Supplementary data

GENERATION OF DIGITAL RESPONSES IN STRESS SENSORS

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Supplementary experimental procedures

In silico analysis

BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/BLAST/) was used to obtain the AMPK orthologs of *Xenopus laevis* from the AMPK human sequences. AMPK sequences from different species were obtained from Entrez Gene Database and a phylogenetic tree was created using CLUSTAL program.

Oocyte isolation and treatment

Oocytes were obtained from sexually mature *Xenopus laevis* females (purchased from Centre d'Elevage de Xenopes, Montpellier, France), anesthetized in 0.02% benzocaine and portions of ovary were removed through a small incision on the abdomen. The incision was sutured and the animal was returned to a separate tank until it had fully recovered from the anaesthesia. It was then returned to a large tank in which all the frogs were kept for at least 4 weeks until the next surgery. The tissue was examined to ensure the ovaries were healthy and dissected in small pieces. Oocytes were defolliculated for 1-1.5 hr at room temperature with collagenase/dispase (0.8 mg/ml (Sigma), 0.48 mg/ml (Roche)) in MBS (5 mM HEPES, 88 mM NaCl, 1 mM KCl, 1 mM $MgSO_4 \cdot 7H2O$, 2.5 mM $NaHCO_3$, 0.7 mM $CaCl_2$, pH 7.8) with agitation. The oocytes were then washed thoroughly with MBS and transferred to petri dishes. Stage VI oocytes were sorted manually and incubated overnight in MBS at 18ºC. The next day, healthy survivors were selected and transferred to a fresh petri dish containing fresh MBS. Pools of oocytes were treated with drugs at the concentrations and times indicated.

RT-PCR

cDNAs were amplified by PCR with specific primers (Sigma).

Sense α1: 5'GCCGTCGACATGCGCAGGCTCAGTTCGCTC3'
Antisense α1: 5'GCCGCGGCCGCGTACAAGCAGCAGCTCAGCACT3'
Sense β1: 5'GCCGGATCCATGGGGAACACGAGCAGTGAACGACC3'
Antisense β1: 5'GCCGAATTCTCATATTGGTTTGTACAGCAAT3'
Sense β2: 5'GCCAAGCTTATGGGTAACACTGCAAGTGATCGCAT3'
Antisense β2: 5'GCCGAATTCCTAAATTGGCTTGTACAATAAA3'
Sense γ1: 5' GCCGGATCCATGGAACCCGGTTCCGTTAT3'
Antisense γ1: 5'GCCGAATTCTTAAATTAAGCTCTTTTCGCCCCC3'
Sense γ2: 5' GCCGGATCCATGGGAAGTACAGTACAGTCATGGA 3'
Antisense γ2: 5'GCCGAATTCTTTGGAGCTGGTCGGGACAA3'
Sense γ3: 5'GCCAAGCTTATGGAGCAAGAGGAGTCTCA3'
Antisense γ3:5'GCCGAATTCTCACAAGGAATTGCGGTCAA3'

Oocyte nuclei isolation

Healthy stage VI oocytes were selected and rinsed in supplemented isolation buffer, which is composed of 20 mM Tris HCl (pH 7.5), 75 mM KCl, 7 mM MgCl2, 0.1 mM EDTA, 0.1 mM EGTA, 2 mM dithiothreitol, with protease and phosphatase inhibitors as previously described. Each nucleus (germinal vesicle) was manually removed and separated from the cytoplasm by using watchmaker's forceps in isolation buffer at room temperature and transferred to a tube kept on ice. After 10 nuclei had been collected they were disrupted by pipetting repeatedly through a 200 µl micropipette tip, suspended in SDS sample buffer, boiled for 5 min, and subjected to electrophoresis on 10% polyacrilamide gel. The cytoplasm from 1 oocyte was also collected and cleared by centrifugation at 14.500 rpm for 5 min and the supernatant processed for immunoblotting.

