JNK signaling: regulation and functions based on complex protein-protein partnerships

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Supplementary File containing:

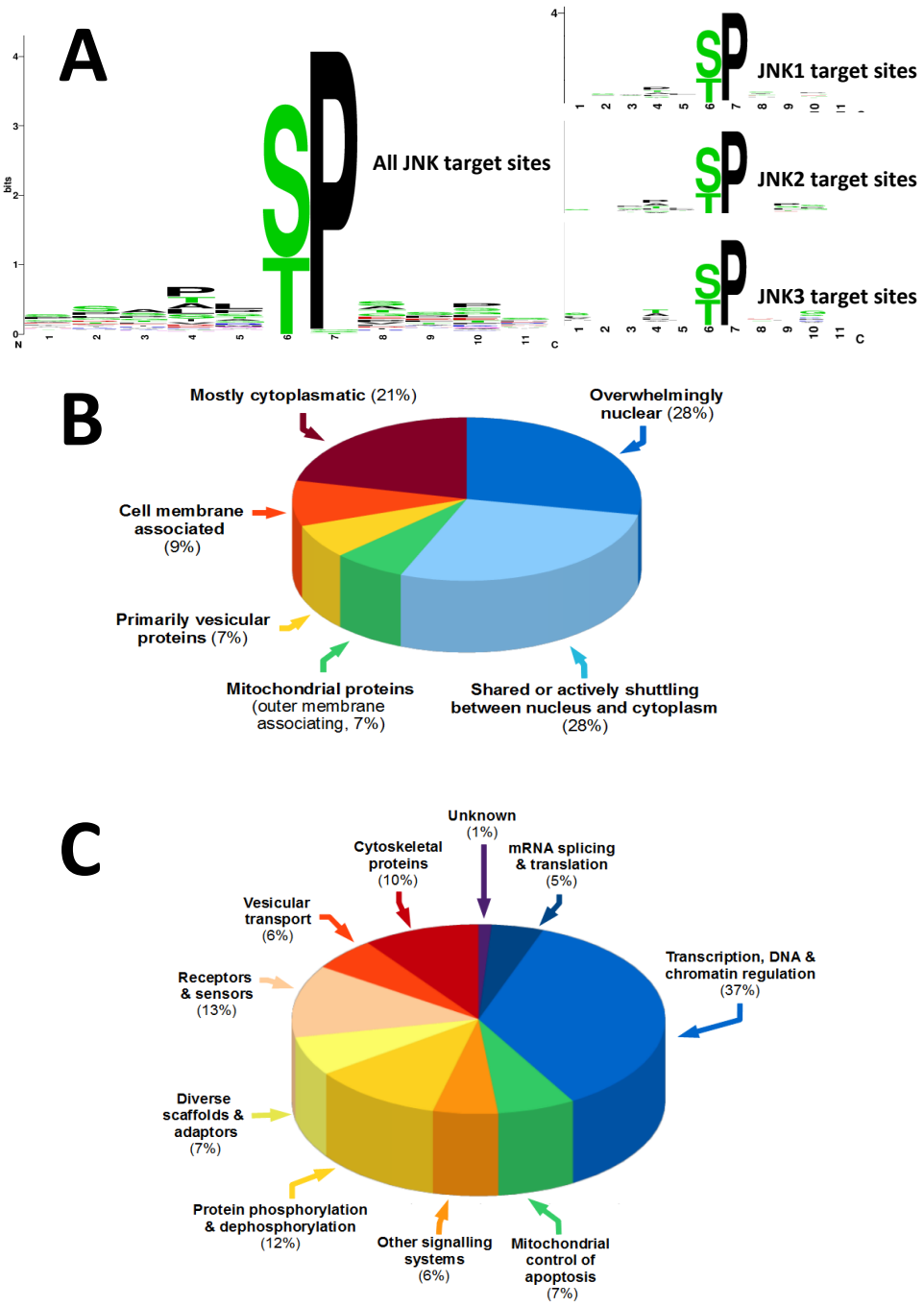

FIGURE S1: Overview of JNK substrate proteins. A. Consensus sequence motif for JNK-mediated phosphorylation. Sequences surrounding confirmed JNK substrate sites were analysed *(i.e.* the sequences encompassing 5 amino acids N-terminal of the targeted Ser/Thr residue followed by the 5 C-terminal amino acids were included for analysis). The analysis included 176 phosphorylation sites in 89 different confirmed well-validated substrate proteins (see **Table 1** for details of which proteins were considered as well-validated substrates so that their JNK-targeted phosphorylation sites were included in this analysis). Where possible, the sites identified for the different JNK isoform, JNK1, JNK2 or JNK3, were also assessed. 62 of the JNK-targeted phospho-sites (from 23 proteins) co-occur with known JNK docking motifs (see **Table 1** for further details). The ratio of known JNK target sites per protein is 1.98 for all JNK substrates (*i.e.* 176 sites in 89 proteins), 2.70 for proteins possessing a known JNK binding D-motif (*i.e.* 62 sites in 23 proteins), and only 1.73 for proteins devoid of known D-motifs (*i.e.* 114 sites in 66 proteins). **B.** Subcellular distribution of confirmed JNK substrates**.** The majority of confirmed JNK substrates either shuttle between nucleus and cytoplasm (28%) or are located in the nucleus (28%) or the cytoplasm (21%). 9% are cell membrane associated, 7% are associated with the mitochondrial outer membrane, and 5% are primarily vesicular proteins. **C.** Functional classification of confirmed JNK substrates. The majority of confirmed JNK substrates are involved in transcription/DNA/ chromatin regulation (37%); other functional groups include receptors and signaling sensor proteins (13%), proteins directly involved in protein phosphorylation/dephosphorylation (12%), and cytoskeleton proteins (10%).

