Chapter 12
Protein Kinase C of Filamentous Fungi and Its Roles in the Stresses Affecting Hyphal Morphogenesis and Conidiation

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Abstract Protein kinase C (PKC) is known to play pivotal roles in the various signal transduction pathways in mammalian cells. Its functions have been extensively explored in mammalian cells, whereas those of the PKC of filamentous fungi remain largely unknown, with the exception that PKC is known to function in the cell wall integrity signaling pathway similar to that in the yeast *Saccharomyces cerevisiae*. Recent advances in the functional analyses of *Aspergillus nidulans* PKC suggest that it has functions in germination, hyphal morphogenesis, and spore formation under heat stress. These functions are suppression of apoptosis induction and the establishment of cell polarity during germination, reestablishment of hyphal polarity after depolarization, and repression of conidiation. In this chapter, we present these functions of PKC and describe them in detail.

Keywords Apoptosis • *Aspergillus* • Cell wall integrity signaling pathway • Conidiation • Morphogenesis • Protein kinase C • Stress response

12.1 Introduction

Fungi grow in nature under many environmental stress conditions. To respond to these stresses, fungi have various signal transduction pathways and change their morphology to adapt to their environment. Protein kinases, such as protein kinase A, G, and C (AGC protein kinases), calmodulin-dependent protein kinase (CAMK),

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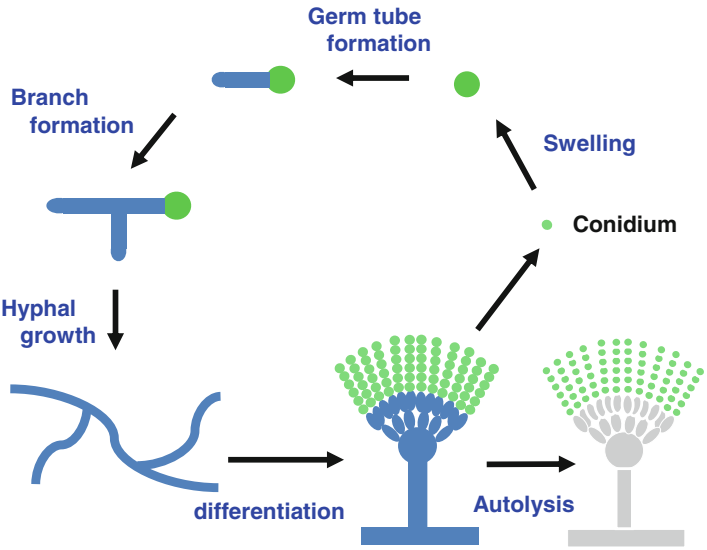

Fig. 12.1 Asexual life cycle of *Aspergillus nidulans*. Details described in text

and kinases involved in the mitogen-activated protein (MAP) kinase cascade, are speculated to have important roles in these processes (De Souza et al. 2013). The functions of these fungal kinases have been investigated primarily using *Saccharomyces cerevisiae*, and little is known about their functions in filamentous fungi.

Filamentous fungi belonging to Ascomycota undergo asexual and sexual life cycles and form asexual spores (conidia) and sexual spores (ascospores). In the asexual life cycle of *Aspergillus nidulans*, conidia grow isotropically at 37 °C for 2 to 3 h and establish cell polarity to form germ tubes. Then, polar growth proceeds with branching followed by hyphae formation. The hyphae differentiate conidiophores, which form conidia after approximately 20 h of cultivation. Finally, autolysis occurs at a late stage of cultivation (Fig. 12.1). These specific structural changes in the life cycle of filamentous fungi are thought to be closely related to both their industrial merits and demerits. Thus, elucidating the mechanisms of conidial germination, hyphal growth, conidiophore, and conidia development at the molecular level is very important for the efficient utilization of filamentous fungi in industry. Fungal cells are surrounded by cell walls, which consist mainly of polysaccharides and proteins and form very rigid structures. The cell wall is crucial for the formation of differentiated structures of filamentous fungi, and the architecture changes during the progression of the life cycle and in response to various cell wall stresses.

Protein kinase C (PKC) is a serine/threonine kinase that is ubiquitous from fungi to mammals. There are more than ten isozymes in mammalian cells that function in various signal transduction pathways in these cells (Steinberg 2008). In contrast to mammalian cells, there are only one or a few PKC-encoding genes in the genomes of filamentous fungi and yeasts. Although PKC is known to have a crucial role in the

signal transduction pathway in *S. cerevisiae* under cell wall stress, its functions in filamentous fungi remain poorly understood.