Supplementary results

AMPK orthologs in Xenopus laevis genome

Since there are no previous publications about *Xenopus* AMPK, we performed in silico analysis of *Xenopus* AMPK subunits and compare them with the mammalian orthologs. The public access to human and *Xenopus* sequences allowed us to search with BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/BLAST/) to obtain the orthologs of *Xenopus laevis* from the human AMPK subunits sequences (Fig. S1A). For *Xenopus* α1 we found two different sequences (BC084741 and AF340021) with a high homology (96% identity in aminoacids) and we considered them as the same subunit. Moreover, a phylogenetic tree was created using CLUSTAL program, showing that *Xenopus* α subunit is closer to α1 than to α2 subunits from other species (Fig. S1B). We have not found the *Xenopus* α2 homolog sequence in Entrez Gene Database, probably due to the incomplete sequence of *Xenopus* genome. In Fig. S1C we schematize the different subunits of *Xenopus* AMPK, the domains and the key aminoacids described to be important for AMPK regulation in mammals. *Xenopus* α1 subunit contains the kinase domain and a Thr184 (equivalent to human Thr172, with the aminoacids surrounding this phosphorylation site well preserved), the autoinhibitory domain (AID) and the aminoacids Val307, Leu337 (important for stabilization of the autoinhibitory conformation (1)) and Ser495, an autophosphorylation site containing also a regulatory site for PKA and PKB/Akt (2). *Xenopus* β1 and β2 subunits contain the glycogen binding domain (GBD) and the complex interaction domain (CIR). Finally, *Xenopus* γ subunits contain four tandem repeats of the CBS motif (responsible of AMP and ATP binding), and the inhibitory pseudosubstrate sequence well preserved (3).

In conclusion, the high similarity of *Xenopus* AMPK subunits with the mammalian orthologs makes of *Xenopus* an adequate model for studies of AMPK signalling.

Supplementary references

1. Pang, T., Xiong, B. Li, J. Y., Qiu, B. Y., Jin, G. Z., Shen, J. K.,and Li, J. (2007) *J Biol Chem* **282,** 495-506
2. Hurley, R. L., Barre, L. K., Wood, S. D., Anderson, K. A., Kemp, B. E., Means, A. R.,and Witters, L. A. (2006) *J Biol Chem* **281,** 36662-36672
3. Scott, J. W., Ross, F. A., Liu, J. K., Hardie, D. G. (2007) *EMBO J* **26,** 806-815

Supplementary figure legends

Fig. S1. *In silico* analysis and subcellular distribution of *Xenopus* AMPK. (A) Similarity grade between human and *Xenopus* AMPK is shown. Using BLAST (Basic local Alignment Search Tool) program the *Xenopus* orthologs sequences were obtained and compared to the human AMPK subunits sequences. The numeric value (P) indicates the coincidence probability at random: minor the value bigger the possibility that the sequences are related significantly. (B) Eukaryotic AMPK α subunits comparison represented in a phylogenetic tree. Aminoacid sequences, obtained from public NCBI data bank, were aligned and a phylogenetic tree was generated with ClustalW program. (C) Conserved domain structures of *Xenopus* AMPK. The α1 subunit contains the kinase domain and a Thr184 residue in its activation loop that must be phosphorylated for activity, an autoinhibitory domain (AID), and the aminoacids Val307, Leu337 and Ser495, which are important for regulation of AMPK activity. Both β subunits contain central glycogen-binding domains (GBD) and complex interaction domains (CIR) that are required for binding the α and γ subunits. All three γ isoforms have a variable N-terminal length, and contain four cystathione b synthase motifs (CBS). Potentially, they are able to act in pairs, forming Bateman domains that can bind AMP or ATP. (D) AMPKα distribution in the cytosol and nucleus. AMPKα was quantified by densitometry from Western blots, as described in Fig. 1D, and expressed as percentage of AMPK in the cytosol or the nucleus referred to total AMPK in the extract. Results represent mean ± SEM of three independent experiments.

