Recombinant Protein Expression and Purification

Mammalian cell expression of PIKK enzymes and enzyme complexes involved in DNA repair: Full-length DNA-PK-ATM and ATR/ATRIP complex were produced using a transient expression system in a mammalian cell line. A simple transfection and feed procedure was developed to permit expression of milligram amounts of recombinant protein from suspension cultures quantified by SDS-PAGE and with good recombinant protein yields, costs per milligram of purified protein are in-line with similar yielding chromatography as described above.

Insect cell expression of recombinant mTOR: Full-length and truncated recombinant mTOR proteins were generated using the BEVS in insect cells. SDI suspension cell cultures are routinely injected at 5 L and 10 L scales using bench-top bioreactors and the recombinant proteins purified by immuno-affinity chromatography as described above.

E.coli expression of PIKK substrate proteins: Two PIKK substrate proteins, full-length p53 and a truncated p73α698 kinase (mTOR substrate), were developed using an E.coli expression system in order to minimize phosphorylation of the recombinant protein products. FRET assays and western blots using specific anti-phospho-residue antibodies revealed that the purified substrate proteins were essentially unphosphorylated on the residues of interest and therefore suitable for PIKK activity assays.

Inhibitor Screening

The enzymes were tested against a panel of small molecules, comprising reported lipid and protein kinase inhibitors. The majority of classical protein kinase inhibitors were shown to have little effect, including the pan-kinase inhibitor staurosporine. This compound was also tested in a concentration response to confirm lack of inhibition (bottom left panel). The reported lipid kinase inhibitors showed a much greater hit frequency. Selected compounds from this set were tested in concentration responses. The panels above show selected data to highlight selectivity between ATM, ATR/ATRIP, and DNA-PK for different compounds. KU55933 has the highest potency towards ATM, while PK757 is selective for DNA-PK, and the ATR inhibitor has highest selectivity for ATR. PI-103 inhibits all the PIKKs tested here with a range of potencies.

Rapamycin is a specific inhibitor of mTOR, but requires the protein FKBP12 to function. By varying the amount of FKBP12 in the assay, the degree of inhibition by rapamycin can be controlled (upper left panel).

Summary

- The PIKK enzymes ATM, ATR/ATRIP, DNA-PK, and mTOR, key regulators of DNA damage and protein synthesis pathways, have been developed for commercial scale production.
- Milligram quantities of each enzyme and two physiological substrate proteins have been generated using inexpensive production processes and enzyme activities have been characterised using FRET based or radiometric assay systems.
- A compound profile for four PIKKs has been conducted using known protein and lipid kinase inhibitors.
- Compound selectivity between DNA-PK, ATR, and ATM has been demonstrated.