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Pakay *et al.*

TBP-1 subunit of the 19S proteasome cooperates with an ERK-regulated degron to regulate turnover of the Fra-1/AP-1 transcription factor

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Running Title: Regulation of Fra-1 turnover.

SUMMARY

The timely synthesis and degradation of short-lived transcriptional regulators is required to orchestrate gene expression responses to extracellular signals. Aberrant turnover of the Fra-1 component of AP-1 transcription factor complexes can result in its overexpression in cancers, which is linked to increased proliferation, migration and invasion. A major unresolved issue is how Fra-1 undergoes proteasomal degradation without requiring prior ubiquitylation. We found that this process involves association of Fra-1 with TBP-1, a subunit of the 19S proteasome. TBP-1 does not bind to, but cooperates with a previously described ERK-regulated degron to regulate Fra-1 degradation. These findings provide a molecular insight into control of Fra-1 turnover and highlight a role for the 19S proteasome in regulating AP-1-dependent gene expression.

INTRODUCTION

Activator Protein-1 (AP-1) transcription factor complexes are dynamic regulators of gene expression programs activated by extracellular signals (XX). They comprise members of the Fos, Jun, ATF and Maf families, which form dimers that recognize TPA-response element (TRE) and other consensus motifs (*e.g.* CRE, ARE, NF κ B, E2F) in the promoter/enhancer regions of specific genes. The products of these genes modulate key cellular processes, including proliferation, survival, differentiation and migration {Chinenov, 2001 #116; Karin, 1997 #39}.

The Fra-1 member of the Fos family is an immediate-early gene product that is transiently induced by growth factor signaling events {Cohen, 1988 #117}. A growing body of work indicates that Fra-1 levels are persistently elevated in several types of

human cancers and tumour cell lines, where they are linked to increased migration, invasion and angiogenesis [Belguise, 2005 #14; Belguise, 2005 #14; Chen, 2009 #32; Doehn, 2009 #33; Luo, #31; Pollock, 2005 #48; Vial, 2003 #10; Young, 2006 #4]. Fra-1 is also an important regulator of oncogenic RAS-induced cell proliferation and malignant transformation [Kakumoto, 2006 #15].

Fra-1 is an intrinsically unstable, short-lived protein, whose turnover is tightly regulated in cells (XX). The major pathway controlling its synthesis and stability is the RAS-ERK pathway, which induces transcription of the *fosl1* gene and phosphorylation of the protein on serines 252 and 265 (XX). These residues are located in a C-terminal degnon known as the destabilizer (DEST) domain, which is functionally inactivated upon their phosphorylation (XX). This post-translational stabilization of Fra-1 is likely to play a critical role in enabling it to accumulation in cells containing oncogenic variants of *RAS* and *BRAF* (XX).

Most cellular proteins are degraded by the 26S proteasome, a multi-subunit complex consisting of a 20S core that is capped at both ends by a 19S regulatory particle (XX). Each 19S regulator has a lid, comprised of eight non-ATPase subunits, and a base, consisting of six AAA-type ATPases and three non-ATPase subunits (XX). The 19S lid is required for protein degradation, while its base interacts with, and modulates access of substrates to the core of the 20S complex.

The best understood mechanism of directing proteins to the proteasome for destruction is the covalent attachment of ubiquitin chains to lysine residues within or in their N-termini (XX). A growing number of proteasomal substrates however do not appear to require prior ubiquitylation to be degraded (XX). Fra-1 is one such protein, which undergoes properly regulated proteasomal degradation even when all its lysine

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residues are substituted with arginines and its N-terminus is blocked by fusion to a myc-epitope tag (XX). Other notable examples of cancer-associated proteins that can undergo ubiquitin-independent proteasomal degradation include c-Fos {*Basbous, 2008 #46*}, c-Jun ({*Takeuchi, 2007 #78*}, p21 {*Chen, 2007 #79*}, Rb {*Kalejta, 2003 #80*}, KLF5 () and p53 {*Asher, 2005 #81*}.

Although not fully understood, ubiquitin-independent degradation of proteasomal substrates can involve specific binding partners and the presence of unstructured regions in the proteins that are recognized by proteasomal components (XX). Many of these insights have come from work on ornithine decarboxylase (ODC), whose ability to undergo ubiquitin-independent degradation is regulated by a 37 residue C-terminal domain known as cODC. This domain consists of two functionally separable regions that are required for efficient degradation of the protein. The first mediates association with the proteasome, and the second is a loosely structured region, that initiates proteolysis (XX). Interestingly, proteasomal association of ODC appears to involve binding to the same region the 19S proteasome that recognizes ubiquitinated substrates (XX).

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To better understand how the turnover of Fra-1 proteins is regulated, we used mass spectrometry to search for proteasomal components that associate with Fra-1 in mammalian cells. We found that Fra-1 associates with TBP-1, a subunit of the 19S proteasome that is involved in the recognition of proteasomal substrates. TBP-1 depletion stabilized Fra-1 and increased its levels in a variety of cells by a mechanism that was distinct to ERK-induced phosphorylation of its DEST domain. These effects on Fra-1 levels correlated with changes in AP-1 activity. Our findings suggest a model in which TBP-1 mediates ubiquitin-independent recognition of Fra-1 by the proteasome,

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while its DEST domain acts as an ERK-regulated degron required for initiating proteolysis.

