The SnRK Protein Kinase Family and the Function of SnRK1 Protein Kinase

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ABSTRACT

The sucrose non-fermenting 1 (SNF1) protein kinase plays important roles in process of biotic growth and development. In yeast (Saccharomyces cerevisiae) and mammals, it regulates the protein-protein interaction, activity regulation and the gene expression of the substrate in response to changing nutrient sources or energy demands and SNF1-related kinase (SnRK1) proteins are present in plants. The sucrose non-fermenting 1-related protein kinase (SnRK) is homologous of SNF1 and AMP-activated protein kinase (AMPK), which widely exists in plant and involves in a variety of signaling pathways. SnRK has been divided into three sub-families: SnRK1, SnRK2 and SnRK3. SnRK is the key switches in plant sugar signal, stress, seed germination and seedling growth. In this review, we summarized the progress of SnRK protein kinase family and the structure, substrate, regulation and gene expression of SnRK1. © 2012 Friends Science Publishers

Key Word: SnRK; Protein kinase family; SnRK1; Signaling pathway; ABA

INTRODUCTION

Protein kinase and protein phosphatase are ubiquitous in organism. They catalyze the processes of protein phosphorylated and dephosphorylated. A series of physiological and biochemical activities including metabolism, mitosis, amitosis, signal transduction are regulated by protein phosphorylation and dephosphorylation. They play important roles in growth and development (Ruben et al., 2011).

SNF1 of yeast, AMPK of mammal and SnRK1 of plant are homologous, belonging to the SNF1 protein kinase superfamily. SNF1 was found in yeast (Saccharomyces cerevisiae) originally (Alderson et al., 1991). The low intracellular glucose level can be perceived by SNF1. In yeast, glucose regulates the protein-protein interaction, substrate specificity and subcellular localization of the SNF1 subunit that modulates SNF1 kinase activity, resulting in the phosphorylation of activators and repressors that control transcription of multiple genes in metabolic pathways required for the utilization of alternative energy sources. In mammals, activation of AMPK due to increases in the AMP to ATP ratio during metabolic stress results in the enhancement of ATP-producing pathways and the inhibition of ATP consuming pathways (Bradford et al., 2003). AMPK complex is directly relevant to obesity and type II diabetes (Rune et al., 2009). In the eukaryote, SNF1 protein kinase is very strongly conservative. Many SNF1 analogues have been identified in plants (Alderson et al., 1991). Through conservative amino acid sequence analysis, it is divided into three subfamilies, SnRK1, SnRK2, and SnRK3, respectively. They participate in a variety of metabolic activities of plants; especially play important roles in the physiological resistance.

The SnRK is a class of Ser/Thr protein kinase, which widely exists in plant and involves in a variety of signaling pathways. They play a pivotal role in plant stress physiology. SnRK family is classified into three subfamilies: SnRK1, SnRK2 and SnRK3. In this review, we summarized the progress of research on SnRK protein kinase family and the structure, substrate, regulation and gene expression of SnRK1.

Protein kinase superfamily of SnRK: Structure of SnRK protein kinase family: A self-regulation kinase domain in the N-terminal is the common structural characteristic of SnRK protein kinase family. The region is a highly variable, and can interact with other protein. Compared with other protein kinase, there is a conserved amino acid-threonine in the activation region. SnRK is composed of α, β and γ composed of three structural domains. The α-domain has a
N-terminal with Ser/Thr protein kinase domain, C-terminal has regulatory sequences of self-inhibitory function (Jiang & Carlson, 1996). β-domain can combine with α-and γ-domain, so β-domain can regulate shape of heterologous trimer complexes (Jiang & Carlson, 1997). Three sub-families have their own structural characteristics, as shown in Fig. 1 (Wang et al., 2010).