In this chapter, we mainly focus on the protein kinase C (PKC)-related signal transduction pathway of filamentous fungi.

12.2 PKC Signaling Pathway

Fungal PKCs have a specific domain organization consisting of two HR1 domains, a C2-like domain, a pseudo-substrate sequence, two C1 domains, a protein kinase domain, and a protein kinase C-terminal domain (Fig. 12.2). The organization of these domains is similar to that of the mammalian novel PKC (nPKC) subclass with the exception of the HR1 domain. The HR1 domain is not present in nPKC but is present in the PKC-related kinase, protein kinase N (PKN) (Mukai 2003). In these domains, the HR1 domain is known to bind to the GTPase Rho (Schmitz et al. 2002) and the C2-like domain is a sequence homologous to the C2 Ca^{2+}-binding domain. However, the amino acids required for Ca^{2+} binding are not conserved in the "C2-like" domain. The C1 domain, a region that binds to diacylglycerol (DAG), is followed by a pseudo-substrate sequence located between C2-like and C1 domains. The pseudo-substrate sequence resembles the amino acid sequence of substrate

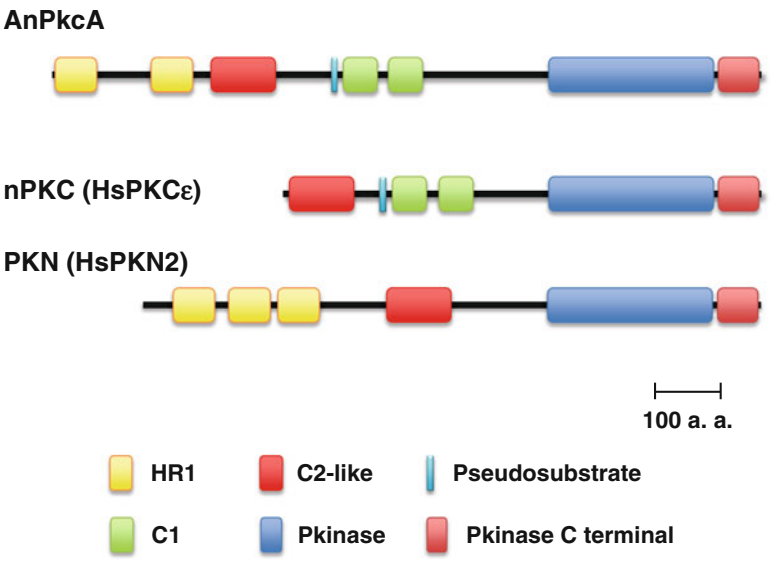

Fig. 12.2 Domain organization of the fungal PKC and human nPKC and PKN. Hs *Homo sapiens*, An *A. nidulans*

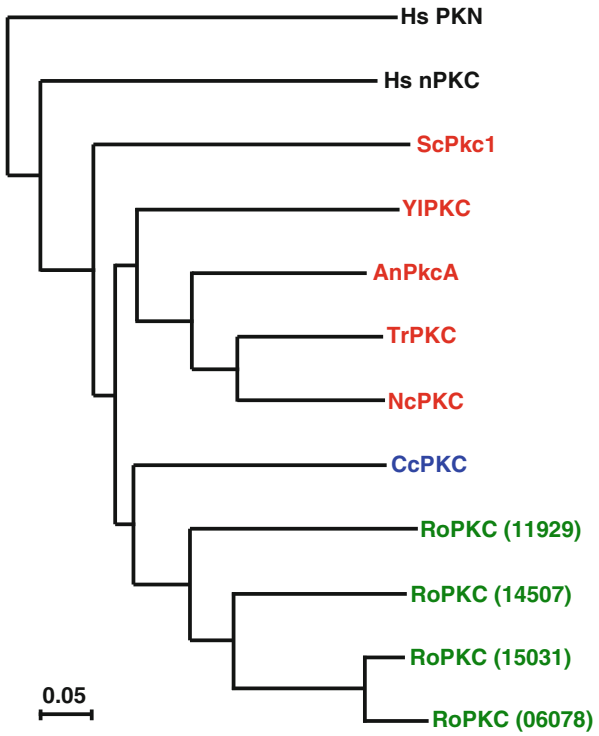

Fig. 12.3 Phylogenetic tree of fungal PKC and human nPKC and PKN. Alignment was performed with ClustalW using the entire amino acid sequence of each PKC and PKN. The tree was drawn using NJprot. Branch length indicates evolutionary distance. PKCs of the fungi belonging to Ascomycota, Basidiomycota, and Mucoromycotina are shown in *red*, *blue*, and *green*, respectively. Hs *Homo sapiens*, Sc *Saccharomyces cerevisiae*, An *A. nidulans*, Tr *Trichoderma reesei*, Nc *N. crassa*, Yl *Yarrowia lipolytica*, Cc *Coprinopsis cinerea*, Ro *Rhizopus delemar*. The numbers in parentheses indicate the gene IDs in the genome database of *R. delemar*

proteins, with the exception of the phosphoacceptor residue, which is replaced by alanine. The pseudo-substrate sequence is thought to bind to the catalytic subunit of PKC and keep the kinase inactive.