RESULTS

Identification of proteasomal components that associate with Fra-1

The molecular mechanism underlying ubiquitin-independent recognition of Fra-1 proteins by the proteasome is unknown. One suggestion is that proteasomes can directly recognize non-ubiquitylated Fra-1 proteins (XX), a possibility that prompted us to search for proteasomal components that interact with Fra-1 in mammalian cells. Because of the unstable nature of wild-type Fra-1 (Fra-1^{WT}), we employed two approaches to improve its expression and thereby generate sufficient bait protein for subsequent MS analysis (Figure S1). First, we deleted three C-terminal residues of the Fra-1 DEST domain (XX), while the second approach involved coexpressing Fra-1^{WT} with the BRAF kinase to induce phosphorylation of the DEST domain (XX).

Using mass spectrometry, we identified the 19S proteasomal subunit, TBP-1 (also known as Rpt5/S6'; Figure S1), as the main proteasomal component in anti-FLAG immunoprecipitates from HEK293 cells expressing FLAG-Fra-1^{Δ3} or FLAG-Fra-1^{WT} and BRAF. No peptides corresponding to TBP-1 were detected in anti-FLAG immunoprecipitates from cells transfected with vector alone.

We first confirmed that TBP-1 associates with Fra-1^{Δ3} and with Fra-1^{WT} in HEK293 cells (Figure 1A). Treatment of the cells with TPA, which induces phosphorylation and activation of Fra-1 (young 2002X), did not alter the abundance of Fra-1/TBP-1 complexes. However, we noted that TBP-1 immunoprecipitates

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contained Fra-1 species of differing mobilities on SDS-PAGE gels, a likely indication that multiple phosphorylation states of the protein were present (XX).

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Next, we determined if endogenous Fra-1 and TBP-1 proteins can form complexes. In HCT116 colon carcinoma cells, which express high levels of endogenous Fra-1 (XX), endogenous TBP-1 copurified with immunoprecipitates prepared using anti-Fra-1 but not control (IgG) antibodies (Figure 1B). These experiments also revealed that the pool of TBP-1 that associated with Fra-1 represented only a small proportion of total TBP-1 in these cells.

To test if Fra-1 and TBP-1 can associate *in vitro*, we purified soluble FLAG-TBP-1 from HEK293 cells and incubated it with immunoprecipitated GFP, GFP-Tam67 (a c-Jun mutant; XX) and GFP-Fra-1 proteins (Figure 1C). In this assay, only GFP-Fra-1 was able to bind FLAG-TBP-1, demonstrating the specificity and potentially direct nature of the interaction. Together, these findings demonstrate that Fra-1 associates with the TBP-1 subunit of the 19S proteasome, thus providing a potential mechanistic link between Fra-1 and proteasomal complexes in mammalian cells.

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TBP-1 modulates Fra-1 turnover

Previous studies have suggested that TBP-1 may form part of a common binding site on the proteasome for both ubiquitylated and non-ubiquitylated substrates (XX). Association with TBP-1 may thus provide a mechanism for proteasomal recognition of Fra-1, and therefore play a critical role during its degradation. To test this possibility, we determined the impact of reducing TBP-1 expression on Fra-1 levels in three tumour cell lines, MDA-MB-231, BE and HCT116. The results show that TBP-1 negatively

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regulated Fra-1 protein levels in all three lines, with a 51-73% reduction in TBP-1 levels resulting in a 1.6-4.1-fold increase in cellular Fra-1 content (Figure 2A-2C).

Next, we examined if TBP-1 regulates Fra-1 stability by monitoring the rate of Fra-1 decay after suppression of protein synthesis using cycloheximide (CHX). In HCT116 cells treated with control siRNA oligonucleotides, Fra-1 had a half-life of ~ 40 min, whereas in TBP-1-depleted cells, 60-70% of the Fra-1 protein remained after 2 hours of CHX treatment (Figure 2D-2E). Interestingly, we noted variations in the degradation kinetics of differentially phosphorylated Fra-1 proteins (Fig. 2D), with the slowest migrating species on SDS-PAGE gels being least susceptible to degradation.

In contrast to TBP-1 depletion, we found that overexpression of TBP-1 reduced Fra-1 protein levels in HCT116 cells (Figure 2F), and that this effect that was prevented when cells were treated with the proteasome inhibitor, MG132. Collectively, these results provide strong evidence that TBP-1 is a critical regulator of Fra-1 degradation via the proteasome.

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Distinct roles of TBP-1 and RAS-ERK signaling during Fra-1 degradation

Our findings that reducing TBP-1 levels had a similar stabilizing effect on the Fra-1 protein as deletion or phosphomimetic substitution of ERK-induced sites in its DEST domain (XX) prompted us to consider whether a common mechanism was responsible. One possibility is that TBP-1 interacts with the DEST domain and that ERK-induced phosphorylation of Ser²⁵² and Ser²⁶⁵ in this region disrupts Fra-1/TBP-1 interactions. We found this not to be the case, as Fra-1 protein lacking their DEST domain (Fra-1^{Δ50}) retained their ability to associate with TBP-1 (Figure 3A & 3B). We also noted that Fra-1 proteins carrying aspartic acid (Fra-1^{DD}) or alanine (Fra-1^{AA}) substitutions of Ser²⁵²

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and Ser²⁶⁵ associated with similar amounts of TBP-1. These results indicate that TBP-1 binding is unlikely to be responsible for the destabilizing effects of DEST domain.

To further explore the relationship between binding of TBP-1 to Fra-1 and ERK-induced phosphorylation of its DEST domain, we determined if, like Fra-1^{WT}, Fra-1^{DD} levels increased upon TBP-1 depletion. Figure 3C shows that silencing TBP-1 expression not only resulted in a robust increase in Fra-1^{WT} levels, but also further enhanced levels of Fra-1^{DD} expression. Thus the mechanisms by which TBP-1 depletion and DEST domain phosphorylation stabilize Fra-1 appear to be distinct.

