

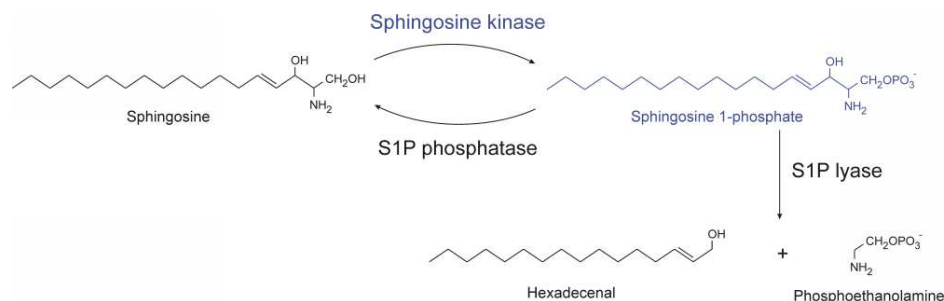
# Molecular Signalling Laboratory

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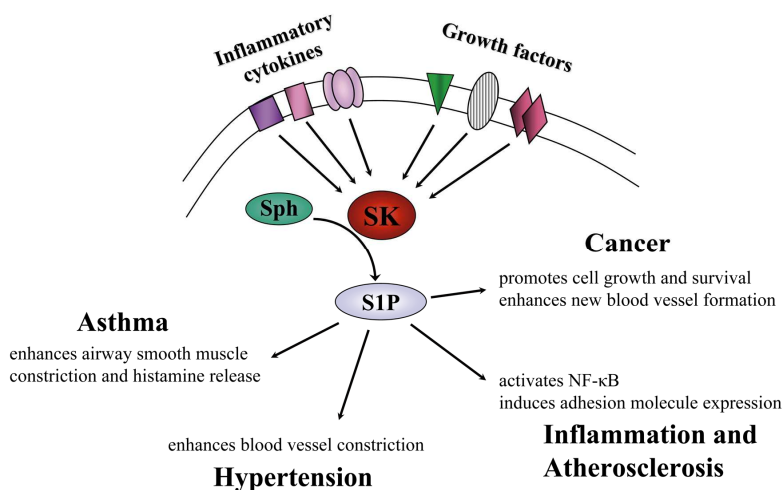
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The Molecular Signalling Laboratory examines sphingolipid-mediated cell signalling pathways, and how they contribute to cancer, inflammatory diseases, hypertension and other medical conditions. In particular, the enzyme sphingosine kinase (SK) is the primary focus of our work. This enzyme catalyses the formation of the phospholipid signalling molecule, sphingosine 1-phosphate.



Sphingosine 1-phosphate regulates a diverse range of cellular processes through its roles as both a ligand for a family of sphingosine 1-phosphate-specific cell surface receptors, as well as an intracellular second messenger. Of greatest interest to our laboratory are findings that elevated cellular SK (and sphingosine 1-phosphate) prevents programmed cell death (apoptosis), enhances cell proliferation, and leads to neoplastic cell transformation (cancerous cell growth). This indicates an oncogenic role for SK, which is further supported by recent data showing elevated SK in a variety of human cancer cells and inhibition of tumor growth *in vivo* by SK inhibitors.

In addition to this role in tumorigenesis, SK and sphingosine 1-phosphate appear central players in many other cellular processes, including; vascular endothelial cell activation, a hallmark of inflammatory diseases; enhancing blood vessel construction, and; enhancing constriction of airway smooth muscle cells. Thus, SK is also a potential target for therapeutic intervention in inflammation and atherosclerosis, hypertension and asthma.



Current work in this laboratory is concentrated on understanding the biochemistry of SK, identifying the mechanisms regulating the activity and localisation of this enzyme, and on the (patho-)physiological functions of signal transduction pathways it controls. Understanding these factors may allow for the development of novel anti-SK therapeutics. Much of our work to date on

SK has focused on the post-translational regulation of this enzyme. SK is activated in cells in response to certain growth factors and other agonists. We have shown that activation of SK occurs through serine-225 phosphorylation which not only enhances its catalytic activity, but also results in its translocation to the plasma membrane. We have made a major breakthrough by demonstrating that this phosphorylation, and especially the subsequent translocation, mediates the oncogenic effects of SK. However, the mechanism(s) regulating the phosphorylation status of SK and its translocation are not known, and are one of the primary foci of our current studies.

### **Some recent and current projects carried out in this laboratory:**

#### **1. Identification of the nucleotide-binding site of sphingosine kinase**

Despite the importance of SK, very little is known regarding its structure or mechanism of catalysis. Moreover, SK does not contain recognisable catalytic or substrate binding sites, based on sequence motifs found in other kinases. We have elucidated the unique ATP-binding site of SK through a combination of site-directed mutagenesis and affinity labelling with an ATP analogue. The uniqueness of this ATP-binding site in SK finding raises the possibility of generating specific inhibitors of SK activity through targeting the nucleotide-binding site.

Pitson *et al.* (2002) The nucleotide-binding site of human sphingosine kinase 1. *J Biol Chem* **277**, 49545–49553.

