The cAMP pathway regulates mRNA decay through phosphorylation of the RNA-Binding Protein TIS11b/BRF1

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**Supplemental Figure S1.** Activation of the cAMP signalling pathway induces the binding of the cAMP-response element (CRE) binding protein CREB to TIS11b promoter. (A-D) Expression and quantification of CREB and phosphoCREB proteins in COS7 cells (A, B) and bovine adrenocortical cells (C, D). Cells were stimulated for the indicated periods of time with forskolin (FSK, 25µM) or ACTH (10nM). Proteins were extracted and 20 µg of each sample were analysed by Western Blot with anti-CREB, anti-phosphoCREB or anti-tubulin antibodies. Graphs represent the PhosphoCREB/Total CREB ratios (means ± S.D) determined from 2 independent experiments. Tubulin was used as loading control. (E-F) Chromatin Immunoprecipitation (CHIP) analysis of PhosphoCREB/TIS11b promoter complex. COS7 or BAC cells were stimulated with forskolin or ACTH, respectively, for the indicated periods of time and CHIP assay was performed as described in details in Supplemental Material and Methods.
Supplemental Figure S2. Phosphorylation Site Predictions in TTP family member sequences according to DISPHOS 1.3 software http://www.dabi.temple.edu/disphos/. Red triangles and blue circles illustrate phosphorylatable serines (S) and threonines (T), respectively. The horizontal green bars represent the zinc finger (Zn) regions. Note that both the N-terminal and C-terminal domains of TTP are enriched in putative phosphorylation sites while the C-terminal domain of TIS11b harbours the majority of putative phosphorylation sites.
**Supplemental Figure S3:** Determination of the phosphopeptide antibodies cross-reactivity in vitro. Phosphorylated peptides were resolved on 16.5% Tris-Tricine gels and analysed by western blot to determine either the antibodies anti-phosphopeptide pS54 could detect the phosphopeptide pS334 (left panel) or the antibodies anti-phosphopeptide pS334 could detect the phosphopeptide pS54 (right panel). Quantification of western blots using Image Lab software (Bio-Rad) indicated that the cross-reactivity of the anti-p54 antibodies with the phosphopeptide pS334 was minimal (less than 2 %) while the anti-pS334 antibodies were highly specific and did not recognize the phosphopeptide pS54 (right panel).

| Phosphopeptide (nmoles): | N-Term pS54 | C-term pS334 | C-Term pS334 | N-term pS54 |
|--------------------------|-------------|--------------|--------------|-------------|
|                          | 5           | 1            | 5            | 25          |
| **WB: Anti-pS54**        | ![Western Blot](image1.png) | ![Western Blot](image2.png) | | |
**Supplemental Material and Methods**

**Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-qPCR)**

Total RNA was extracted using the Nucleospin RNA kit according to the manufacturer’s recommendations (Macherey-Nagel, Hoerdt, France). 1 µg of total RNA was reverse-transcribed with the iScript System (Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer’s guidelines. cDNAs were diluted in a 50 µL final volume. Quantitative PCR was performed using the GoTaq qPCR Master Mix (Promega, Charbonnières Les Bains, France) and 2 µL aliquots of the RT reaction. Amplification of TIS11 family member and RPL27 transcripts was performed with the primers reported in Supplemental Table S2, in a final volume of 20 µL using a CFX96 Real-Time System thermocycler (Bio-Rad, Marnes-la-Coquette, France) with the following program: Initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 10 sec and annealing at 60 °C for 30 sec. Amplification of VEGF transcript was carried out using the forward 5’-AAG GAG GAG GGC AGA ATC AT-3’ and reverse 5’-ATC TGC ATG GTG ATG TTG GA-3’ primers with the following program: Initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 10 sec and annealing at 60 °C for 30 sec. The primers for HPRT amplification were as follows: 5’-ATG GAC AGG ACT GAA CGT CTT GCT -3’ and 5’-TTG AGC ACA CAG AGG GCT ACA ATG-3’.