Table S1. Critical assessment of scaffold proteins implicated in the mammalian JNK pathway

Name and primary function of proposed scaffold	Evidence for binding of multiple JNK pathway members	Mapping of binding sites	Enhancement of JNK activation	Mechanism of JNK pathway enhancement	Selected references
JIP1 *vesicular transport adaptor*	Yes (MLK3/DLK, MKK7, JNK1) (I)	Yes, but some sites may overlap	Yes (in cells only)	Explored (transport based with some details unclear, see main text)	(1)
JIP3 *probable Rho-GEF protein*	Yes (ASK1, MKK4, JNK1)	Yes, except for MKK4	Yes (in cells only)	Partly explored (either small GTPase based feedback or shared with JIP1, see main text)	(2)
Beta arrestins *receptor endocytosis adaptors*	Yes (ASK1, JNK3, do not seem to bind MKK4 directly) (II)	Yes, but some sites may overlap	Yes (Arrestin β3)	Not addressed	(3, 4)
Filamin A & Filamin B *actin crosslinking proteins*	Yes (Rac1, MEKK1, MKK4, JNK1) for Filamin B only	Yes, but problematic due to binding site overlaps (IV)	Yes (in cells only)	Not addressed	(5, 6)
RACK1 *ribosomal protein (a 40S subunit component)*	Incomplete (JNK1/2 and PKC only)	Yes, but in conflict with structure (V)	Yes (in cells only)	Explored (VIII) (but in conflict with structure)	(7)
Crk (isoform II) *SH2/SH3 adaptor protein*	Incomplete (JNK, but others are indirect only)	Yes, but in conflict with structure (VI)	Yes (in cells only)	Not addressed	(8)
POSH *E3 ubiquitin ligase (RING type)*	Questionable (III) (strong binder: MLK2, MLK3, DLK, weak binder: MKK4,	Not addressed	Yes (in cells only)	Not addressed	(9-11)

