

**DEVELOPMENT AND USE OF A PEPTIDE INHIBITOR OF THE
C-JUN N-TERMINAL KINASE (JNK) SUBFAMILY OF
MITOGEN-ACTIVATED PROTEIN KINASES**

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The c-Jun N-terminal kinases (JNKs) comprise one subfamily of Serine/Threonine protein kinases within the Mitogen-Activated Protein Kinase (MAPK) family. Traditionally characterised by their activation by extracellular stresses such as UV irradiation or osmotic stress, JNKs have now been shown to be activated following the exposure of cells to a variety of growth factors and cytokines. Whilst the signalling pathways leading to JNK activation have been extensively studied, confusion remains as to how JNKs can effectively mediate apparently opposing cellular events. For example, is it possible that JNKs can mediate both cell proliferation and cell differentiation? Alternatively, how can JNKs be involved in both cell survival and cell death?

To begin to address these questions, we have looked to known JNK-interacting proteins for potential motifs that may mediate interaction and inhibition of JNK. In this way, we have aimed to exploit short peptide sequences as highly-specific inhibitors of the JNK MAPK subfamily.

We have found one such sequence of 11 amino acids within the JNK scaffold protein, JIP-1. This peptide, that we now call TI-JIP, has the sequence RPKRPTTLNLF and thus conforms to the consensus sequence R/K-X₄-A-X-B-X (where A and B are hydrophobic residues) found in a number of upstream activators, scaffold proteins, and substrates of the MAPK cascades [1]. Alanine-scanning replacement within the TI-JIP sequence has highlighted the critical importance of the conserved amino acids of this consensus sequence to the JNK inhibitory activity of TI-JIP. Importantly, we have shown that TI-JIP does not inhibit either ERK or p38 MAPKs, and thus is a specific inhibitor of the JNKs.

Ongoing research in our laboratory is taking a number of approaches to further characterise the significance of the TI-JIP interaction with JNK.

First, the coupling of TI-JIP to the TAT-protein-transduction domain, is allowing intracellular delivery of TI-JIP. In this way, we can assess the biological efficacy of this peptide inhibitor. We have found that TAT-TI-JIP at micromolar concentrations is effective in preventing apoptotic cell death following simulated ischemia/reperfusion damage of neurones in primary culture.

In parallel, we are interested in the molecular determinants of the interaction of TI-JIP with JNK. With the recent co-crystallisation of related MAPKs complexed with small peptides [2], we can now begin to model the interaction of TI-JIP with JNK. This allows predictions on the nature of the specificity of interaction with the potential to reveal ways to improve the interaction between TI-JIP and JNK with the view to produce an improved peptide inhibitor.

We are now mapping experimentally the sites within the JNK protein structure required for interaction with TI-JIP. This has required the use of a yeast two-hybrid screening strategy. First, in forward screening protocols we have demonstrated that TI-JIP and JNK1 interact. Then we have produced a mutant JNK1 library through PCR mutagenesis. Using a reverse two-hybrid yeast approach we have screened for JNK mutants that failed to interact with TI-JIP. Following validation of each non-interacting clone for expression of full-length JNK1, we have then performed sequence analysis.

Sequence analysis of seventeen non-interacting mutants expressing full-length JNK proteins has revealed changes to various regions of the JNK molecule. The mutant pool has therefore been revised to those containing five or less mutations to reduce noise, and this has revealed a series of mutational “hot-spots” on the JNK structure. We have constructed point mutants to address the importance of these regions and better define the TI-JIP-JNK binding interface. Specifically, after determining which of these single point mutations prevent the TI-JIP-JNK binding by our two-hybrid approach, we are then expressing these mutant JNK proteins in mammalian cells. We are now testing the activities and activation profiles of these mutant JNKs, as well as confirming their loss of inhibition by TI-JIP.

Our combined strategies should help clarify the mechanism by which TI-JIP inhibits JNK. This has the potential to highlight a novel region of JNK to target for drug design.

REFERENCES

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2. Chang, C., Xu, B., Akella, R., Cobb, M.H. and Goldsmith E.J. Crystal structures of MAP kinase p38 complexed to the docking sites on its nuclear substrate MEF2A and activator MKK3b. **Mol. Cell** 9 (2002) 1241-1249.