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The JNK1/JNK3 interactome – Contributions by the JNK3 unique N-terminus and JNK common docking site residues

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ABSTRACT

The c-Jun N-terminal Kinases (JNKs) play important roles in cell responses to stress or growth factor stimulation. The JNK1α1 isoform shares >90% identity with a predominantly neuronal JNK3α1 isoform, but JNK3α1 also includes a distinctive 38 amino acid N-terminal sequence. To address the outstanding question of the potential for these JNK isoforms to have different binding partners that mediate different biological actions, the work presented here refined the yeast two-hybrid approach to identify and categorize binding partners for JNK1α1 and JNK3α1. Specifically, site-directed mutagenesis of the JNK1α1 common docking (CD) domain that mediates typical JNK-binding domain (JBD)-dependent interactions, truncation of the distinctive JNK3 N-terminal domain (*i.e.* ΔN JNK3α1), and interaction evaluation in the yeast two-hybrid system defined the interacting partners as either JNK1-specific interactors (ATF7, FUS, KCNE4, PIAS1, SHANK1, TKT), typical JBD-dependent interactors shared by JNK1α1 and JNK3α1 (AKAP6, BMPR2, EEF1A1, GFAP, GRIP2, GTF2F1, HDAC2, MAP1B, MYO9B, PTPN2, RABGAP1, RUSC2, SUMO1, SYPL1, TOPBP1, ZNF668), or JNK3-specific partners (ATXN1, NNAT, PTGDS) dependent on interaction with the JNK3 N-terminal extension. The interacting partners ATF7, AKAP6, and ATXN1 were explored further as representatives of these different classes. Two potential JBDs were identified in ATF7 as important for its interaction with JNK1α1, but additionally an interaction between ATF7 and ΔN JNK3α1 was shown to be JBD-dependent, suggesting that the JNK3α1 N-terminus prevents interaction with some proteins. For the shared partner AKAP6, one of the multiple potential JBDs predicted by sequence analysis was important for the AKAP6-JNK interaction in the yeast screening system as well as in mammalian cells. Finally, the ATXN1-JNK3α1 interaction was dependent on the JNK3α1 N-terminus in a mammalian cell context. These studies therefore highlight a diversity of potential JNK-interacting partners with both JBD-dependent as well as JBD-independent modes of interaction.

1. Introduction

The c-Jun N-terminal Kinases (JNKs) are a subfamily of the mitogen-activated protein kinases (MAPKs) encoded by the genes *jnk1* (*MAPK8*), *jnk2* (*MAPK9*), and *jnk3* (*MAPK10*) [1]. Whilst JNK1 has been implicated in physiological roles [2–4], JNK3 has been described with roles in a pathological context, especially because *Jnk3*−/− mice showed neuroprotection against insults including ischemic stroke and Alzheimer's disease [5–7]. Despite the important roles for JNKs in diverse physiological and pathological contexts, the basis for JNK isoform specificity of functions remains relatively poorly explored at a molecular level.

Previous structural and biochemical studies have defined specific residues within JNK1 required for interaction with its protein partners [8–10]. Specifically, a complex of JNK1 with a JIP1 scaffold-derived substrate inhibitory peptide revealed the requirement for two JNK1 residues (*i.e.* a common docking (CD) site residues E329 and R127) for binding, and thus JNK1 E329A and/or JNK1 R127A mutants failed to interact with this peptide [9]. JNK1 R127 and E329 are also required for JNK1 interaction with peptides derived from NFAT4 and JIP1 [11] whereas the equivalent JNK3 residues (E367 and R165) are required for interactions with peptides derived from JIP1 and the JNK mitochondrial binding protein Sab [12]. Comparable analyses of the related MAPKs have revealed

Abbreviations: CD site, common docking site; JBD, JNK-binding domain; JNK, c-Jun N-terminal Kinase; MAPK, mitogen-activated protein kinase.

