The role of ATF2 in insulin action

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Appendix

Full-colour Illustrations

Chapter 3

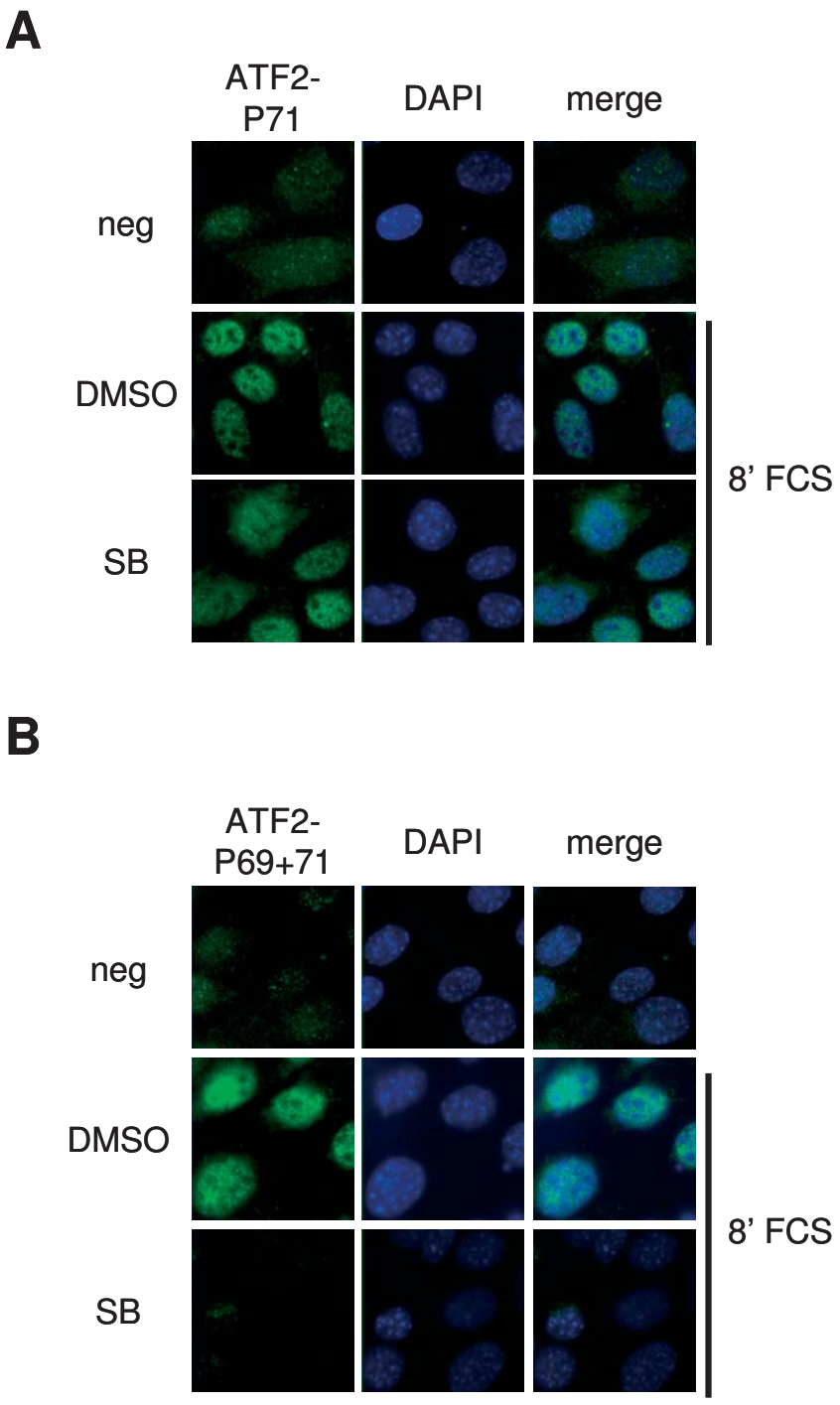

Figure 3. Serum-induced ATF2-Thr69+71, but not Thr71-phosphorylation is SB203580-sensitive in JNK-/- cells. Serum-starved JNK-/- cells were treated with SB203580 (SB) for 30 minutes prior to stimulation with 20% serum (FCS) for 8 minutes. Cells were fixed and stained with antibodies for (A) Thr71- or (B) Thr69+71-phosphorylated ATF2 followed by FITC-conjugated secondary antibodies (green). DNA was stained with DAPI (blue).