TBP-1 modulates AP-1-dependent transcription

The ability of TBP-1 to negatively regulate Fra-1 levels suggested that it could act as an endogenous modulator of Fra-1-induced gene expression. We examined this issue by performing AP-1 reporter gene assays. TBP-1 overexpression reduced basal and Fra-1-induced AP-1 reporter gene activation by ~ 30% (Figure [4A](#) and results not shown). TBP-1 depletion had the opposite effect, increasing basal and Fra-1^{WT}-induced AP-1 transcriptional activation (Figure [4B](#)). TBP-1 siRNA treatment also increased AP-1 reporter gene activation by Fra-1^{DD}. Interestingly, the fold increase of AP-1 activity upon TBP-1 depletion was similar in cells expressing Fra-1^{WT} and Fra-1^{DD}, despite the more dramatic effect on Fra-1^{WT} protein levels (Figure XX).

As indicated in Figure 4B, we noted a significant increase in basal AP-1 reporter gene activity upon TBP-1 depletion. Using two different approaches, we demonstrated that this increase was Fra-1 dependent. First, the increase in basal AP-1 activity was suppressed when TBP-1 siRNA was combined with Fra-1 siRNA (Figure 4C). Secondly, we found that expression of a DNA binding-defective Fra-1 variant prevented

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the increase in AP-1 activity upon TBP-1 depletion (Figure 4A). Together, these findings suggest the TBP-1 is as an endogenous modulator of Fra-1 induced activation of AP-1 target gene transcription.

DISCUSSION

Overexpression of the Fra-1 component of AP-1 transcription factor complexes has been linked to increased tumour cell proliferation, migration, invasion and angiogenesis (XX). While previous studies have established that RAS-ERK signaling induces the synthesis and stabilization of Fra-1, a major unresolved issue is how Fra-1 proteins undergo proteasomal degradation, a process that does not require its prior ubiquitylation (XX). In this study, we identified the 19S proteasomal subunit, TBP-1, as a Fra-1-associated protein, and provide evidence that TBP-1 negatively regulates Fra-1 protein levels in cells by modulating its degradation.

The intrinsically unstable nature of the Fra-1 protein has been shown to depend on its C-terminal DEST domain, which upon undergoing ERK-induced phosphorylation on serines 252 and 265, reduces the susceptibility of Fra-1 to proteasomal degradation (XX). This post-translational stabilization of Fra-1 plays a critical role in enabling it to accumulate in tumour cells harboring oncogenes of the RAS-ERK pathway (e.g. *KRAS*, *BRAF*; XX). We found that depletion of TBP-1 in tumour cell lines containing these oncogenes further increased Fra-1 levels and stability. However, the mechanism by which TBP-1 depletion stabilized Fra-1 appeared to be distinct from that of ERK-induced phosphorylation because TBP-1 depletion also increased the levels of Fra-1 proteins when Ser252/Ser265 were substituted with phosphomimetic residues.

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Previous studies have suggested that that TBP-1 forms part of a common binding site on the proteasome for both ubiquitylated and non-ubiquitylated substrates (XX). Thus, a likely explanation of our findings is that TBP-1 regulates Fra-1 degradation by mediating its ubiquitin-independent recognition and association with the proteasome. This process does not depend on the ability of Fra-1 to dimerize with other AP-1 proteins or to bind DNA because TBP-1 depletion also increased levels of Fra-1 proteins that defective in both these functions (Figure S2).

In addition to requiring a mechanism to associate with the proteasome, efficient degradation of proteasomal substrates can also require the presence of poorly structured regions, often in their termini, from which unfolding or degradation is initiated (Prakash et al 2004, XX). During ubiquitin-independent degradation of the enzyme ODC, both these requirements are supplied by a stretch of 37 residues in its C-terminus. Like this region in ODC, the DEST domain of Fra-1 is poorly structured and consists of ~ 40 residues (XX). While deletion of this domain stabilizes Fra-1 (XX), we found that it did not affect the ability of Fra-1 to associate with TBP-1. These findings indicate that TBP-1 must interact with residues outside the Fra-1 DEST domain, and that TBP-1 binding alone is not sufficient to induce Fra-1 degradation.

While not required for TBP-1 binding, the DEST domain may play an important role in initiating proteolysis of Fra-1 once it is localized to the proteasome. Consistent with this idea, deletion of two or three residues of the DEST domain is sufficient to partially stabilize Fra-1 (XX). Similarly, ERK-induced phosphorylation may lead to structural changes in the DEST domain that interfere with the initiation of proteolysis. Thus, we propose a model of Fra-1 degradation in which TBP-1 regulates proteasomal association of Fra-1 while its DEST domain controls the initiation of proteolysis. This

model also predicts that TBP-1 and dephosphorylation of Ser252 and/or Ser265 (XX) function cooperatively to mediate proteasomal degradation of Fra-1.

Apart from their bZIP domains, the DEST domain represents the second most highly conserved region amongst FOS family proteins (c-Fos, FosB, Δ FosB, Fra-1, Fra-2). The spatial separation of Fra-1 residues that are required for TBP-1 binding from the DEST domain may provide a mechanism for differentially regulating the turnover of individual FOS family members. The identification of Fra-1 residues required for it to associate with TBP-1 will be critical to test this idea. These studies are currently underway.