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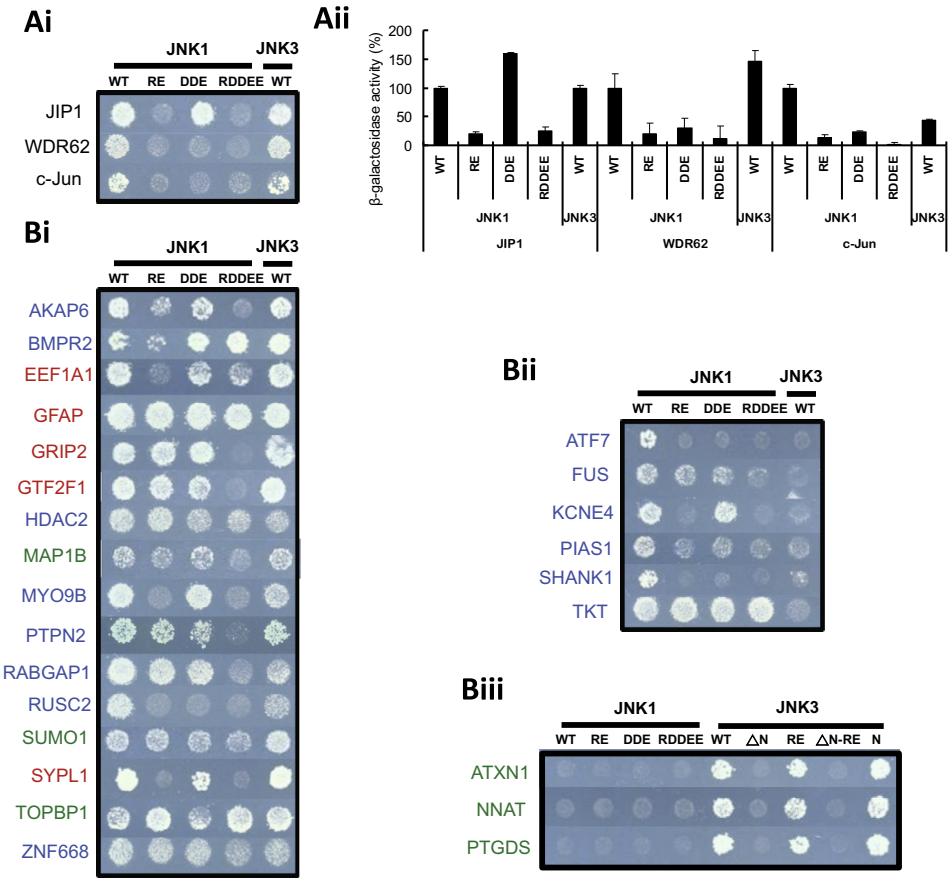

Fig. 1. Evaluation of JNK1/3-interacting partners in a directed yeast two-hybrid analysis. (A) Yeast containing the indicated JNK bait constructs were mated with yeast containing prey protein constructs (JIP1(127–218), WDR62(968–1523), or c-Jun(1–135)) for testing of interactions as assessed by yeast growth on stringent QDO plates over 6 days (Ai). Growth on DDO plates confirmed the presence of mating yeast in each sample (results not shown). Quantitative assessment of yeast two-hybrid system for protein–protein interaction profiles was determined by measuring the levels of β-galactosidase activity (Aii). (B) Definition of the binding mode of JNK1- and JNK3-binding partners. Yeast containing the indicated bait constructs were mated with yeast containing prey protein constructs for testing of protein–protein interaction as assessed by yeast growth on QDO plates with growth examined over 3–6 days, depending on the strength of protein–protein interactions. Evaluation of typical JBD binding partners and atypical binding partners was based on interaction analyses with JNK1 RE, DDE, and RDDEE mutants that disrupt residues typically involved in JBD-dependent binding (Bi and Bii). For JNK3-specific binding partners, deletion of N-terminal 38 amino acids of JNK3 (ΔN) as well as JNK3 RE mutant, ΔN-RE, and N-terminal 38 amino acids alone were tested to classify binding partners (Biii). Partners indicated in blue are those identified in the JNK1 screen, those in green identified in the JNK3, and those in red identified in both screens. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the requirement for a cluster of acidic residues in forming an additional interaction motif for their substrate-derived peptides [8,9,13,14]. Thus, the equivalent JNK1 residues D162, D326, and E331 may also contribute to a substrate interaction site conserved across the MAPK family [8,13].