Chapter 3

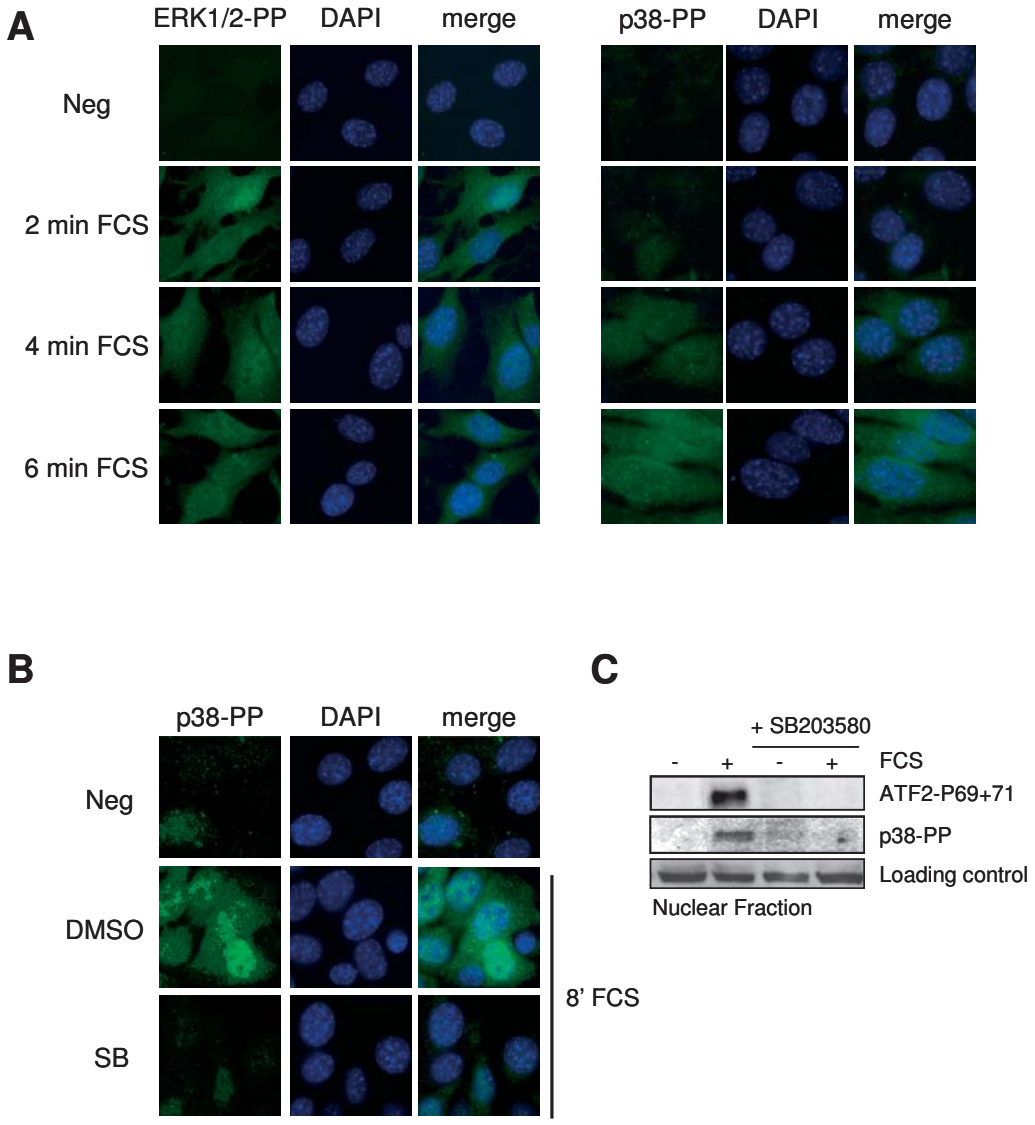

Figure 5. The onset of ATF2-Thr71 and ATF2-Thr69+71-phosphorylation associates with nuclear translocation ERK1/21/2 and p38. (A) Serum-starved JNK-/- cells were stimulated with 20% serum (FCS) and fixed at the times indicated. The fixed cells were stained with antibodies for phospho-ERK1/2 (ERK1/2-PP) or phospho-p38 (p38-PP) followed by FITC-conjugated secondary antibodies (green). DNA was stained with DAPI (blue). (B) Serum-starved JNK-/- cells pre-treated with vehicle or SB203850 for 30 minutes prior to 8 minute FCS-stimulation were fixed and stained for phospho-p38 (p38-PP) as described above. (C) Serum-starved JNK-/- cells, pre-treated with vehicle or SB203850 were serum-stimulated for 8 minutes, after which cellular fractions were prepared and the nuclear fractions examined for p38- and ATF2-Thr69+71 phosphorylation by immunoblotting with phospho-specific antibodies as described.