**Classification and Related Function of SnRK**

**SnRK1 subfamily:** The SnRK1 was discovered initially in the rye (*Secale cereale* L.). The rye SnRK1 encodes a polypeptide with a relative molecular mass of 57.7 kDa and 502 amino acids. The identities of yeast SNF1 and mammalian AMPK were up to 42-45% (Alderson et al., 1991). At present, some members of the SnRK1 subfamily have been found to form a variety of model plant and some important crops, such as *Arabidopsis thaliana*, rye, barley (*Hordeum vulgare*), potatoes (*Solanum tuberosum*), tobacco and beet, and so on. It may exist in all plants (Halford & Hardie, 1998; Halford et al., 2003). According to the similarity of amino acid sequences, SnRK1 is divided into SnRK1a and SnRK1b. SnRK1a expressed throughout the developmental period of the plant. But expression of SnRK1b in seeds is very high quality, and other parts of the plant are relatively low. This gene exists only in dicotyledon (Halford & Hardie, 1998). Studies have shown that SnRK1 is the key switches in plant sugar signal. And the regulation of glucose metabolism, hormonal regulation and sugar signal are directly related to signal transduction (Mathieu et al., 2009; Kleinow et al., 2009).

**SnRK2 subfamily:** SnRK2 sub-family is unique in plants. SnRK2 is a relatively small plant-specific protein family. It is activated by osmotic stress (Sandra et al., 2010). According to the similarity of amino acid sequence, SnRK2 is divided into SnRK2a and SnRK2b. Comparing with SnRK1, SnRK2 protein contains a relatively short C-terminal, characterized of the C-terminal acidic with a short “patch.” In gaoliang (*Sorghum vulgare*), SnRK2 contains a kinase domain, binding domain, ATP binding domain, Ser/Thr active site and four N-fourteen sites. The acidic patch of SnRK2a is rich in aspartic acid residues, while that of SnRK2b enriches in glutamic acid residues (Zhang et al., 2011).

The SnRK2 was first isolated from wheat embryo cDNA library by ABA treatment that is PKABA1 (Anderberg & Walker-Simmons, 1992). PKABA1 is induced by ABA and hypertonic stress. The transient overexpression of PKABA1 gene in barley aleurone inhibits GA-induced promoter activity, indicating that the ABA signal transduction inhibits the GA signal transduction (Johnson et al., 2002). Under drought stress, the expression of PKABA1 and intracellular level both were increased, and the former was found in roots and skin scales (Laurie & Halford, 2001). Another study found that PKABA1 could combine with ABA-responsive element factor-TaABF. This combination could regulate the level of expression of the ABA. Meanwhile, studies have shown that SnRK2 sub-family members regulate ABA signaling, for example, SnRK2.6 can regulate ABA signaling in guard cells (Yoshida et al., 2002). Ten members of SnRK2 have been discovered, namely SnRK2.1-SnRK2.10 in the Arabidopsis. Boudsocq studies (2004) showed that 10 SnRK2 members in Arabidopsis, in addition to SnRK2.9, the other SnRK2 members can be mannose and NaCl-induced activation. SnRK2.2, SnRK2.3, SnRK2.6, SnRK2.7 and SnRK2.8 can be activated by ABA (Boudsocq et al., 2004). Meanwhile, SnRK2 family members have a very important role in plant stress resistance. SnRK2.4 overexpression in Arabidopsis plants, compared with the control group, revealed that the abilities to salt resistance, drought resistance and frost resistance have greatly improved (Man et al., 2010). The overexpression of SnRK2.8 improved a certain ability of resisting drought intimidation. Under nutritional deficiency, the growth conditions of transgenic plant were better than the control group in Arabidopsis (Shin et al., 2007).

**SnRK3 subfamily:** SnRK3 is a rare protein kinase in plant, called calcineurin B-like calcium-sensor-interacting protein kinases (calcineurin B-like calcium sensor-interacting protein kinases, CIPK) (Kim et al., 2000). CIPK interacts with calcium-binding protein SOS3, SCaBPS and CBL (calcineurin B-like calcium sensor, CBL). The interaction region is called NAF/FISL motif, which is made up of 22 amino acid residues (Albrecht et al., 2001; Guo et al., 2001). CIPK kinase region contains a binding site, calcium-binding proteins and calcium-sensitive CBL in the C-terminal inhibitory, which combine to activate protein kinase (Guo et al., 2001). Studies have shown that CIPK and CSLs complex of upstream interactions protein involved in salt stress, sucrose and ABA signal transduction (Imamura et al., 2008).