JNK1 and JNK3 can share many protein substrates, acting redundantly as a consequence of their high sequence similarity [14]. However, the distinctive N-terminal 38 amino acid extension of JNK3 may mediate JNK3-specific interactions [15]. Indeed, site-directed mutagenesis targeting the JNK3 N-terminus indicated a critical requirement for a number of hydrophobic residues for interaction with β-arrestin 2 [15] and so the distinctive JNK3 N-terminus can act as a binding platform. In this study, we addressed the possibility that additional JNK3-specific binding partners could be identified and recruited via this unique N-terminus. Thus, we defined a JNK3 interactome by interaction screening using JNK3α1 as bait in the yeast two-hybrid system. Our counterscreening with JNK1α1 and a number of JNK mutants revealed binding partners predominantly shared by these JNK isoforms, but also three JNK3-specific protein partners. Whilst the JNK3 N-terminus appeared to mediate interaction with these JNK3-specific binding partners, this region may also prevent the recruitment of JNK1-specific JBD-dependent partners. Thus, the JNK3 N-terminus plays at least two important roles in defining the JNK3 interactome.

2. Materials and methods

2.1. JNK mutant expression constructs for use in the yeast two-hybrid system

pGBKT7-JNK1α1 or pGBKT7-JNK3α1 bait constructs were prepared for the yeast two-hybrid system, then mutants created by site-directed mutagenesis (primers in Table S1): JNK1/3 CD mutants [9] were created as JNK1α1 R127/E329A (**JNK1 RE** mutant) or JNK3α1 R165A/E367A (**JNK3 RE** mutant), an extended JNK1 CD mutant JNK1α1 D162T/D326N/E331N was created to parallel mutations made in related MAPKs [8,13] (**JNK1 DDE** mutant), and a combination JNK1α1 mutant R127A/D162T/D326N/E329A/E331N (**JNK1 RDDEE** mutant) was also created. Additional JNK3 mutants were made to explore the contributions by the N-terminus and the CD domain: an N-terminal truncated form (*i.e.* JNK3(39–422); **ΔN JNK3**), the first 38 amino acids of

JNK3 (**JNK3 N**), and a mutant combining the RE mutation and N-terminus truncation, JNK3(39–422) R165A/E367A (**ΔN JNK3 RE** mutant).

2.2. Partner/prey deletion mutant constructs for validation/analysis in the yeast two-hybrid system

The pGADT7 prey vector (Clontech) was modified to contain inserts of JNK partners, JIP1(127–218), WDR62(968–1523), or c-Jun(1–135). To study the contributions by potential JBD sequences to interactions with JNK1/JNK3, ATF7(1–411) mutants were created using primers summarized in Table S2. For ATF7, one putative JBD (JBD1) is comparable to that characterized in ATF2 [16,17], and another (JBD2) was predicted by sequence similarity. Thus, ATF7(1–411)Δ26–40 (*i.e.* **ATF7ΔJBD1** mutant), ATF7(1–411)Δ151–165 (*i.e.* **ATF7ΔJBD2** mutant), and ATF7(1–411)Δ26–40/151–165 (*i.e.* **ATF7Δ2JBDs** mutant) were created. For evaluation of the JBD contributions for JNK interaction with AKAP6(372–782), the following truncation mutants were created: AKAP6(372–635), AKAP6(372–521), AKAP6(372–431), AKAP6(648–782), AKAP6(636–647), AKAP6(560–568) and AKAP6(434–441) using primers summarized in Table S3.

2.3. Yeast two-hybrid analyses

Yeast two-hybrid analyses, using the JNK1 or JNK3 baits described above and expressed in the MATa (AH109) yeast strain cells, were performed in the Clontech Matchmaker system according to the manufacturer's instructions for screening a human fetal brain cDNA library (Clontech) (Fig. S1A) and directed detection of protein–protein interactions (Fig. S1B). Trp/Leu drop-out (double drop-out (DDO)) plates were used to pick diploids (3–4 days, 30 °C). Interactions of bait and prey constructs were then tested in Trp/Leu/His/Ade drop-out (quadra drop-out (QDO)) plates (3–4 days at 30 °C). For quantitative assessments of interactions, diploids on DDO plates were subject to liquid β-galactosidase assays using Chlorophenol red-β-D-galactopyranoside (Sigma) as substrate with monitoring at 595 nm.