Chapter 4

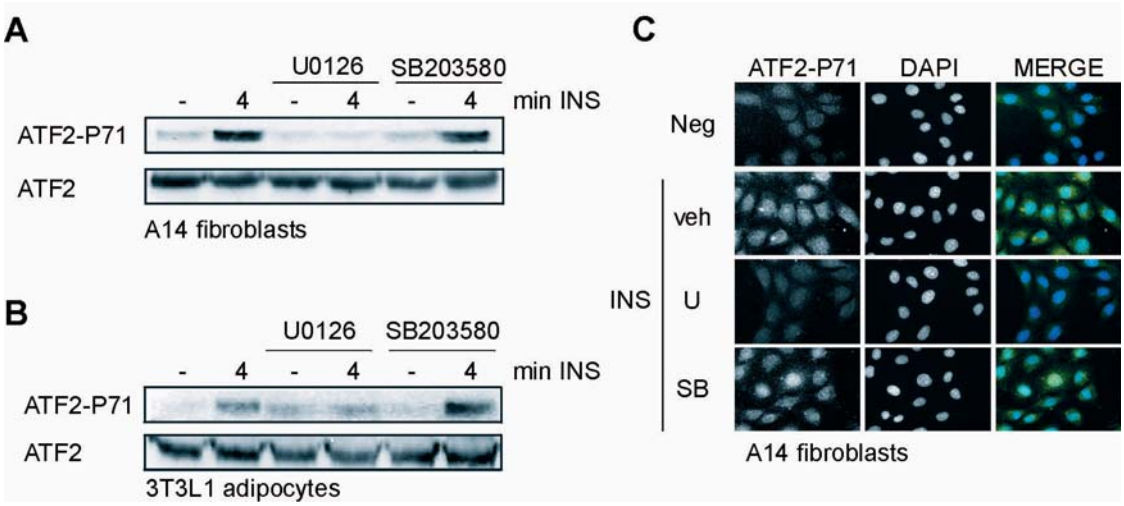

Figure 3. Early insulin-induced ATF2-Thr71 phosphorylation is sensitive to inhibition of the MEK1/2-ERK1/2 pathway. Serum-starved A14 cells (A) or 3T3L1 adipocytes (B) were incubated with 10 µM U0126 (U; 15 minutes) or 2.5 µM SB203580 (SB; 30 minutes) before insulin-stimulation (INS; 10 nM) for 4 minutes. Total ATF2 and ATF2-Thr71-phosphorylation levels were determined by immunoblotting with specific antibodies. (C) A14 cells were treated with inhibitors as described above, prior to stimulation with 10 nM of insulin for 4 minutes. Subsequently, cells were fixed and stained with phospho-specific ATF2-Thr71 antibodies followed by FITC-conjugated secondary antibodies (green). DNA was stained with DAPI (blue).