Name and primary function of proposed scaffold	Evidence for binding of multiple JNK pathway members	Mapping of binding sites	Enhancement of JNK activation	Mechanism of JNK pathway enhancement	Selected references
	MKK7, JNK2)				
WDR62 *microtubule-binding protein*	Yes (MKK7: MKK7-β1 isoform only, JNK1, JNK2)	Yes	Not addressed	Not addressed	(12)
DUSP19 *dual specificity phosphatase*	Yes (MKK7, JNK2)	Yes, but in conflict with structure (VII)	Not addressed	Not addressed	(13)
GRASP1 *ion channel anchoring protein*	Yes (MEKK1, JNK1)	Yest	Yes (in cells only)	Not addressed	(14)

(I) *JIP1 has been reported to binding several other signalling components including Notch1 (15), the Ser/Thr kinase Akt1 (16, 17), the Tyr kinase c-Abl (18), the Src family of Tyr kinases (19, 20) and the Ras-like small G-protein RalA and its GTP exchange factor RLF (21) .*

(II) *Beta-arrestins interact with the N-terminus of JNK3 (long isoform only), and also with ASK1, however the relation of these binding sites (on beta arrestin) is obscure: comparable binding was seen for all kinases with both the N-terminal and the C-terminal arrestin domains. The experiments of Song et al. might even suggest competition between ASK1 and JNK3 (3). No direct interaction was found with MKK4: this kinase was shown to be recruited indirectly, through the D-site of JNK3.*

(III) *POSH interacted with MAP3Ks directly, but not with other components of the JNK pathway. The latter interactions were only detectable in cells, but not in pull-downs: thus, they were likely indirect.*

(IV) *MEKK1, MKK4 and JNK all appeared to bind to largely overlapping site(s) on filamin B. However, the constructs created for these mapping studies would structurally disrupt the rod domain (repeats 16-23) of filamin B, so the conclusions drawn from these studies appear problematic. Furthermore, GTP-Rac1 is probably not a direct regulator of MEKK1 activity (22).*

(V) *The mapping of binding sites was problematic, since all WD40 repeats of RACK1 would normally fold together to form a single domain. Most known WD40 domains only have a single partner binding site, in the center of the WD40 domain "wheel" (the complete, closed solenoid domain) (23).*

(VI) *CrkII was proposed to bind to a segment of the JNK1 kinase domain that is normally inaccessible, as most of its side chains forming the SH3 binding motif consensus are buried under native conditions.*
(VII) *Interactions were not mapped on DUSP19, only on MKK7. However, the latter bound very weakly as an intact protein; strong bindings were only obtained with fragments where the N-terminal lobe of the kinase domain was disrupted.*

(VIII) *The authors proposed a novel pathway in which PKC-dependent phosphorylation would activate JNKs. Because the site S129 is deeply buried in the kinase domain of JNK1, S129 phosphorylation appears unlikely in its native state whereas the mutation S129A likely disrupts JNK folding and enzymatic activity.*

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Table S2. Sequences of mutually exclusive exons for the human JNK genes
The different amino acids in the corresponding exons of the same gene are underlined. The *α* and *β* splice variants can be obtained by exchanging the shown sequences.

Protein (gene) name	**Exon 6a** - amino acid sequence	**Exon 6b** - amino acid sequence
JNK1 (MAPK8)	VDLWSVGCIMGEMVCHKILFPGRDY	VDIWSWGCIMGEMIKGGVLFPGTDH
JNK2 (MAPK9) *	VDIWSVGCIMAEMVLHKVLFPGRDY	VDIWSVGCIMGELVKGCVIFQGTDH
JNK3 (MAPK10)**	VDIWSVGCIMGEMVRHKILFPGRDY	VDMWSVGCIMGEMIKGAVLFPGTDH

** In the case of JNK2, the names of the exon 6a- and 6b-incorporating isoforms are swapped due to historic reasons (i.e. exon 6a in the β isoforms and exon 6b in the α isoforms)*
*** The sequence of exon 6b-incorporating JNK3 proteins (JNK3 β-isoforms) is based on mRNA sequences retrieved from ENSEMBL and NCBI and not featured in protein sequence databases (such as SwissProt/UniProt).*