2.4. Novel JNK-binding partner co-immunoprecipitation

Ad293 cells were maintained in growth medium (DMEM containing 10% (v/v) fetal calf serum and 1% (v/v) Penicillin–Streptomycin, then transfected (Lipofectamine 2000) with mixtures of expression plasmids to evaluate JNK1 interaction with AKAP6: (pXJ40-myc-JNK1 (1 μg), pEGFPN3-AKAP6 or variants (ΔJBDs, R/KA, 3LA, and R/K3LA) (1 μg) (Table S4), or the interaction of JNK3 with ATXN1: pXJ40-myc JNK3 (6 μg) or pXJ40-myc-JNK3ΔN (2 μg) and pEGFPC1-ATXN1 (1 μg). Cells were returned to growth medium 4 h after transfection and grown for a further 24 h. Where indicated, cells were treated with arsenite (0.3 mM, 1 h), or were left untreated as a control.

Cells were lysed in buffer (50 mM Tris–HCl, pH 7.3, 150 mM NaCl, 1% sodium deoxycholate, 0.1 mM EDTA, 1% Triton X-100, 0.2% sodium fluoride and 100 μM sodium orthovanadate containing protease inhibitors (Roche)) then subjected to immunoprecipitation with myc-specific antibody/protein A-agarose (Santa Cruz sc-40/Pierce) or GFP-Trap (ChromoTek). The resulting pellets were extensively washed with buffer, then the precipitated complexes subjected to immunoblotting with antibodies to myc (Santa Cruz sc-789), AKAP6 (Abcam 24639), or GFP (Invitrogen).

3. Results and discussion

3.1. Yeast two-hybrid counterscreening defines novel JNK1-specific, JNK1/JNK3-shared, and JNK3-specfic partners

We began with a validation of the yeast two-hybrid system for the study of JNK interaction partners by demonstrating a robust interaction of both JNK1 and JNK3 with the known JNK pathway scaffold JIP1 [18], the WDR62 protein [19], and the substrate c-Jun [20] (Fig. 1Ai). Furthermore, the mutation of common docking (CD) site residues JNK1 R127 and E329 was sufficient to disrupt these interactions with JNK1 (Fig. 1A) as expected from previous studies showing the importance of these residues in mediating interactions between JNKs and its substrate/scaffold proteins [9,19]. Conversely, disruption of residues JNK1 D162, D326, and E331 did not disrupt JNK1–JIP1 interaction consistent with the structural information from the analysis of the JNK1–JIP1 peptide complex [9,11,14], but this mutation did disrupt JNK1–WDR62 and JNK1–Jun interactions, emphasizing the possibility that a more extended JNK1 surface comparable to that identified for the related ERK MAPKs makes important contributions to the interactions with these partners [8,13]. Quantitative analysis of binding strength using a liquid β-galactosidase assay further confirmed these observations (Fig. 1Aii).

To identify protein partners specific to each JNK isoform, we performed parallel yeast two-hybrid screens of a fetal human brain cDNA library with either JNK1α1 or JNK3α1 as bait according to the overview presented in Fig. S1. For each bait, >10 million transformants were analyzed. Sequence analysis followed by database searching revealed that 145 positive clones matched 69 different proteins in the screening for JNK1-interacting partners (Table S5), whereas 96 positive clones in the screening for JNK3-interacting partners matched 36 different proteins (Table S6). A number of these proteins have been reported previously as either direct interacting partners of JNKs (e.g. BMPR2 [21], MAPBP1 [22]) or subject to JNK-dependent regulation (*e.g.* ATF7 [17], MAP1B [23]). Analysis of prey sequences for the JBD consensus sequence $[R/K]_{1-3}X_{1-6}$-[LIVFM]X[LIVFM] revealed that most preys, but not NNAT, POMP