Chapter 4

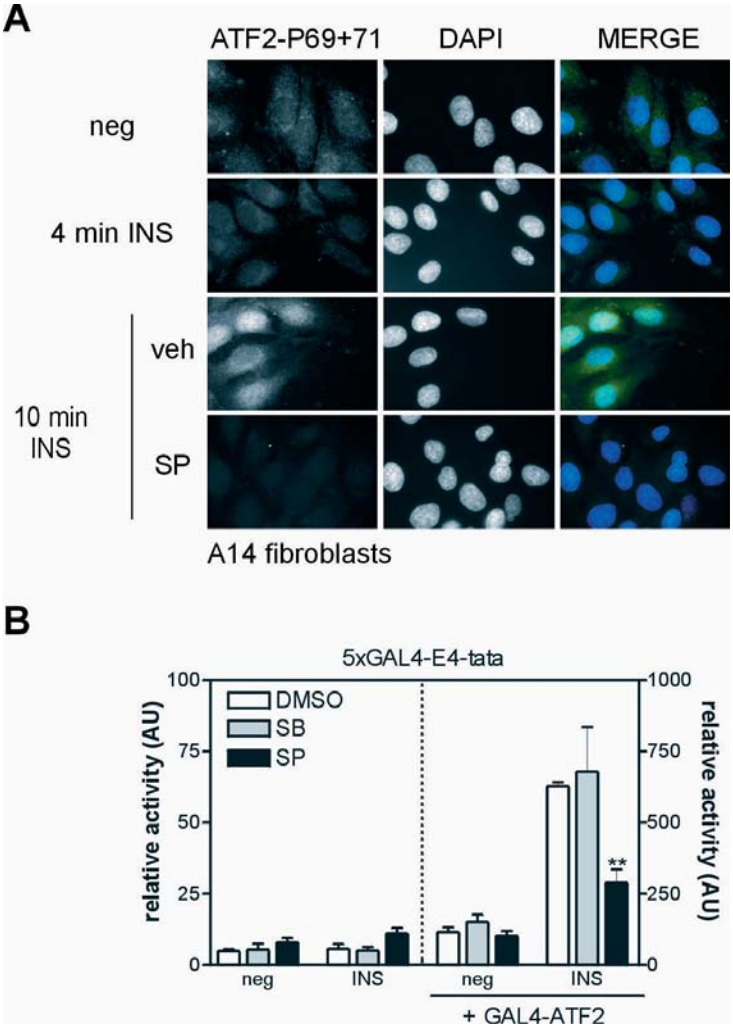

Figure 6. Inhibition of JNK, but not p38, abrogates the insulin-induced ATF2 phosphorylation and activation. (A) Serum-starved A14 cells were treated with inhibitors as described above prior to stimulation with 10 nM insulin (INS) for the indicated times. Cells were fixed and stained with antibodies for Thr69+71-phosphorylated ATF2 followed by FITC-conjugated secondary antibodies (green). DNA was stained with DAPI (blue). (B) Insulin-induced ATF2 transcriptional activity was examined in a GAL4-dependent luciferase reporter assay using the activation domain of ATF2 fused to the GAL4 DNA binding domain (19). Cells were transiently transfected and grown for 8 hours, subsequently serum-starved in DMEM containing 0.5% FBS O/N and treated for 30 minutes with vehicle or inhibitors SB203580 or SP600125 before adding insulin (INS; 10 nM). Cells were lysed 16 hrs later and luciferase activity was determined. The relative firefly luciferase activity is depicted as the mean enhancement of promoter activity in the absence or presence of insulin and/or inhibitors +/- the SD of three independent experiments performed in triplicates. Note the different scaling of the left and right *y*-axis. ** P = 0.0097 in a Student t-test.