#### **2. The catalytic and functional activation of sphingosine kinase 1 by phosphorylation**

We have identified that phosphorylation of SK at serine-225 by a member of the extracellular signal regulated protein kinase (ERK) family directly results in its activation. Strikingly, the oncogenic effects of SK are blocked by mutation of this phosphorylation site. This is despite this non-phosphorylatable mutant retaining full basal catalytic activity. More recently we have established that this single phosphorylation of SK not only directly increases its catalytic activity but also results in its translocation from the cytosol to the plasma membrane. Furthermore, we have shown that this phosphorylation-induced change in localisation of SK is critical in driving oncogenic signalling by this enzyme.

Pitson *et al.* (2003) Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. *EMBO J* **22**, 5491–5500.

Pitson *et al.* (2005) Phosphorylation-dependent translocation of sphingosine kinase to the plasma membrane drives its oncogenic signalling. *J Exp Med* **201**, 49–54.

#### **3. Identification of the calmodulin-binding site of sphingosine kinase**

We have examined the known Ca<sup>2+</sup>-dependent interaction of SK with calmodulin (CaM), and using a combination of limited proteolysis, peptide interaction analysis and site-directed mutagenesis, have identified the unique CaM-binding site of this enzyme. Furthermore, using the CaM-binding-deficient version of SK we have shown that this site is essential for translocation of SK from the cytoplasm to the plasma membrane. Since this translocation is a process that is important in cancer development, these studies have identified the interaction of SK with CaM as a potential target for anti-cancer therapies.

Sutherland *et al.* (2006) The calmodulin binding site of sphingosine kinase and its role in agonist-dependent translocation of sphingosine kinase 1 to the plasma membrane. *J Biol Chem* **281**, 11693–11701.

#### **4. Identification of a sphingosine kinase-interacting proteins that may play a role in sphingosine kinase 1 regulation**

We have identified several proteins that interact with SK through the use of a yeast two-hybrid screen. We are currently examining some of these SK-interacting proteins to establish their apparent roles in the regulation of SK activity and function.

**Other selected publications from this laboratory:**

- Pitson *et al.* (2000) Human sphingosine kinase: purification, molecular cloning and characterisation of the native and recombinant enzymes. *Biochem J* **350**, 429–441.
- Pitson *et al.* (2000) Expression of a catalytically inactive sphingosine kinase mutant blocks agonist-induced sphingosine kinase activation: a dominant-negative sphingosine kinase. *J Biol Chem* **275**, 33945–33950.
- Bolz *et al.* (2003) Sphingosine kinase modulates microvascular tone and myogenic responses through activation of RhoA/Rho kinase. *Circulation* **108**, 342–347.
- Thompson *et al.* (2005) Sphingosine kinase 1 (SK1) is recruited to nascent phagosomes in human macrophages. Inhibition of SK1 translocation by *Mycobacterium tuberculosis*. *J Immunol* **174**, 3551–3561.
- Wattenberg *et al.* (2006) The sphingosine and diacylglycerol kinase superfamily of signaling kinases: localization as a key to signaling function. *J Lipid Res* **47**, 1128–1139.
- Leclercq and Pitson (2006) Cellular signalling by sphingosine kinase and sphingosine 1-phosphate. *IUBMB Life* **58**, 467–472.
- Pébay *et al.* (2007) Stem cell regulation by lysophospholipids. *Prostaglandins & Other Lipid Mediators* **84**, 83–97.
- Leclercq *et al.* (2008) Eukaryotic elongation factor 1A interacts with sphingosine kinase and directly enhances its catalytic activity. *J Biol Chem* **283**, 9606–9614.

**Funding:**

National Health and Medical Research Council  
Cancer Council of South Australia  
Fay Fuller Foundation

**Available Student Projects:****1. The molecular mechanisms of sphingosine kinase regulation**

SK becomes rapidly and transiently activated in cells in response to growth factors and other regulatory agonists. This activation is critical in the signalling functions of this enzyme, and its dysregulation can lead to tumor formation. Thus, knowing how this activation occurs is important for understanding the function of this enzyme. As noted above, we have recently made a major advance in this area by establishing that phosphorylation of SK at serine-225 by a member of the ERK family directly results in its activation. Much is still not known, however, regarding how this phosphorylation is regulated, and whether other alternative regulatory mechanisms also control the activity and cellular location of this protein. Indeed, we have recently identified several proteins that interact with SK through the use of a yeast two-hybrid screen. We are currently examining some of these proteins to establish their possible roles in the regulation of SK activity and function.

**2. The cell signalling pathways controlled by sphingosine kinase**

SK is involved in the development of a number of disease states, including cancer, inflammation and atherosclerosis, asthma and hypertension. The exact mechanisms whereby SK exerts these effects, however, are relatively poorly understood. Thus, we are undertaking studies to better understand which cell signalling pathways are controlled by SK and its product, sphingosine 1-phosphate, and how this regulation is achieved. This involves both (i) biochemical studies to directly identify the specific intracellular targets regulated by SK and sphingosine 1-phosphate (ie. sphingosine 1-phosphate binding proteins), as well as (ii) microarray and phosphoprotein array studies to identify the broader pathways regulated by SK and its activation. For these latter experiments we have developed a large number of important molecular tools to definitively dissect the signalling pathways regulated by SK, its activation, and its agonist-induced translocation to the plasma membrane.