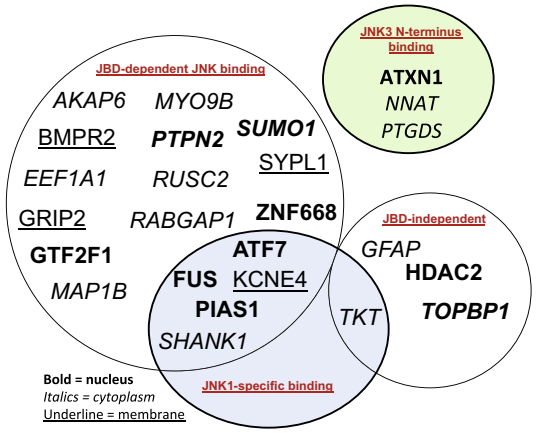

Fig. 2. Schematic summary of the categories of JNK1- and JNK3-binding partners. Counterscreening in the yeast two-hybrid system allowed further categorization of identified partners. JBD-dependent JNK-binding partners were those with interactions disrupted by the JNK1 RDDEE mutation whereas JBD-independent partners continued to interact with the JNK1 RDDEE mutant. A group of JNK3-specific binding partners dependent on the unique JNK3 N-terminus was identified (green circle) as well as a number of JNK1-specific partners (blue circle). Notably, the binding partners have been annotated previously as showing localization to the nucleus (bold), cytoplasm (italics) and plasma membrane (underlined). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

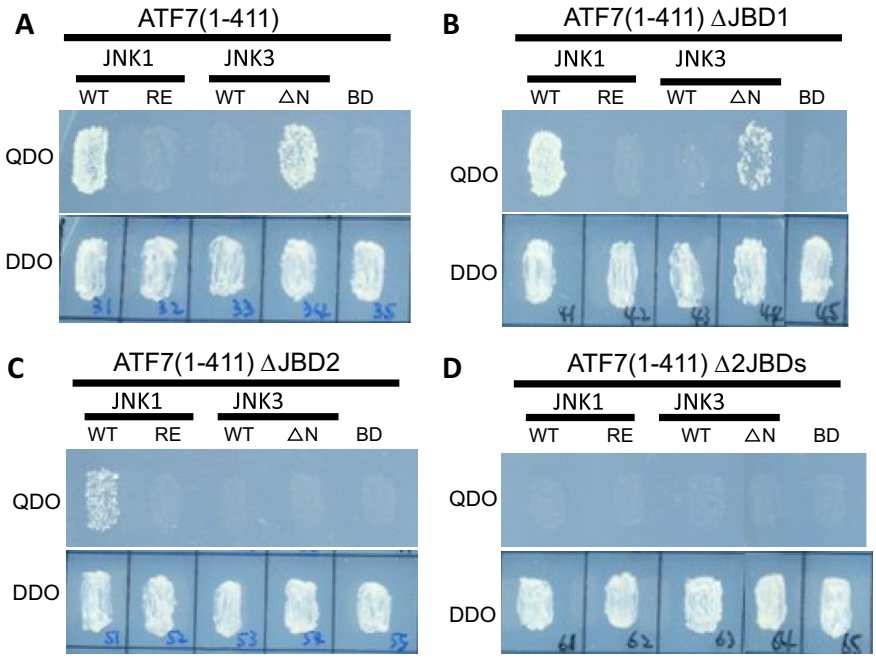

Fig. 3. Evaluation of ATF7 as a JNK1-specific, JBD-dependent interacting protein. In the ATF7 sequence (NM_006856.2), two sequences conform to JBD predictions: residues 26–40 (JBD1) and 151–165 (JBD2). Yeast containing (A). ATF7(1–411), (B). ATF7(1–411)Δ26–40 (ATF7ΔJBD1), (C) ATF7(1–411)Δ151–165 (ATF7ΔJBD2), or (D) ATF7Δ26–40/151–165 (ATF7Δ2JBDs), were mated with yeast expressing JNK variants or the empty bait (BD) vector as indicated. The DDO plates were used to demonstrate mating yeast whereas QDO plates, which contained the yeast stamp replicated from the DDO plate, showed growth as an indication of bait-prey protein interaction. All yeast shown were grown on the same plate, and images obtained on the same day.

and PTGDS from the JNK3 screen, contained at least one putative JBD, suggesting an alternative binding mode by JNK3 that warranted further investigation.

