight nor (B) fat and (C) lean mass. For the nutritional protocol (top left panel), a quarter circle represents a period of 6 h. Black arrows indicate time points for body parameters measurements.







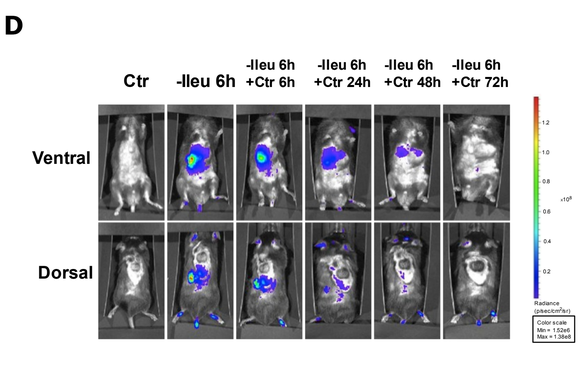


Figure 5 : The reversible AARE-Gene system is induced the most in response to diets lacking Isoleucine, Valine or Threonine and can be used for transgene delivery in the liver, pancreas or the brain (A) Process for generation of the AARE-LUC mice strain. (B) Inducibility test of the AARE-Gene system in response to a diet devoid of one Essential Amino Acid (EAA) or one Non-Essential Amino Acid (NEAA) in the AARE-LUC transgenic mice. (C) Identification of tissues sensitive to EAA deficiency. (D) Demonstration of the reversibility of transgene expression following the consumption of a complete diet

3 Demonstration of functional proofs on concept by controlling transgene expression through the AARE-Gene system .

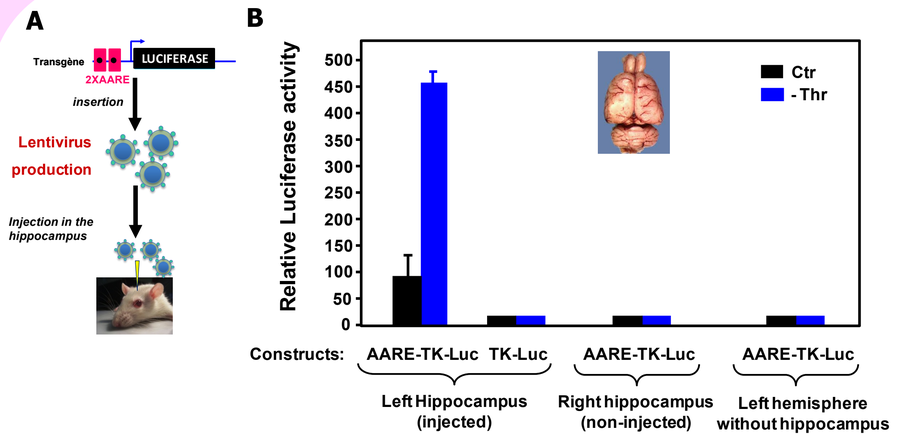


Figure 6 : Validation of the AARE-Gene system functionality after tissue injection *in vivo*. (A) Lentiviral vectors were injected into the hippocampus of the left hemisphere of rats. (B) Two weeks after injection the animals were fasted overnight and then fed either a control (Ctr) or threonine-deficient diet (-Thr). Six hours after the beginning of the meal, the animals were sacrificed, brains dissected and luciferase activity was measured.

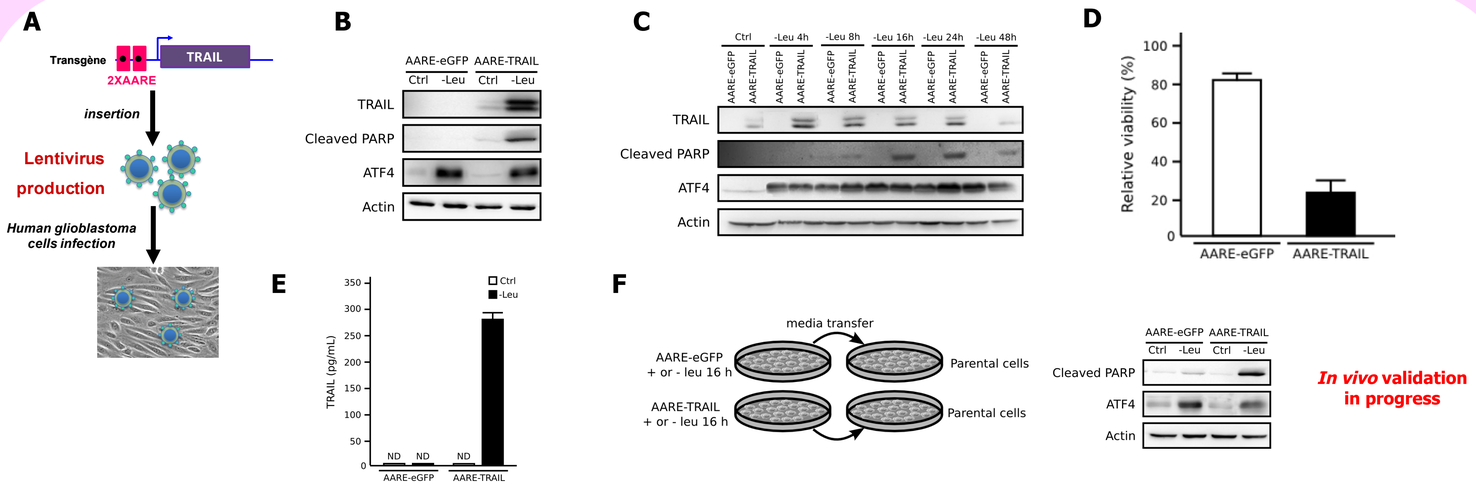


Figure 7 : Induction of the AARE-controlled expression of TRAIL in response to an EAA starvation leads to apoptosis in glioblastoma cell line (A) Gli36-Luc cells were infected with lentiviral vectors containing the AARE-TRAIL sequence. (B) EAA induction of AARE-TRAIL system expression leads to apotosis. (C) Kinetic analysis of EAA induction of AARE-TRAIL system expression. (D) Viability assay of Gli36-Luc cells infected with lentiviral vectors containing AARE-TRAIL or AARE-GFP sequences in leucine-starved Gli36-Luc cells (16 h). (E) TRAIL release measurement (Elisa kit) in culture media, 16 h post leucine starvation. (F) Apotosis induction in parental cells caused by paracrine effect of TRAIL.

CONCLUSION: Our lab has develop a new gene regulation system controlled by nutrition displaying multiple advantages : (1) use of endogenous molecular mechanisms meaning no requirement of pharmacological inducers and expression of regulatory proteins, (2) no toxicity of an EAA limitation in a short time (3) possibility to target the brain (no blood-brain barrier restriction) and other tissues (pancreas, liver...), (4) system reversibility allowing transgene deliveries by pulses, avoiding development of therapy resistance following a prolonged exposure to the therapeutic protein.

We are looking for collaborations and partners interested in developing and testing this AARE-Gene system for gene therapy applications in different pathological models.

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