In addition to being proteasome-bound, TBP-1 has been reported to associate with transcriptional complexes on chromatin. The presence of TBP-1 in Fra-1 complexes may facilitate their rapid proteasomal association and turnover, thus providing a dynamic mechanism to regulate AP-1 activity. Consistent with this possibility, we found that TBP-1 also repressed Fra-1-induced AP-1 reporter gene activation, an effect that correlated with its effects of Fra-1 levels.

While it is presently not known if decreased TBP-1 expression correlates with elevated Fra-1 levels in cancer, a previous study has shown that TBP-1 levels expression is repressed by oncogenic ErbB receptor kinases (XX). This study also showed that TBP-1 has tumour suppressive properties, a finding confirmed by other groups. Several proteins, whose cellular levels are regulated by TBP-1, may be involved in mediating the tumour suppressive effects of TBP-1. These include p14ARF (XX), HIF1 α (XX) and Rb (XX). Our findings suggest that suppression of Fra-1 levels may also contribute to the ability of TBP-1 to act as a tumour suppressor.

EXPERIMENTAL PROCEDURES

Cell culture and reagents

HEK293, MDA-MB-231, HCT116 and BE cells (a gift from Prof. David Gillespie, Beatson Institute, UK) were grown in DMEM supplemented with 2 mM L-glutamine and 10% foetal calf serum. Cells were transfected with expression constructs using Lipofectamine 2000 or Lipofectamine LTX (Invitrogen). Cycloheximide and MG132 were from Merck, while TPA was purchased from Sigma.

The expression constructs pCDNA3-FLAG-Fra-1^{WT} (XX) and FLAG-TBP-1 (XX) have been described previously. The pCDNA3-HA-Fra-1^{WT} and pCDNA3-HA-Fra-1^{A3} constructs were generated using standard PCR-based methods. Mutations to generate dimerization-defective (XXX) and DNA binding-deficient (XXXX) variants of Fra-1 were introduced by site-directed mutagenesis. All constructs were verified by sequencing.

The siRNA oligonucleotides used in this study were from Dharmacon (TBP-1^{#1}, GGACAAUGCUGGAGCUUCU; TBP-1^{#2}, CAAAGACUCCUAUCUGAUC) or Qiagen (AllStars negative control siRNA), and were introduced into cells using DharmaFECT 1 reagent (Dharmacon).

Analysis of Fra-1 complexes by mass spectrometry

Eight 10 cm diameter dishes of HEK293 cells were transfected with pCDNA3, pCDNA3-FLAG-Fra-1^{A3} or pCDNA3-FLAG-Fra-1^{WT} and wild-type BRAF. The cells were lysed after 48 h by passaging through a 23G needle in Buffer A (20 mM Hepes pH 8.0, 150 mM NaCl, 2 mM EDTA, 0.5% NP-40, 1 mM PMSF, 2 mM NaF, 1 mM Na

vanadate, 5 µg/mL leupeptin and 2.2 µg/mL aprotinin). The cell extracts were incubated on ice for 20 min, clarified by centrifugation, and incubated with 80 µL of α-FLAG M2 affinity gel (Sigma) for 2h. Immunoprecipitates were washed four times with Buffer A before elution of the FLAG-Fra-1^{Δ3} complexes with FLAG peptide (0.1 mg/mL). Eluted proteins were separated on 4-12% NuPAGE gels (Invitrogen) and stained with Colloidal Coomassie (BioRad). Gel lanes of interest were sliced into multiple pieces and processed for analysis by mass spectrometry as described previously (XX).

Immunoprecipitation and immunoblotting

Cells were lysed for immunoprecipitation analysis by passaging through a 23G needle in Buffer A. HEK293 cell extracts from each 10 cm diameter dish were incubated with 2 µg of α-GFP (Invitrogen) or α-HA (Roche) antibodies and 20 µL of protein A-agarose beads (Sigma). Anti-Fra-1 immunoprecipitates were prepared from two 10 cm diameter dishes of HCT116 cells using and 6 µg of α-Fra-1 antibody (R-20; Santa Cruz Biotechnologies) and 20 µL of protein A-agarose beads.

SDS-PAGE and transfer of proteins onto PVDF membranes (GE Healthcare) was performed using standard protocols. The membranes were incubated with the following antibodies: α-FLAG M2 (Sigma); α-Fra-1, α-14-3-3 and α-tubulin (Santa Cruz Biotechnologies), α-HA (Roche), α-TBP-1 (Upstate Inc.), anti-GFP (Invitrogen) and anti-phospho ERK1/2 (Cell Signaling). Protein bands were visualized using HRP-labelled secondary antibodies and detected with ECL reagents (Pierce).

Reporter gene assays

HEK293 were transfected in quadruplicate in 24-well dishes with a total of XX ug of pCDNA3, Fra-1 and/or TBP-1 expression constructs (XX ug) together with XX ug pfLuc 5xAP-1 (XX) and pSV40-RL (as internal control). pFLUC 5xAP-1 is based on the pFLUC reporter construct with the minimal c-Fos promoter (Saksela et al., 1993), but contains five copies of the TPA response element (TRE) from the intronic enhancer of the S100A4 gene cloned in the HindIII site in a head-to-tail orientation. Jeannine: Also what about sequential si and plasmid transfxn. 48 h after transfection, luciferase activity was measured using the Dual-Luciferase Reporter Assay (Promega). The normalized activities were expressed as fold of pCDNA3-transfected cells \pm SD.