To extend the results from the parallel JNK1α1 or JNK3α1 screens, a counterscreening protocol was performed for 25 randomly selected interacting proteins to define JNK1-specific partners, JNK3-specific partners or JNK1/JNK3-shared partners, *i.e.* partners identified in the JNK1 screen were directly tested for interaction with JNK3, and vice versa. The identities of these 25 proteins, as well as the growth characteristics typical of their interactions as prey proteins with JNK1 and/or JNK3 bait proteins on QDO plates, are shown in Fig. 1B. This counterscreening defined the largest population of binding partners as shared by both JNK1 and JNK3 (16/25 proteins tested, Fig. 1Bi), but also revealed a smaller number of JNK1-specific (6/25, Fig. 1Bii) or JNK3-specific binding partners (3/25, Fig. 1Biii). A survey of the curated cellular distributions of these JNK1- and/or JNK3-binding partners suggested they would be classified as cytosolic proteins (11/25), nuclear proteins (7/25), nuclear-cytoplasmic proteins (3/25) or plasma membrane proteins (4/25), reinforcing the roles played by JNKs cross multiple intracellular locations (Fig. 2). Further classification of these 25 JNK-interacting proteins by gene list analysis (http://www.pantherdb.org/) for their reported functions showed these to be dominated by proteins having catalytic activities (10/25) as well as protein- and nucleic acid-binding (10/25).

3.2. Direct interaction testing with JNK mutants defines modes of interaction with JNKs

To define the patterns of interactions, we extended our protocol by direct interaction testing with JNK mutants. Specifically, binding domain-defective JNK1 and/or JNK3 mutants were tested for interaction with each of the 25 proteins from the counterscreen. This analysis with JNK1 RE, DDE, and RDDEE mutants further confirmed the complexity of interactions for JNK1-specific or JNK1/JNK3-shared partners (Fig. 1B). For most partners, the JNK1 RE mutant was sufficient to disrupt the interaction, but there were exceptions, such as FUS (JNK1-specific, Fig. 1Bii) or GRIP2, GTF2F1, PTPN2, RABGAP1, SUMO1 and ZNF668 (JNK1/JNK3-shared partners, Fig. 1Bi) that required the combined 5 site mutation to disrupt binding. Thus, as summarized in Fig. 2, across the population of JNK binding partners evaluated in this manner, 72% (*i.e.* 18/25 proteins tested) interactions were disrupted by the JNK RE mutant and/or combined RDDEE mutant. Thus, in agreement with previous observations that most JNK binding partners are JBD-dependent [reviewed by [24]], this JNK interactome analysis also showed predominantly JBD-dependent binding. As the multisite JNK1 RDDEE mutant retained binding with some JNK1-specific (TKT) or JNK1/JNK3-shared (GFAP, HDAC2 and TOPBP1) partners, this suggests that different residues in the expressed JNK1 RDDEE mutant mediate its interactions with these partners.

In addition to the classical JBD-dependent interaction pattern, three proteins including ATXN1, NNAT, and PTGDS were classified as JNK3-specific binding partners (Figs. 1Biii and 2). Not only was interaction no longer observed for the ΔN JNK3 mutant, but interaction was retained for a JNK3 CD mutant (JNK3 R165A/E367A mutant) or the 38 amino acid JNK3 N-terminus (Fig. 1Biii). A further, but unexpected, observation was that some JBD-dependent binding partners such as ATF7 were classified as JNK1-specific partners despite the similarity in the interacting residues in both JNK1 and JNK3, and this is further considered in the following section.

3.3. Analysis of ATF7 as an example JNK1-specific binding partner

The JNK1/JNK3 CD site sequence identities suggest that JNK1-binding proteins would also interact with JNK3. The ATF7(1–411) prey construct was identified in the screen for JNK1 interactors, but cross-screening unexpectedly showed no interaction with JNK3. ATF7 was therefore examined further as a JNK1-specific

