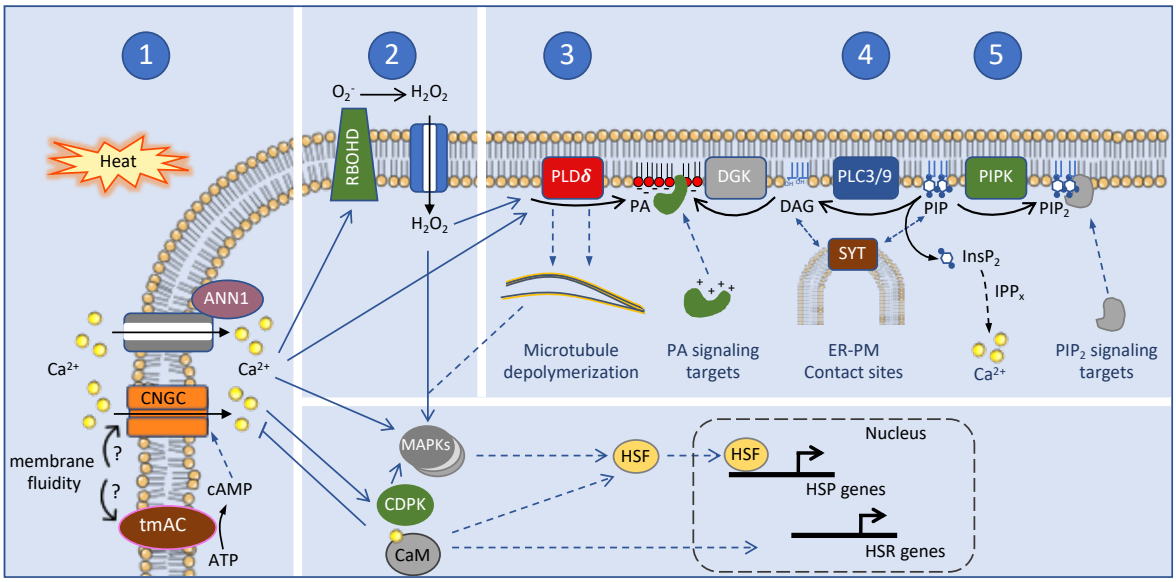

Figure 4. Sensing and primary signaling events of heat stress at the plasma membrane.

In response to heat stress, several plasma membrane-linked protein activities are triggered which lead to intracellular signals that collectively regulate the heat stress response in plants. 1. Heat perception gives rise to increases in Ca^{2+}, which can enter the cytosol from the apoplast through channels such as CNGC6. This channel might be activated by cAMP, which is generated by an unknown transmembrane adenylyl cyclase (tmAC), perhaps activated under heat stress as membrane fluidity increases. Through association with calmodulin (CaM), Ca^{2+} can negatively regulate CNGC6, and promote the function of HSFs. HSFs are the primary regulators of the heat response leading to transcriptional induction of HSPs and other genes. Apart from CNGC6, Annexin 1 (ANN1) is required for cytosolic increases in Ca^{2+}. 2. The second major factor in the heat stress response is H_2O_2, which is generated by the plasma membrane microdomain NADPH oxidase, RBOHD, whose activity is modulated by several factors, including Ca^{2+} and PA. After H_2O_2 enters the cell, it modifies the PLDδ protein such that it becomes sensitive to activation by Ca^{2+}. 3. PLDδ generates PA, which has a myriad of signaling functions which are mediated by its interaction with cytosolic target proteins. PLDδ

is attached to microtubules and its activity leads to microtubule depolymerization. Moreover, H_2O_2 can activate HSFs through MAPK signaling. 4. PLC3 and PLC9 are required for sHSP induction and thermotolerance. Most likely, they hydrolyze PIP to generate DAG, releasing the inositol-bisphosphate (IP_2) headgroup. DAG can be phosphorylated to PA by diacylglycerol kinase (DGK). In plants, rather than IP_2 or IP_3, inositol's more highly phosphorylated derivatives are the likely inducers of cytosolic release of Ca^{2+}. DAG could associate with synaptotagmin (SYT) in the ER at ER-PM contact sites, which may function to stabilize the plasma membrane under stress, and facilitate the exchange of lipids between the plasma membrane and the cortical ER. 5. Besides PA, also PIP_2 accumulates under heat stress, through PIP kinase (PIPK) activity, first only in the plasma membrane, later also in internal membranes, including the nuclear envelope. PIP_2 regulates effector proteins through specific lipid-binding domains.