**TIS11b promoter cloning and plasmid constructions**

All constructs were cloned into pGL3-basic vector (Promega) containing the luciferase reporter gene. Wild type (WT) zfp36l1 promoter was amplified by PCR from human genomic DNA using the primers 1 and 2 (Supplemental Table S3) and Advantage GC Genomic LA polymerase (Clontech). The obtained fragment of 1088 nucleotides was purified by Qiaquick gel extraction kit (Qiagen). Blunt-ends were generated with T4 DNA polymerase (Fermentas) then the fragment was inserted into pGL3-basic vector previously digested by SmaI. The consensus CRE sequence TGACGTCA was mutated into TCTCGAGA (mutCRE) using overlapping PCR. A first fragment was amplified from human genomic DNA with primers 1 and 3 (S3 Table). A second fragment was generated with primers 2 and 4. After purification, a new PCR using both fragments as matrices was performed with primers 1 and 2. The insertion into pGL3-basic vector was performed as described above. All constructs were verified by sequencing (Cogenics, Takeley, UK).

**TIS11b promoter-driven luciferase assay**

COS7 cells (10⁴ cells/well) were plated in 12 well-plates the day before transfection. Subconfluent cells were transfected with 500 ng of WT or mutCRE constructs and 25 ng of pRL-TK (Promega) using Lipofectamine Reagent (Invitrogen). 24h post-transfection, cells were stimulated with 25 µM of forskolin (FSK, Sigma) or 10 nM of ACTH (Neosystems) for different periods of time in medium containing 0.1 mM Isobutylmethylxanthine (Sigma). The PKA inhibitor Rp-cAMP (Sigma) was used at 10 nM. Luciferase activity of pWT or pmutCRE was measured in cells lysates using the Dual-luciferase reporter assay system (Promega) according to the manufacturer’s instructions. Results are expressed as relative light units of Firefly luciferase activity over relative light units of renilla luciferase activity, and are represented as a percentage of luciferase activity in control cells. Each transfection condition was performed in triplicate.
Chromatin Immunoprecipitation assay (ChiP)

Cells were plated at 10⁶ cells per 10cm-dish and serum-starved one day before stimulation. They were subsequently stimulated with 10 nM of ACTH (BAC) or 25 µM of FSK (COS7) for the indicated periods of time. Protein/DNA crosslinking was performed by adding formaldehyde (1% final concentration) for 10 minutes at 37°C. Formaldehyde was quenched by 0.125M glycine for 5 min at room temperature. Cells were washed twice with PBS containing protease inhibitors (PI: 1 mM phenylmethylsulfonyl fluoride, 1 µg/mL aprotinin, 1 µg/mL peptatin and 1 µg/mL leupeptin) and re-suspended in 200 µL SDS lysis buffer (50 mM Tris, 10 mM EDTA, 1% SDS, PI). Samples were sonicated using 12 sets of 10 seconds (Sonimasse S20) at 30% of maximum power to generate fragments between 100 to 1000 base pairs. The supernatant obtained after centrifugation at 10 000 rpm for 10 min at 4°C was diluted 10-fold in ChIP dilution buffer (1.2 mM EDTA, 16.7 mM Tris-HCl pH8.1, 167 mM NaCl, 0.01% SDS, 1.1% Triton X-100) with PI. At this point, an aliquot corresponding to 10% of the sample was taken off for further use as input/starting material. Samples were incubated with 75 µL of protein A Agarose/salmon sperm DNA (50% slurry) for 30 min at 4°C to avoid non-specific interactions. Supernatant was then immunoprecipitated overnight at 4°C using a monoclonal anti-CREB antibody (1/250). A negative control was performed with non-immune rabbit IgG. The immune complex was collected by addition of 200 µL protein A Agarose/salmon sperm DNA for 1 h at 4°C. After centrifugation at 1500 rpm for 5 min, the complex was washed once with low-salt buffer (2 mM EDTA, 20 mM Tris-HCl pH8.1, 150 mM NaCl, 0.1% SDS, 1% Triton X-100), then with high salt buffer (2 mM EDTA, 20 mM Tris-HCl pH8.1, 500 mM NaCl, 0.1% SDS, 1% Triton X-100) and finally with 20 mM Tris-EDTA pH 8. Protein/DNA complexes were eluted with 250 µL of 0.1M NaHCO₃ containing 1% SDS at room temperature for 15 min. Crosslinks were reversed by addition of 20µL of 5M NaCl and by heating the samples at 65°C for 4 h. DNA samples were further incubated with 10µL of 0.5M EDTA, 20 µL of 20 mM Tris-HCl pH 6.5 and 1 µg/mL of protease K for 1h at 45°C. After ethanol precipitation, samples were used for PCR with the primers sets indicated in Supplemental Table S4.
**Supplemental Table S1.** PCR primers used for TIS11b mutagenesis