In Arabidopsis, PKS3, PKS18 and CIPK3 of SnRK3 family can regulate plant growth, stomatal opening and closing and seed germination treated by ABA (Kim et al., 2003). AtCIPK1 of Arabidopsis form complexes with AtCBL1 and AtCBL9 regulates ABA-independent and ABA-dependent pathways, respectively (Angelo et al., 2006). AtCIPK3 regulates ABA and cold signal transduction pathways (Kim et al., 2003). Girdhar's study showed that CBL9 interacted with CIPK3 to regulate the ABA pathway, and this point got validation in the yeast two-hybrid experiment (Pandey et al., 2008). Comparing with cb19/cipk3 double mutant plant and single mutant of two genes, three mutants could response to ABA signaling. Under salt and mannitol stress, seed germination and seedling growth of three mutants are inhibited (Pandey et al., 2004).

**Structure and Function of Snr1k**

**SnR1k structure:** The AMPK/SNF1/SnRK1 protein kinase functions as heterotrimeric complexes require α-, β-subunit and γ-subunits. For example, in yeast (*Saccharomyces cerevisiae*), SNF1 contains a catalytic subunit (Snf1), three β-subunits (Sip1, Sip2 & Gal83)
SnRK1 substrates and activity regulation: The first plant protein identified as a substrate for SnRK1 was HMG-CoA reductase in Arabidopsis (Dale et al., 1995). At the same time, sucrose phosphate synthase, nitrate reductase and trehalose-5-phosphate synthase had been identified as SnRK1 substrates (Fig. 2). In Arabidopsis, Snf1 and AMPK α are KIN10/KIN11 with a similar structure and function, while there may be a pseudogene of KIN12 (Gustavo et al., 2011). Other three genes KINδ1/KINβ2/KINβ of Arabidopsis 3 and β-subunit of yeast and mammalian are sharing the same feature. Meanwhile, a SNF4/AMPKγ homologue called AtSNF4 has been cloned from Arabidopsis by complementation of a snf4 mutant (Kleinow et al., 2000).

SnRK1 can directly phosphorylate HMG-CoA reductase (HMGR) catalyzes the conversion of HMG-CoA into mevalonate (MVA), which is the limiting step in the biosynthesis of isopentenyl pyrophosphate. SPS is a key enzyme in the pathway of sucrose biosynthesis in plants. Nitrate reductase is a molybdoenzyme that reduce nitrate (NO$_3^-$) to nitrite (NO$_2^-$). It plays an important role in the synthesis of nitro-organic compounds. The achievement as follows about interaction of sugar and nitrogen signal: as mentioned above, sucrose synthase, the key enzyme in carbon metabolism is controlled by SnRK1 protein kinases, at the same time the kinase also regulates the activity of NR. When tobacco (Nicotiana tabacum) leaves were fed with sucrose in vitro, NR activity, nitrogen assimilation and amino acids were significantly enhanced. These results showed that the SnRK1 kinase plays an important role in carbon-nitrogen interactions (Halford et al., 2004; Li et al., 2010). SnRK1 can regulate the activity of those important physiological effects of substrate, which gives us a new way of thinking about the co-regulation of sugar, nitrogen compounds and the variety of secondary metabolites. SnRK1 can also control the activity of other enzymes. ADP-glucose pyrophosphorylase (AGPase) is a key enzyme in starch synthesis in growing potato tubers. AGPase activity depends on the redox regulation, to make own cells to respond to sucrose, but AGPase oxidation does not occur in the antisense expression of potato tubers.

SnRK1 plays important roles in the transcription regulation of gene expression. In the developing tuber of potato, the expression level of SnRK1 was higher, lower in stem and lowest in leaf. The experiment on potato provided evidence for SnRK1 to regulate the transcription.

The enzyme 3-hydroxy-3-methyl glutaryl coenzyme A. (HMG-CoA) reductase (HMGR) catalyzes the conversion of HMG-CoA into mevalonate (MVA), which is the limiting step in the biosynthesis of isopentenyl pyrophosphate. SPS is a key enzyme in the pathway of sucrose biosynthesis in plants. Nitrate reductase is a molybdoenzyme that reduce nitrate (NO$_3^-$) to nitrite (NO$_2^-$). It plays an important role in synthesis nitro-organic compounds. The achievement as follows about interaction of sugar and nitrogen signal: as mentioned above, sucrose synthase, the key enzyme in carbon metabolism is controlled by SnRK1 protein kinases, at the same time the kinase also regulates the activity of NR. When tobacco (Nicotiana tabacum) leaves were fed with sucrose in vitro, NR activity, nitrogen assimilation and amino acids were significantly enhanced. These results showed that the SnRK1 kinase plays an important role in carbon-nitrogen interactions (Halford et al., 2004; Li et al., 2010). SnRK1 can regulate the activity of those important physiological effects of substrate, which gives us a new way of thinking about the co-regulation of sugar, nitrogen compounds and the variety of secondary metabolites. SnRK1 can also control the activity of other enzymes. ADP-glucose pyrophosphorylase (AGPase) is a key enzyme in starch synthesis in growing potato tubers. AGPase activity depends on the redox regulation, to make own cells to respond to sucrose, but AGPase oxidation does not occur in the antisense expression of potato tubers.