Fig. S2. ACC phosphorylation with antimycin and hyperosmolar sorbitol. **(**A) Time course response of ACC and AMPK to 1.5 μg/ml antimycin. (B) Time course response of ACC and AMPK to 400 mM

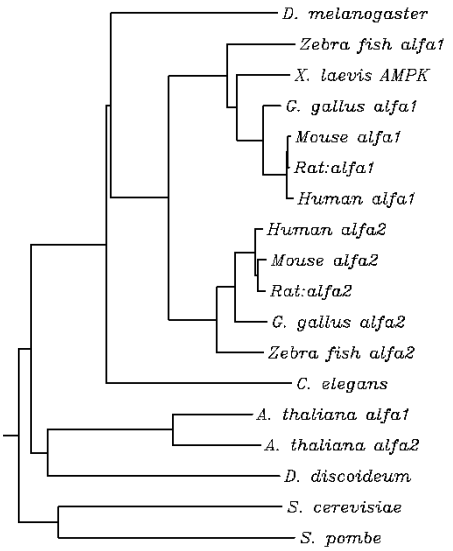
sorbitol. (C) Dose response of ACC and AMPK to antimycin. (D) Dose response of ACC and AMPK to sorbitol. Oocytes (stage VI) were treated with different concentrations of antimycin or sorbitol, collected and lysed at 4 hr to analyze pACC, ACC, pAMPK, and AMPK by Western blot. (E) ACC phosphorylation in individual oocytes treated with 0.1 μg/ml antimycin. (F) ACC phosphorylation in individual oocytes treated with 200 mM sorbitol. Oocytes were incubated for 4 hr and pACC and ACC were measured by Western blot. Results are represented as pACC/ACC ratio, taken as maximum activity (100% value) the oocytes treated with 1.5 μg/ml antimycin or 400 mM sorbitol (M). Each box represents one individual oocyte.

Figure S1

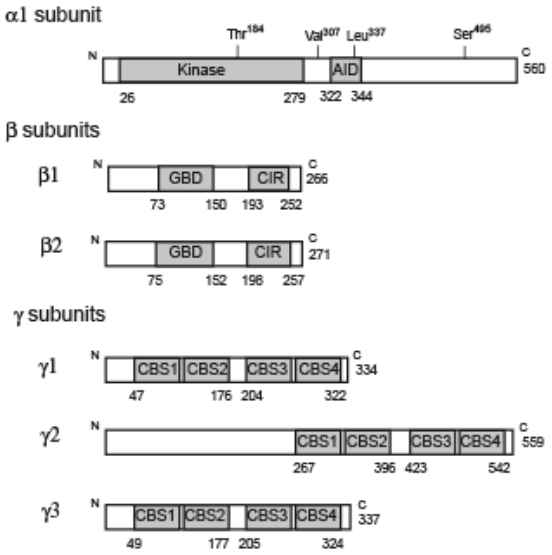
A

Human sequences	*Xenopus* sequences	P value (DNA)	P value (protein)
α1 NP_996790	BC084741 α1	$4x10^{-93}$	0
α1 NP_996790	AF340021 α1	$1x10^{-90}$	0
β1 NM_006253	BC072961 β1	$3x10^{-24}$	$2x10^{-119}$
β2 NM_005399	BC053787 β2	$8x10^{-53}$	$1x10^{-129}$
γ1 NM_002733	BC073621 γ1	$3x10^{-17}$	$1x10^{-154}$
γ2 NM_016203	BC060444 γ2	$1x10^{-138}$	0
γ3 NM_017431	BC043738 γ3	$3x10^{-5}$	$4x10^{-135}$