Chapter 4

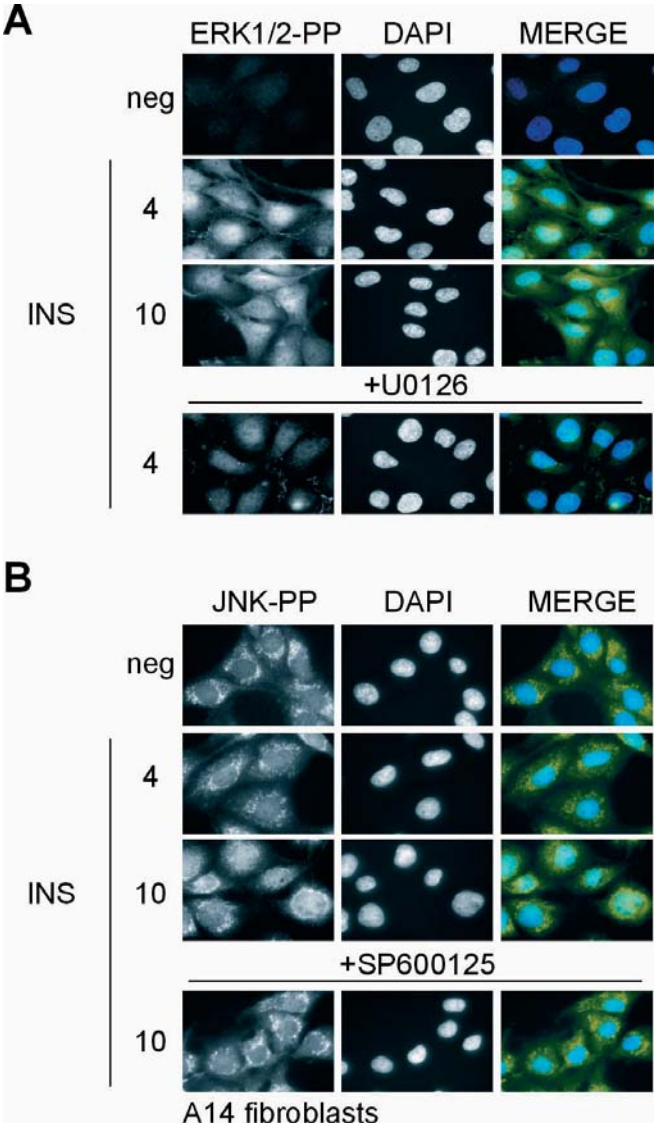

Figure 7. Time course of nuclear translocation of ERK1/2 and JNK. Serum-starved A14 cells were incubated with DMSO, 10 μM U0126 (U) for 15 minutes or 10 μM SP600125 (SP) for 30 minutes prior to stimulation with 10 nM insulin (INS) for the indicated times. Cells were fixed and stained with antibodies for (A) phosphorylated ERK (ERK-PP) or (B) phosphorylated JNK (JNK-PP) and FITC-conjugated secondary antibodies (green). DNA was stained with DAPI (blue).

Chapter 6

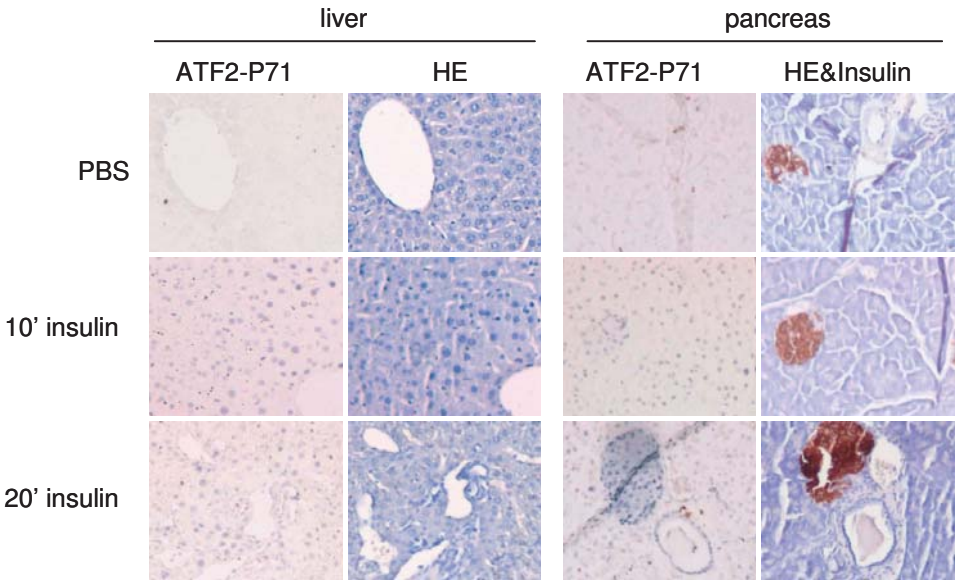

Figure 1. Immunohistochemical staining of mouse liver and pancreas tissue sections with phospho-ATF2-Thr71 antibodies (ATF2-P71), or hematoxylin (HE; blue) in combination with insulin (brown). Mice were infused with either PBS or insulin for the indicated times. Photographs are representative of 2-3 independent animals.

Appendix