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SnRK1 is at the heart of the control of carbon/nitrogen partitioning in plants, regulating the activity of important enzymes by affecting their activation state or the activity of the genes that encode them (Adriano et al., 2010). The black arrows indicate reactions; yellow arrows indicate increases in expression of SnRK1. Red arrows are positive sign, symbolize protein activation and in blue, with negative sign, protein inhibition. Glc, glucose; Fru, fructose; Suc, sucrose; G6P, glucose-6-phosphate; G1P, glucose-1-phosphate; T6P, trehalose-6-phosphate; Glu, glutamate; Gln, glutamine; AGPase, ADP-glucose pyrophosphorylase; UGPase, UDP-glucose pyrophosphorylase; SPS, sucrose phosphate synthase; SuSy, sucrose synthase; NR, nitrate reductase; NITR, nitrite transporter. For simplification, neither sub-cellular compartmentalization nor mechanistic information is presented.

expression inhibits a-amylase promoter activity in the wheat germ (Laurie et al., 2003). These accounts for that SnRK1 influence starch biosynthesis via regulating the expression of sucrose synthase and the activity of AGPase. SnRK1 activity can respond to sucrose appropriately (Halford & Paul, 2003). According to the studies, currently, SnRK1 family is a key switch in sugar signal transduction and metabolic pathways of plant (Fig. 3) (Polge & Thomas, 2007).

The role of SnRK1 in signaling and plant development: Antisense gene expressions in different plant indicate that SnRK1 has very important roles in plant growth and development processes. For instance, sprouting process of potato tuber was inhibited by the SnRK1 antisense gene (Halford et al., 2003). Barley pollen development can be inhibited by antisense SnRK1 and Pollen is very small, pear shaped, containing little or no starch and no life. But antisense SnRK1 gene cannot be passed to the next generation. Also it inhibited the ovule development. Pollen dysplasia is related to the lack of starch accumulation and non-active sugar (Zhang et al., 2001). Male sterility in rice and wheat caused the decrease of activity of acid instead of non-sucrose synthase activity. SnRK1 and the invertase regulation have not discovered the positive connection but in tobacco's anthers. Antisense SnRK1 of transgenic barley was similar to that antisense expression of invertase in extracellularly causes abnormal pollen (Goetz et al., 2001). Currently, there are evidences on the SnRK1 involving in cell metabolism and circulation. The overexpression of plant SnRK1 in yeast could make the body of yeast decrease in size significantly. It implied that SnRK1 makes cell cycle completely earlier in yeast. The possible reason is that the normal function of the SnRK1 or SNF1 is coupled with the interaction between metabolism and cell cycle signals. However, the overexpression of exogenous SnRK1 can cause system disturbance of yeast. On the basis of the above resolution, we can conjecture that SnRK1 family is a key switch in sugar signal transduction and metabolic pathways of plant. At present, we need further study in order to clarify the operation of carbon and nitrogen metabolites, and their signal perception and transduction.

CONCLUSION

As the SNF1 function research work went further and more detailed, especially SNF1 is related with sugar metabolism and sugar signaling, which has become hot area of research. But there is little attention for the research on SnRK1 in plant. With the discovery of new SnRK1 and the isolation of more substrates, more and more studies suggest that SnRK1 plays an important role in the plant growth and signal transduction pathway. At present, the majority of researchers is focused on SnRK1 in plant distribution, physical and chemical properties, physiological substrates, correlation between protein kinase and the position of the signal transduction and so on. At the same time, the application of transgenic technology and antisense RNA technology, SnRK1 determines the enzyme in the position of plant regulatory networks, regulation mechanisms. Development of effective products was applied to agricultural production, the promotion of plant growth and development, enhancing plant stress resistance and improving crop quality and yield, which is very significant.

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