REFERENCES

ACKNOWLEDGEMENTS

We thank Drs Nishida, Satoh and Gillespie for generously providing reagents used in this study. **Dr Willie Bienvenut ??** This work was supported by grants from the National Health and Medical Research Council of Australia (to ASD and RDH), AICR (to ET) and XX (Walter??).

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FIGURE LEGENDS

Figure 1. Association of TBP-1 with Fra-1 in mammalian cells. (A) Coimmunoprecipitation analysis of FLAG-TBP-1 association with HA-Fra-1^{WT} and HA-Fra-1^{A3} in asynchronously growing and TPA-treated (100 ng/mL for 30 min) HEK293 cells. EV, empty vector. (B) Coimmunoprecipitation analysis of endogenous Fra-1/TBP-1 complexes in HCT116 colon carcinoma cells. (C) Analysis of *in vitro* FLAG-TBP-1 binding to GFP-Fra-1, GFP-Tam67 and GFP. The lower panel shows the relative amount of each GFP protein used in the binding assay.

Figure 2. TBP-1-mediated regulation of Fra-1 degradation and expression levels. (A)-(C) Fra-1 protein levels in asynchronously growing MDA-MB-231 breast carcinoma cells, and HCT116 and BE colon carcinoma cancer cells transfected with control and TBP-1 siRNA oligonucleotides. 14-3-3 or α -tubulin was used as loading control. (D) Effect of control and TBP-1 siRNA oligonucleotides on the rate of Fra-1 disappearance in HCT116 following treatment with cycloheximide (CHX; X mM for X h). (E) The data from (D) were quantified by densitometry and plotted after normalization relative to tubulin levels in each sample. (F) Effect of TBP-1 overexpression on endogenous Fra-1 levels in HCT116 cells treated with DMSO or MG132 (X mM for Xh). Coexpressed GFP was used as a transfection control.

Figure 3. Roles of TBP-1 and ERK-induced phosphorylation of the DEST domain in regulating Fra-1 levels. (A) Schematic illustration of the Fra-1 deletion mutants analysed, highlighting the major known functional domains and ERK-regulated

phosphorylation sites in the C-terminus of Fra-1. (B) Coimmunoprecipitation analysis of FLAG-TBP-1 association with truncated and Ser²⁵²/Ser²⁶⁵ mutants HA-Fra-1 proteins in HEK293 cells. (C) Effect of control or TBP-1 siRNA oligonucleotides on levels of wild-type Fra-1 (HA-Fra-1^{WT}) and Fra-1 proteins containing phosphomimetic substitutions of Ser²⁵²/Ser²⁶⁵ (HA-Fra-1^{DD}).

Figure 4. TBP-1 modulates Fra-1-induced AP-1 activity. (A) Effect of TBP-1 overexpression on Fra-1-induced AP-1 reporter gene activation in HEK293 cells. (B) Effect of control or TBP-1 siRNA oligonucleotides on AP-1 reporter gene activation in HEK293 cells induced by empty vector (EV), wild-type Fra-1 (WT), a DNA-binding-defective Fra-1 mutant (DBD) and Fra-1 proteins containing phosphomimetic substitutions of Ser²⁵²/Ser²⁶⁵ (DD). (C) The increase in basal AP-1 reporter gene activation by TBP-1 siRNA treatment of HEK293 cells is Fra-1-dependent.

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Search pubmed/google “ubiquitin-independent 19S”

