SUMMARY

The AMP-activated protein kinase family (AMPK) shows a remarkable structural and functional conservation in eukaryotes. AMPKs are activated by cellular energy depletion (i.e. when the cellular AMP/ATP ratio is increased) during stress, such as glucose starvation. Compared to yeast Snf1p and animal AMPKs, our knowledge on the regulatory functions of Snf1-related plant AMPKs (type I SnRK1 kinases) is limited. SnRK1s, as other members of the AMPK family, are trimeric enzymes consisting of a catalytic α, a substrate targeting β, and an activating γ subunit. N-terminal domains of the catalytic α-subunits carry a conserved T-loop, a threonine residue of which undergoes phosphorylation during SnRK1 activation. Yeast SNF1 and mammalian AMPKs are exclusively activated by upstream kinases. Plant SnRK1s is partially activated by autophosphorylation, but orthologues of Snf1-activating Elm1p/Tos3p/Sak1p kinases are also present in plants. The activity of α-subunits is also regulated by autoinhibitory sequences present in their C terminal domains that interact with the nonphosphorylated N-terminal catalytic domain. The substrate targeting β-subunits serve as anchors for the assembly of α and γ subunits, and are characterized by two conserved domains, the kinase interacting sequence (KIS), which is also known as glycogen/glucose-binding domain (GBD), and the kinase association domain (ASC). The activating γ-subunit carries AMP-binding CBS (cystathionine-β-synthase/Bateman 2) domains and is required for AMP-dependent conformational change and activation of the α-subunits. Uniquely, the α subunits of plant SnRK1 enzymes contain ubiquitin-associated (UBA) domains, which may play a role in their degradation and/or interaction with the proteasome α7 subunit, whereas the N-terminus of plant γ/SNF4 subunits carry a GBD domain found in other organisms only in the AMPKβ subunits. Plant SnRK1s appear to be activated by sugar starvation, osmotic stress and ABA. Activation of SnRK1s results in reprogramming the transcription, as well as phosphorylation of several key metabolic enzymes, including 3-hydroxymethyl-3-methylglutaryl-CoA reductase (HMGR), nitrate reductase (NR), sucrose phosphate synthase (SPS), trehalose phosphate synthase 5 (TPS5) and 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (F2KP). This leads to cellular adaptation to altered energetic status and corresponding metabolic adjustments to cope with environmental stress.

The main goal of this work was to analyse the transcription regulatory functions of *Arabidopsis thaliana* SnRK1 kinases, and to study their potential roles in the crosstalk between glucose, ABA and ethylene signalling. Subcellular localisation of SnRK1 subunits was used to examine their nuclear localization. To generate SnRK1 subunit fusions with fluorescent reporter proteins (GFP, RFP, CFP and YFP), a recombineering technique was adapted to simple modification of genes in Arabidopsis BAC clones. Using a *galK* exchange recombination technique in *E. coli* (Warming et al., 2005), C-terminal protein fusions to each SnRK1 subunits were generated by replacing the genes' stop codons with GFP/CFP/YFP coding sequences. Subsequently, the modified genes, along with their native transcription regulatory regions, were transferred into novel Agrobacterium binary vectors that were designed to perform gap repair recombination with BACs.

Upon transferring these constructs into Arabidopsis, the expression of SnRK1-GFP/YFP/CFP fusions was characterized throughout the plant life cycle in different tissues. Genetic analysis of available T-DNA insertion mutants revealed that individual or combined inactivation of genes encoding the myristoilated membrane-bound AKINβ1 and β2 SnRK1 subunits has no apparent effect on sugar and ABA responses. By contrast, insertion mutations in the single copy *SNF4/AKINβγ* gene that encodes the nuclearly localized SnRK1 activating subunit prevented male transmission by causing a chromosome separation defect in meiosis II. T-DNA insertion mutations do not exist in the SnRK1 catalytic subunit genes *AKIN10* and *AKIN11* and the male transmission defect in the gamma/SNF4 subunit gene prevented us to determine the phenotype of a homozygous mutant. Therefore, we have designed artificial microRNAs to silence these and all other SnRK1 subunit genes individually, or in case of the catalytic subunits, simultaneously. (Schwab et al., 2006). To avoid the lethal effects resulting from constitutive repression of SnRK1 α and γ subunit genes, an estradiol-inducible system for conditional down-regulation of SnRK1 subunits was developed by expression of amiRNAs with the pER8 vector in plants (Zuo et al., 2000). amiRNA lines for individual down-regulation of AKIN10, AKIN11, AKIN12, AKINβ1, AKINβ2 and SNF4/AKINβγ, and simultaneous silencing of AKIN10/11/12 α-subunits were obtained and characterized at molecular and phenotypic levels. The development of amiRNA lines in the light and dark, and at high sugar concentrations was followed and their responses to ABA, ethylene, red, far-red and blue light was characterized, in order to elucidate the role of each SnRK1 subunit in known signalling pathways. Knock-down of the AKINβγ/SNF4 subunit conferred ABA insensitive seed germination, partially activated the ethylene triple response in the dark and impaired cell elongation in the dark, as well as in red and far-red light causing growth arrest, induction of cell death and ultimate seedling lethality seven days after germination. Affymetrix microarray transcript analysis during the time course of SNF4/AKINβγ amiRNA induction under both dark and light conditions revealed the activation of pathogenic defence responses, ethylene and jasmonate signalling pathways. Parallel genetic studies showed that the ethylene effect of inducible SNF4/AKINβγ amiRNA construct is suppressed by the *ein6* mutation in the dark. These data demonstrate a key role of SnRK1 kinases in coordinate regulation of several biotic and abiotic stress response pathways.