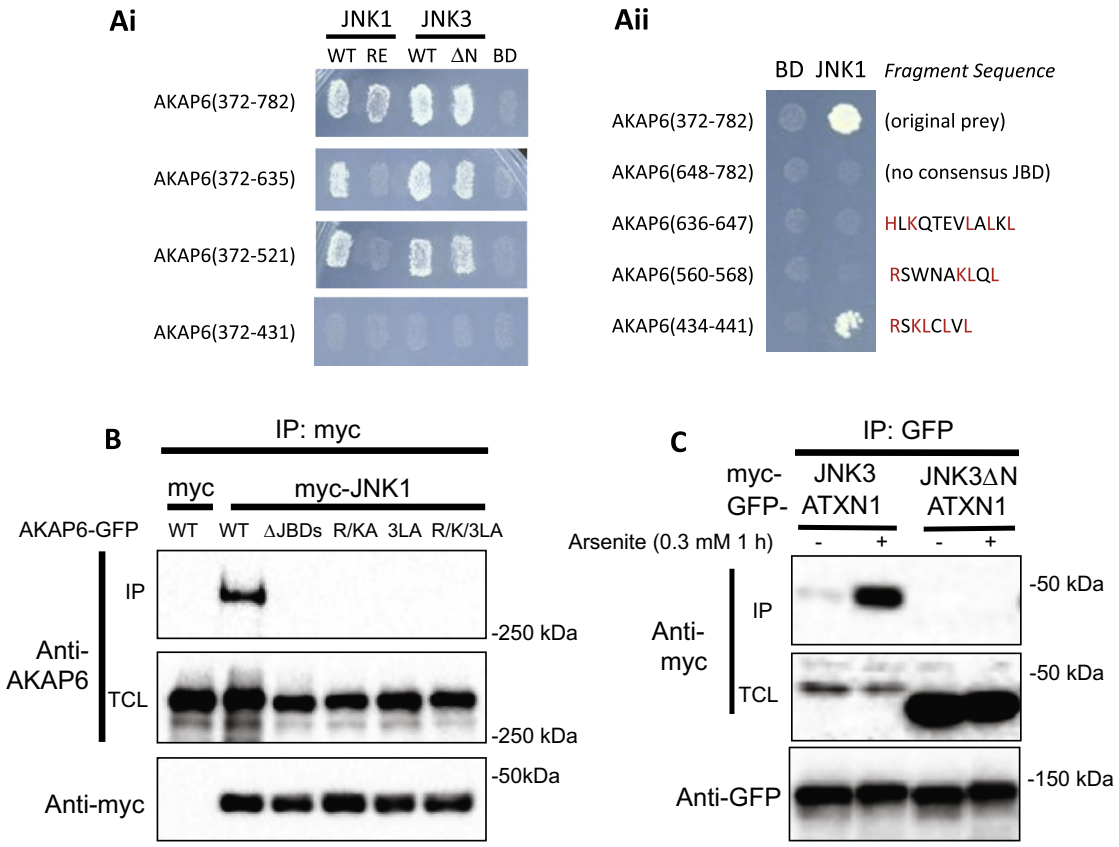

Fig. 4. Evaluation of AKAP6 as JNK1/JNK3-shared interacting partner but ATXN1 as a JNK3-specific interacting partner. (A, B) Evaluation of JNK–AKAP6 interaction. In the isolated AKAP6 sequence, AKAP6(372–782), five potential JBDs were predicted. (A) Yeast containing further C-terminal truncations of AKAP6 (Ai) or smaller AKAP6 sequences (Aii) were mated with yeast expressing JNK variants or the empty bait (BD) vector as indicated. DDO plates (data not shown) were used to demonstrate mating whereas QDO plates replicated from the DDO plates showed growth as an indication of bait-prey protein interaction. All yeast shown were grown on the same plate, and images obtained on the same day. (B) JBD-targeted mutation disrupts AKAP6–JNK1 interaction in mammalian cells. Ad293 cells were transfected to express myc-vector or myc-JNK1 with AKAP6-GFP or AKAP6 mutants as indicated. Total cell lysates (TCL) were prepared 16 h following transfection followed by immunoprecipitation (IP) with a myc-specific antibody, then immunoblotting with myc- and AKAP6-antibodies. (C) ATXN1–JNK3 interaction in mammalian cells requires the JNK3 N-terminus. Ad293 cells were transfected to express myc-JNK3 or myc-ΔN JNK3 and GFP-ATXN1, then treated with arsenite as indicated. TCL were prepared 16 h following transfection followed by immunoprecipitation (IP) with a myc-specific antibody, then immunoblotting with myc- and GFP-antibodies.

interacting partner. Importantly, no interaction was observed for the ATF7 prey and the JNK1 RE mutant, indicating a JBD-dependent ATF7–JNK1 interaction (Fig. 3A). Furthermore, whilst no interaction was detected for ATF7 and JNK3 constructs, the ΔN JNK3 mutant did show interaction with ATF7 (Fig. 3A), suggesting that the JNK3 N-terminus can serve to exclude some JBD-dependent binding partners.