Figure 1 - Pakay *et al.*

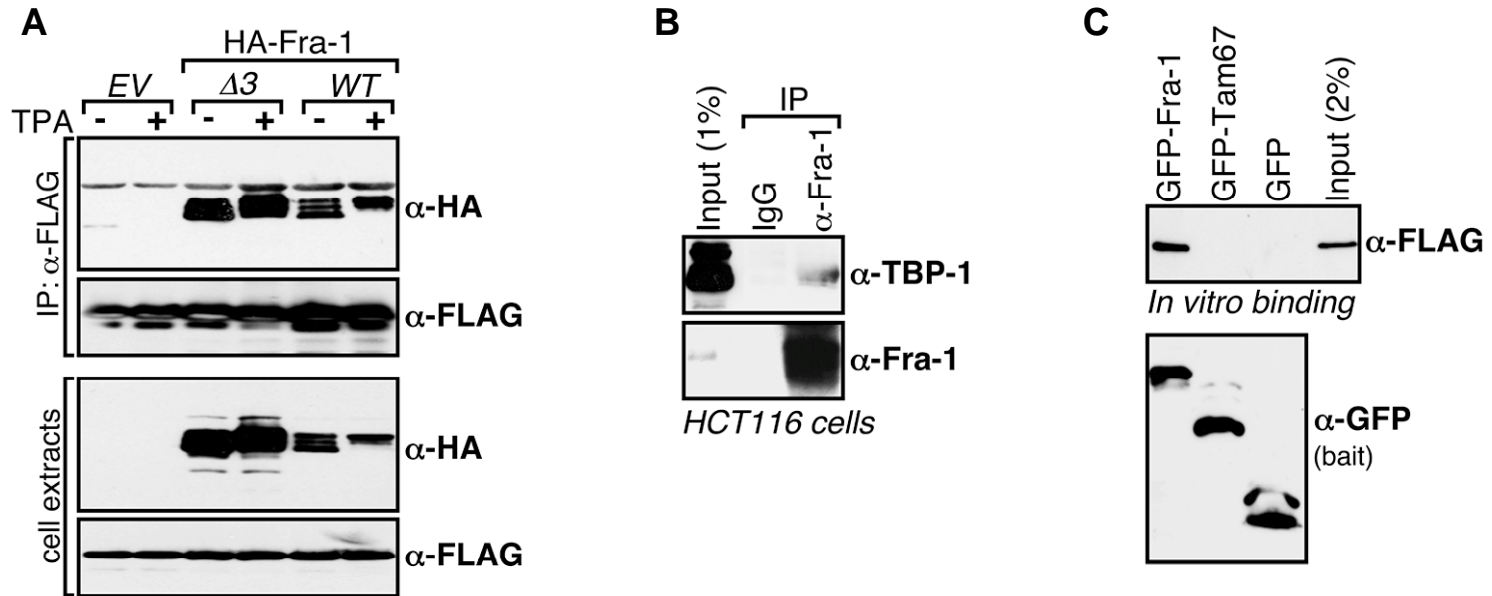


Figure 2 - Pakay *et al.*

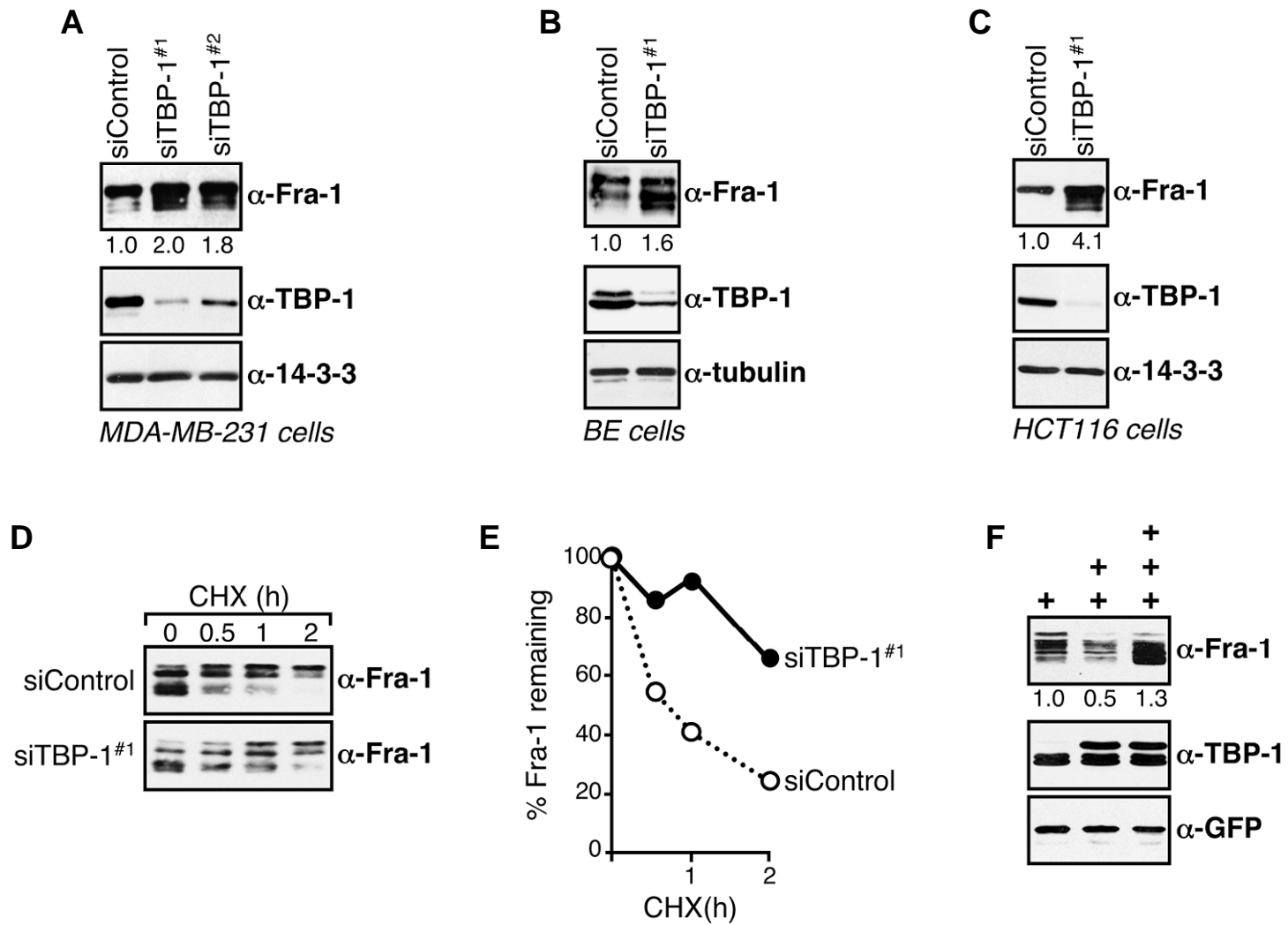


Figure 3 - Pakay *et al.*

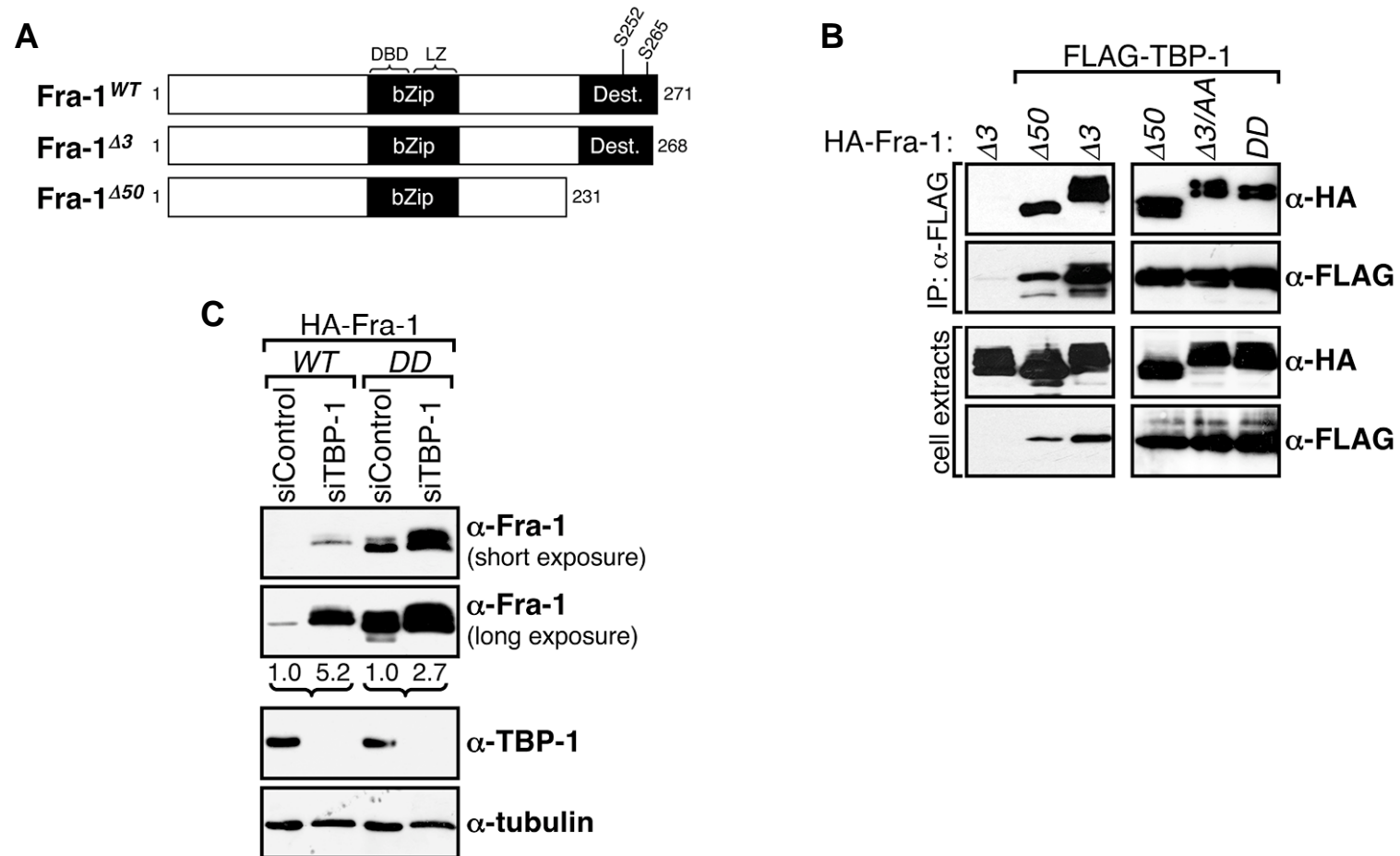


Figure 4 - Pakay *et al.*

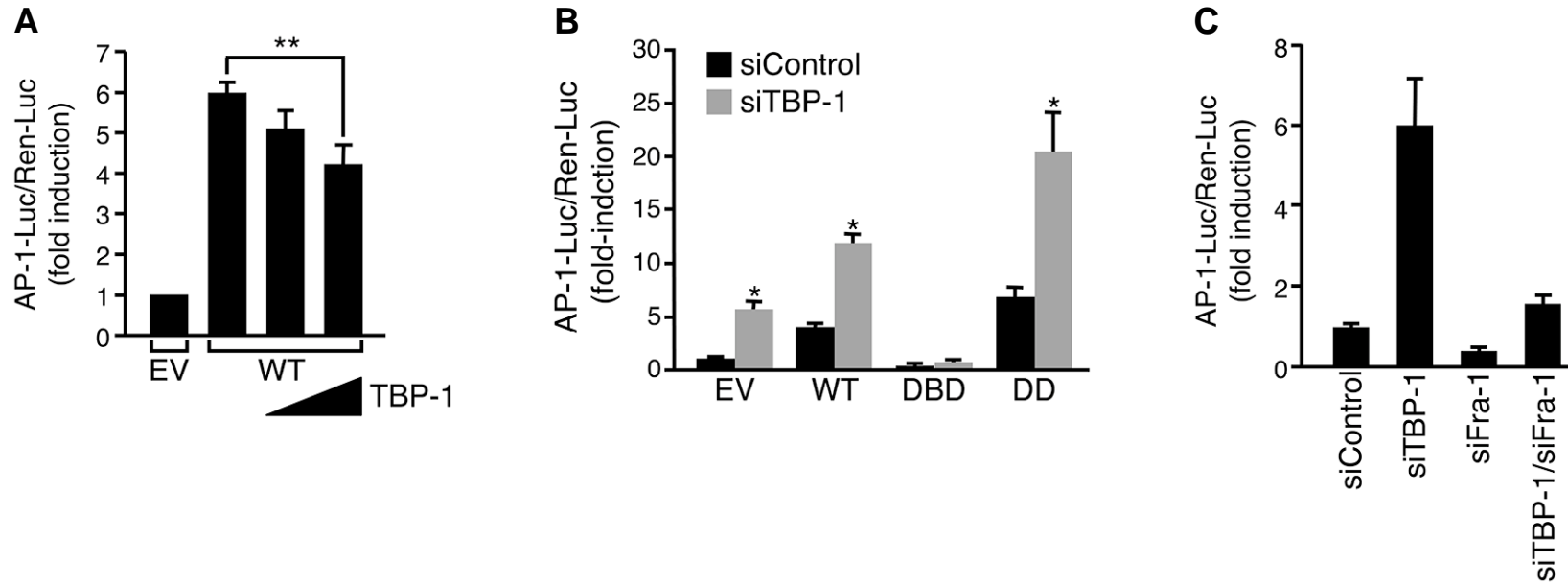


Figure S1 - Pakay *et al.*

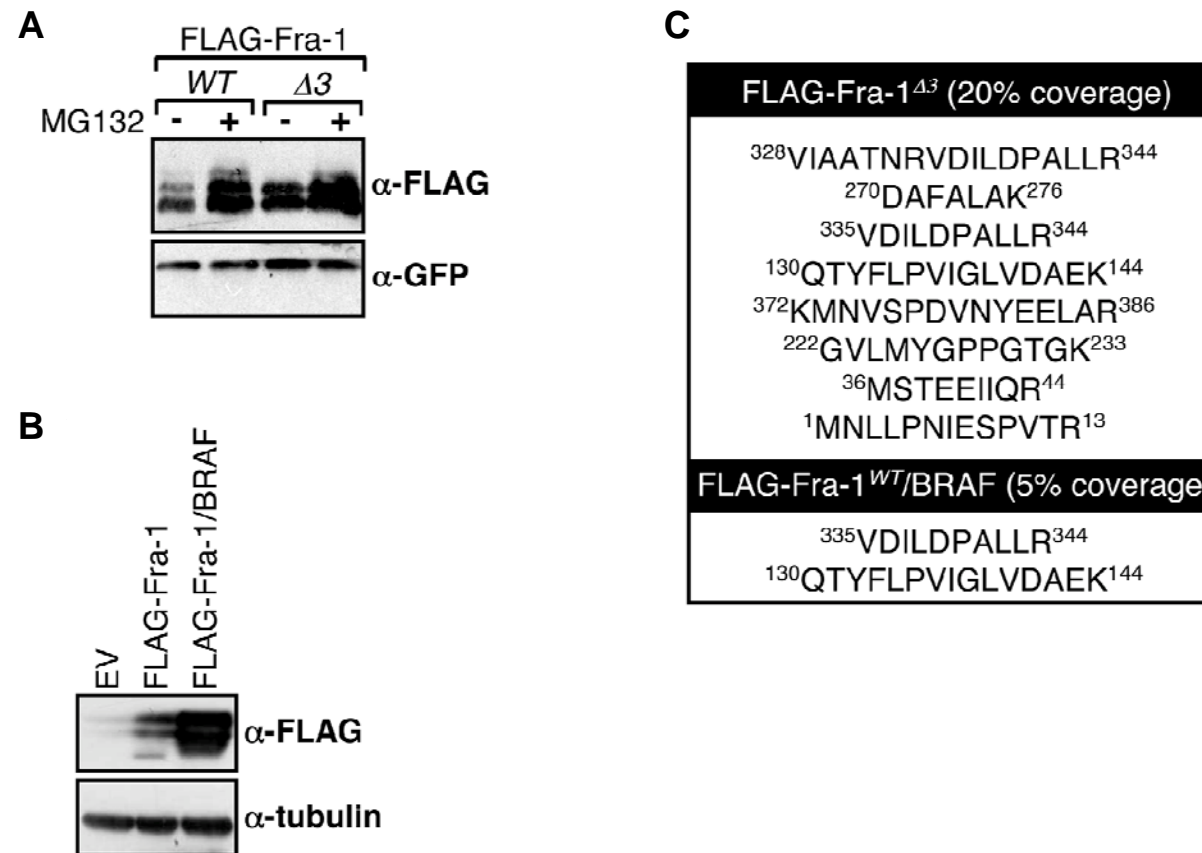


