Sphingolipids are complex, ubiquitous lipids commonly believed to protect the cell surface against harmful environmental factors by forming a mechanically stable and chemically resistant outer leaflet of the plasma membrane lipid bilayer. Over the last decade relatively simple sphingolipid catabolites, such as ceramide and sphingosine-1-phosphate (S1P), have been identified as signal-transducing molecules involved in the regulation of cell growth, viability, mitosis, cytoskeletal rearrangement, immunity and angiogenesis in a variety of cells and tissues [1]. Ceramide and S1P are products of the membrane phospholipid sphingomyelin, and they appear to exert opposing biological actions; the sphingosine precursor ceramide is associated with cardiomyocyte apoptosis, whilst S1P, derived from phosphorylation of sphingosine by sphingosine kinases (SphK), may exert an anti-apoptotic effect. Activation of SphK is the final and rate-limiting step in the cellular synthesis of S1P, and accordingly is an important modulator of both ceramide and S1P levels. SphK exists as two isoforms which may have opposing roles; thus, activation of SphK1 appears to be protective, whereas SphK2 is considered to be pro-apoptotic [2]. Hence, the actions of S1P may in part be determined by the SphK directing its downstream action.

The actions of S1P are mediated either extracellularly, through a family of specific high-affinity G-protein-coupled receptors (SIP1-5), or intracellularly, where S1P functions as a direct second messenger. The diverse G-proteins that couple to S1P receptors, in combination with the extra- and intracellular modes of action, are likely to contribute to the various biological responses ascribed to S1P. However, considerable uncertainty remains as to the specific direct intracellular targets and the exact signaling pathways downstream of the individual S1P receptors.

In this issue of *Cardiovascular Research*, Jin et al. present additional evidence to show that SphK1 deficiency in the whole heart sensitizes the myocardium to ischemia/reperfusion (I/R) injury and abrogates ischemic preconditioning [3]. This group has already made important contributions towards unravelling some of the mechanisms and roles of sphingolipid signalling in the heart. In earlier studies they demonstrated that exogenous S1P is cardioprotective in cultured neonatal rat cardiomyocytes [4] and the isolated Langendorff-perfused mouse heart [5]. Subsequently, they showed that cardioprotection by ischemic preconditioning was associated with an increase in endogenous S1P levels, an effect blocked by the SphK inhibitor N,N'-dimethyl sphingosine (DMS) [6]. The current study is thus a logical extension of previous work.

Although the SphK-S1P pathway has been implicated in cell survival and ischemic preconditioning, experimental data to date has relied principally on pharmacological modulation with all the usual caveats regarding selectivity. The current manuscript provides the first insight using a model of global ischemia-reperfusion in Langendorff-perfused hearts to demonstrate significant increases in infarct size, creatine kinase release, cytochrome C content and apoptosis in SphK1-null, compared to wild-type, hearts. Detrimental effects on infarct size and left ventricular developed pressure (LVDP) were rescued by recomplementation with exogenous S1P. As expected, total SphK activity in the mutant hearts was significantly reduced but not absent; this corresponded with compensatory upregulation and expression of the proapoptotic SphK2 enzyme. To determine whether the increased susceptibility to ischemia-reperfusion injury in SphK1-null mice results from deficient SphK1 or increased SphK2, they used the SphK inhibitor DMS and were able to confirm that pretreatment at 3 μM decreased infarct size and increased LVDP.
recovery in null hearts such that they were indistinguishable from wild-type controls. The authors also report that ischemic preconditioning failed to protect null hearts, even when the index ischemia duration was reduced, implying an obligatory role of sphingolipid signaling. Akt phosphorylation was increased at baseline in the SphK1-null mice compared to wild type, and this decreased with ischemic preconditioning. Conversely, Akt phosphorylation was seen to increase in the preconditioned wild-type animals to the same value as the preconditioned SphK1-null mice.

Interpretation of these findings requires some caution. DMS, for example, is not isoform selective and the IC50 for wild type, and this decreased with ischemic preconditioning. Increased at baseline in the SphK1-null mice compared to index ischemia duration was reduced, implying an obligatory preconditioning failed to protect null hearts, even when the recovery in null hearts such that they were indistinguishable (demonstrates a biphasic effect whereby lower concentrations (~1 μM) activate SphK [8]). The increase in basal Akt phosphorylation seen in the SphK1-null mice is not explored further in this context, but may confound differences in infarct size and resistance to preconditioning between groups, independent of SphK activity, as chronically elevated levels of phosphorylated Akt have previously been associated with deleterious outcomes in I/R injury [9]. This contrasts with the acute increase in phosphorylated Akt seen in wild-type animals subjected to ischemic preconditioning, which is associated with protection.

The interplay between the SphK-S1P axis and more established preconditioning signaling pathways has yet to be fully elucidated. One such interaction is with PKCe, which is thought to be an obligate upstream mediator of SphK activation [5]. Downstream of this, intracellular S1P generated by SphK1 is exported from cardiomyocytes and binds to S1P receptors, exerting autocrine and paracrine effects [10]. The S1P3 receptor, in particular, has been identified as crucial to the protection mediated by HDL [11], a finding consistent with the high S1P content of this lipid subfraction. SphK activity is not only regulated through phosphorylation, but through association of a variety of interacting proteins, including platelet endothelial cell adhesion molecule (PECAM)-1, tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2), RPK118 and four and a half LIM domain 2 (FHL2). This latter protein has been shown to inhibit SphK1 activity, whereas siRNA-knockdown of FHL2 markedly increases SphK1 activity, protecting cardiomyocytes from apoptosis [12]. These observations provide further evidence for the pro-survival function of SphK1.

The potential therapeutic application of sphingolipid modulation is attractive but faces important hurdles at present, including the potential for arrhythmogenicity [13,14] and intracellular calcium overload [15]. Future strategies will therefore be guided not only by a better understanding of the SphK1-S1P signalling pathway, emphasised in the current manuscript [3], but also the identification and highly selective targeting of the relevant downstream kinases.

References