ATF7 is highly related to the ATF2 transcription factor which is a classical JBD-dependent JNK substrate [25]. The ATF2 JBD corresponds to residues 45-V**HKHKH**E**M**T**L**K**F**GPA-59 (JBD consensus motif in bold). Although ATF7 shows an identical sequence 26-V**HKHKH**E**M**T**L**K**F**GPA-40 (known as JBD1), previous *in vitro* studies indicated the JNK-mediated phosphorylation of an N-terminal truncated ATF7 mutant lacking JBD1 [17], thus suggesting another JBD can direct JNK-mediated phosphorylation. As ATF7 151-V**R**PGS**L**P**L**H**L**GYDPL-165 also follows the expected JBD consensus and is located within the region (100–180) thought to be responsible for JNK-mediated phosphorylation [17], this was denoted JBD2. Interaction analysis with a series of ATF7 deletion studies was therefore undertaken to explore the contributions by ATF7 JBD1 and JBD2 to interactions with JNK1 and JNK3. Notably, JNK1α1 continued to show interaction with ATF7(1–411)Δ26–40 (*i.e.* ATF7ΔJBD1) (Fig. 3B). ATF7(1–411)Δ151–165 (*i.e.* ATF7ΔJBD2) showed weaker interaction with JNK1 but no detectable interaction with ΔN JNK3 (Fig. 3C). To explore whether these two JBDs together contribute to the total interaction with JNK, the double deletion mutant ATF7(1–411)Δ26–40/151–165 (*i.e.* ATF7Δ2JBDs) was tested and, as this did not interact with any of the JNK variants (Fig. 3D), these results were consistent with a requirement for both JBDs to mediate maximal interaction with JNK1 but with JBD2 directing the interaction with ΔN JNK3.

3.4. AKAP6 and ATXN1 as example JNK1/JNK3-shared and JNK3-specific partners

AKAP6 belongs to the A Kinase Anchor Protein family of scaffold proteins [26], and an AKAP6 fragment corresponding to residues 372–782 that was identified in the JNK1 screen was shown to be a JNK1/JNK3-shared partner (Fig. 1Bi). Multiple sequences conforming to the JBD consensus were determined within this fragment, prompting evaluation of their contributions to the observed interaction. Thus, of 3 tested truncations, AKAP6(372–431) no longer showed interactions with JNK1 and JNK3 (Fig. 4Ai). Furthermore, testing of AKAP6(434-R**S**K**L**C**L**V**L**-441) showing high similarity to the previously characterized JBD of NFAT4(146-R**D**H**L**Y**L**P**L**-152) [11], indicated this was sufficient for interaction (Fig. 4Aii). A multiple JBD deletion mutant AKAP6Δ393–445/635–651 and AKAP6 multi-site alanine-substitution JBD mutants were therefore tested for JNK1 interaction by co-

immunoprecipitation, these results reinforcing the importance of AKAP6434-**RSKLCLVL**-441 in mediating JNK interaction (Fig. 4B).

Lastly, ATXN1 is an RNA-binding protein that has been implicated in the disease progression of the ataxia SCA1 [27]. As the interaction between ATXN1 and JNK3 appeared to be JBD-independent (Fig. 1Biii), the interaction was also further evaluated by co-immunoprecipitation. Thus, myc-JNK3 and GFP-ATXN1 were co-expressed, ATXN1 was immunoprecipitated using GFP-Trap beads, and its association with JNK3 was detected following cell exposure to arsenite (Fig. 4C), pointing to the essential require for stress in allowing this interaction in a mammalian cell context. Furthermore, no interaction was observed between ΔN JNK3 and ATXN1 under either control or stress conditions (Fig. 4C), consistent with the requirement for the JNK3 N-terminus in mediating this interaction.

Taken together, these studies reinforce the importance of the JNK CD site in mediating interactions with most, but not all, protein partners. Further analysis should thus be directed to defining at a molecular level how the JNK3 N-terminus can serve as an interaction platform, but also how this 38 amino acid sequence can prevent JNK3 interactions with some protein partners characterized as JNK1-specific. Notably, these isoform specific differences in protein partners begin to provide a molecular explanation for the differences in JNK1 and JNK3 functions across a range of physiological and pathophysiological settings.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.09.122.

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