Figure S1. Identification of TBP-1 in Fra-1 complexes. (A) Comparison of Fra-1^{WT} and Fra-1 ^{$\Delta 3$} expression in HEK293 cells. GFP was used transfection control. (B) Effect of BRAF on Fra-1^{WT} expression in HEK293 cells. (C) TBP-1 peptides identified by mass spectrometry in FLAG-Fra-1 ^{$\Delta 3$} and FLAG-Fra-1^{WT}/BRAF, but not control (vector alone) immunoprecipitates from HEK293 cells.

Figure S2 - Pakay *et al.*

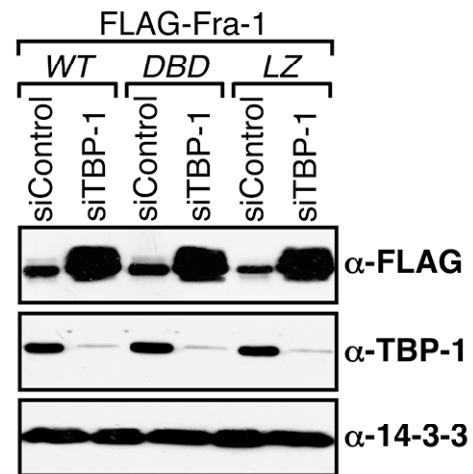


Figure S2. TBP-1 negatively regulates expression of Fra-1 bZIP domain mutants. Effect of control or TBP-1 siRNA oligonucleotides on levels of wild-type, DNA binding-defective and dimerization-defective FLAG-Fra-1 proteins.