| Mutant    | Forward primer                                      | Reverse primer                                      |
|-----------|-----------------------------------------------------|-----------------------------------------------------|
| TIS11b-S54A | 5'-CCCTCGGAGGCAAGCGATCACCCTGCCCAGC-3'              | 5'-GCCGCTTAGTCATCTGAGATGAGCAG-3'                    |
|           |                                                     | 5'-GCTGGGCAGGGTGACTCGTGCCTCCGAGGG-3'                |
| TIS11b-S54D | 5'-CCTCGGAGGCAAGCGATCACCCTGCCTCCGAGGG-3'           | 5'-CTGGGGGAGGGTGACGTCGTGCCTCCGAGGG-3'               |
| TIS11b-S334A | 5'-GCCCATCTTCAGCAAGACTTCCATCCTCAGATGACTAAGCGGC-3' | 5'-GCCCGCTTAGTCATCTGAGATGGCAG-3'                    |
|           |                                                     | 5'-GCCGCTTAGTCATCTGAGATGAGCAG-3'                    |
| TIS11b-S334D | 5'-CTGCCCATCTTCAGCAAGACTTGGACAAGTCTGCTGAGATGAGG-3' | 5'-GCCGCTTAGTCATCTGAGATGAGCAG-3'                    |
|           |                                                     | 5'-GCCGCTTAGTCATCTGAGATGAGCAG-3'                    |
**Supplemental Table S2. PCR primers used for gene expression analysis**

| Gene | GenBank accession number | Forward primer | Location | Reverse primer | Location |
|------|--------------------------|----------------|----------|----------------|----------|
| TTP  | NM_003407                | 5’-TCCTGGTGCTCAA ATTACCCCTCA-3’ | 1455     | 5’-ATACAAGGGAGCACAGAC GACCCCAA-3’ | 1585     |
| TIS11b | NM_0011012 34            | 5’-ATTACCTCTTCAG CGCAGA-3’ | 1575     | 5’-AACCAGTGCTGGAACAC AC-3’ | 1768     |
| TIS11d | NM_006887                | 5’-AACATGTGACCC ACACTTACGGTC-3’ | 289      | 5’-CGCCCTCTCGTCCAGCAT GTTGT-3’ | 373      |
| RPL27 | NM_0010340 51            | 5’-GAACATGTGATGAGGGACCCACCTC-3’ | 121      | 5’-GGGGATATCCACAGAGTA CC-3’ | 291      |
Supplementary Table S3. PCR primers used for TIS11b promoter cloning and mutagenesis

| Number | Construct | Primer | Location | Commentary          |
|--------|-----------|--------|----------|---------------------|
| 1      | WT        | 5’-GGAAAGTAGTGAGTTGCTCGGTG-3’ | -1166    |                     |
| 2      | WT        | 5’-CCTGTCTCGAGTCCCACACG-3’    | -98      | Contains XhoI site  |
| 3      | mutCRE    | 5’-GGGAGTGGAATGAGTCTCGAGACGCGCGCTCTGAG-3’ | -544 | Contains CRE mutation |
| 4      | mutCRE    | 5’-CTCAGAGCGCGGCTGTCTCGAGACTCATTCACCTC-3’ | -544 | Contains CRE mutation |
**Supplemental Table S4.** PCR primers used for TIS11b promoter amplification in Chromatin Immunoprecipitation assays

| Promoter | Species | GenBank accession number | Used in | Forward primer | Location | Reverse primer | Location |
|----------|---------|--------------------------|---------|----------------|----------|----------------|----------|
| TIS11b   | human   | NM_004926                | COS7    | 5’-GGAAAGTA GTGAGTTGCTC GGTG-3’ | -1166    | 5’-CTCAGAGC GCGGCTGTGACG TCACTCATTCCAC TCCC-3’ | -547     |
| TIS11b   | bovine  | NM_001101234             | BAC     | 5’-TTAGGACG GGCTTCTTTCTG -3’ | -541     | 5’-AGTCCGCT CGCCTGTCTAC-3’ | -292     |