B



C



D

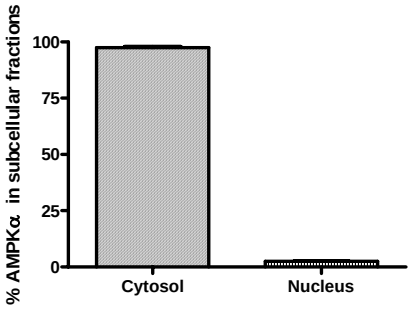
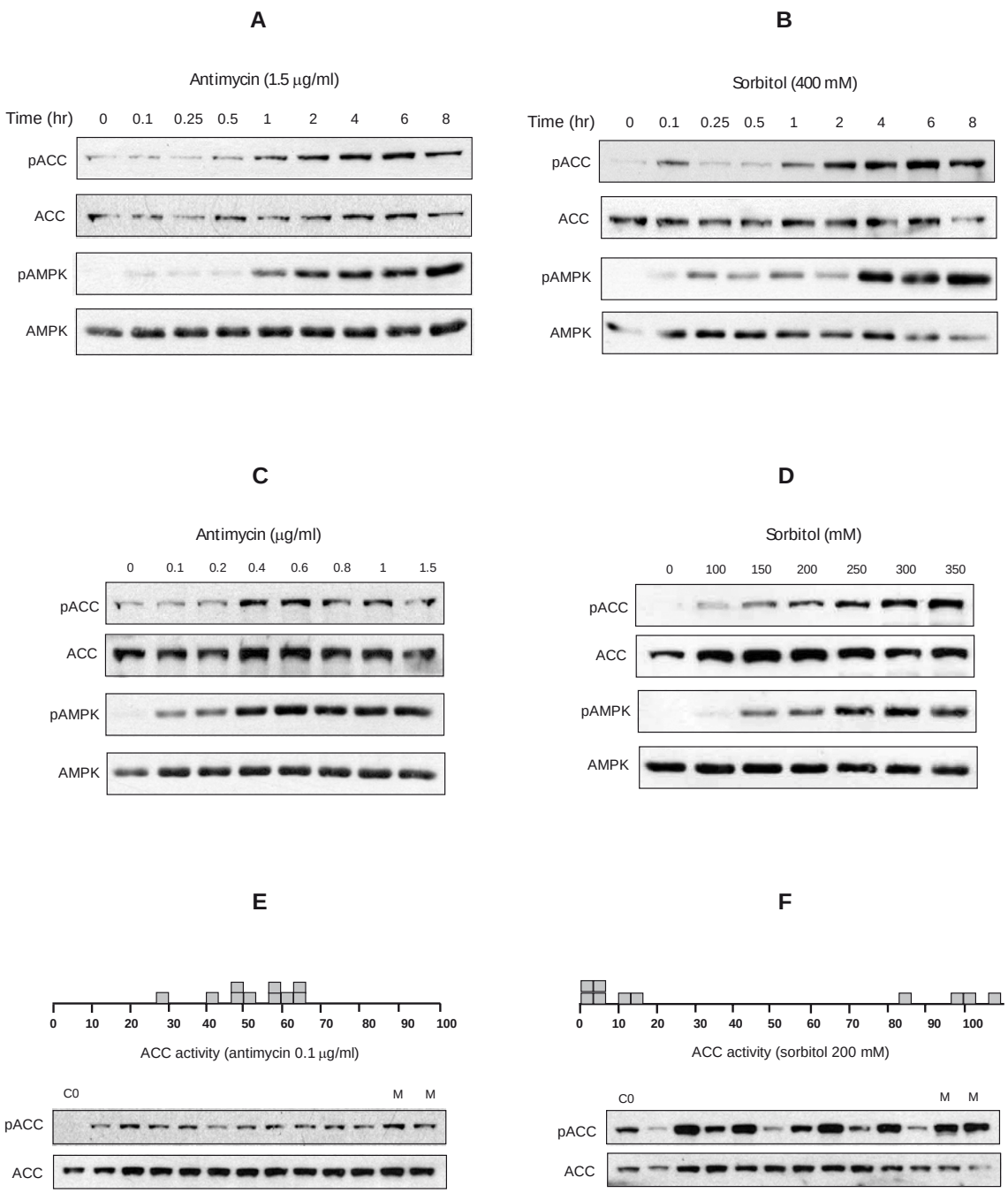


Figure S2


A
Antimycin (1.5 μg/ml)
Time (hr) 0 0.1 0.25 0.5 1 2 4 6 8
pACC
ACC
pAMPK
AMPK
B
Sorbitol (400 mM)
Time (hr) 0 0.1 0.25 0.5 1 2 4 6 8
pACC
ACC
pAMPK
AMPK
C
Antimycin (μg/ml)
0 0.1 0.2 0.4 0.6 0.8 1 1.5
pACC
ACC
pAMPK
AMPK
D
Sorbitol (mM)
0 100 150 200 250 300 350
pACC
ACC
pAMPK
AMPK
E
0 10 20 30 40 50 60 70 80 90 100
ACC activity (antimycin 0.1 μg/ml)
C0 M M
pACC
ACC
F
0 10 20 30 40 50 60 70 80 90 100
ACC activity (sorbitol 200 mM)
C0 M M
pACC
ACC