The phylogenetic relationships among fungal PKCs and human nPKC and PKN are shown in Fig. 12.3. In general, fungi belonging to Ascomycota or Basidiomycota have one or two PKCs, whereas those belonging to Mucoromycotina (formerly known as Zygomycota) (Hibbett et al. 2007) have three or four PKCs.

Saccharomyces cerevisiae has the only PKC-encoding gene, *PKC1*. The gene product of *PKC1*, Pkc1, has the typical domain organization of fungal PKC (Fig. 12.2), and its activity is not stimulated by Ca^{2+} or DAG (Kamada et al. 1996). Amino acids that are thought to be required for binding to DAG are not conserved in the C1 domain of fungal PKCs (Schmitz and Heinisch 2003). The C1 domain of Pkc1 has also been reported to bind Rho1 in *S. cerevisiae* (Nonaka et al. 1995).

Pkc1 is known to have pivotal function in the cell wall integrity (CWI) signaling pathway. The *pkc1* deletion mutant could not form colonies unless an osmotic stabilizer was added to the medium. It has been suggested that *PKC1* was involved

in various cellular processes, such as the cell wall integrity (CWI) signaling pathway, cell-cycle progression, and phospholipid synthesis (Levin 2005). However, its functions at the molecular level remain poorly understood except for the function in the CWI signaling pathway. In the *Aspergillus nidulans* genome, there is a PKC-encoding gene, *pkcA*. *pkcA* was cloned and its functions have been investigated. Deletion of *pkcA* caused frequent hyphal lysis and its deletion mutant did not form colonies, indicating that *pkcA* is essential for hyphal growth (Ichinomiya et al. 2007). This growth defect was not remedied by the addition of osmotic stabilizers to the medium, which suggests that PkcA has other functions besides the function in the CWI signaling pathway (see following). It was reported that *pkcA* was involved in penicillin biosynthesis and was crucial for the nuclear localization of the transcription factor AnBH1 (Herrmann et al. 2006).

Pkc1 of *S. cerevisiae* localizes to polarized growth sites. However, Denis and Cyert showed that the deletion of the HR1 domain causes the relocalization of Pkc1 to the mitotic spindle. They also determined a nuclear localization signal (NLS) and a nuclear export signal (NES) in Pkc1. These results suggest that the small portion of Pkc1 is shuttled between the nucleus and cytoplasm (Denis and Cyert 2005). Recently, Pkc1 has been reported to disappear at the polarized growth site and to accumulate in damage sites caused by lasers (Kono et al. 2012).

PkcA of *A. nidulans* mainly localizes to polarized growth sites, such as hyphal tips, forming septa, and tips of phialides (Teepe et al. 2007). The PKC of *Neurospora crassa* also localizes at some hyphal tips and subapical membranes in actively growing hyphae and forming septa. The PKC in the cytoplasm accumulates in the plasma membrane after treatment with the phorbol ester, 12-myristate 13-acetate (Khatun and Lakin-Thomas 2011).

12.2.1 Function of PKC in the Cell Wall Integrity Signaling Pathway

Fungal cells sense cell wall stresses and transduce signals inside the cells. The signals are transmitted to transcription factors that induce the expression of certain genes. This signal transduction pathway is called the CWI signaling pathway. In this pathway, transmembrane sensor proteins, Wsc1–3, Mid2, and Mtl1, which localize in the plasma membrane, sense cell wall stresses and transmit signals to the downstream effectors Rom1 and 2. Rom1 and 2 are GDP/GTP exchange factors (GEFs) for the GTPase Rho1, a small G protein that is active when it binds to GTP. The GTP-bound form of Rho1 interacts with Pkc1 and activates it, and Pkc1 phosphorylates MAP kinase kinase kinase Bck1. Signals are transduced to transcription factors Rlm1 and the Swi4/Swi6 SBF complex through the MAP kinase cascade. Because the genes encoding almost all the components involved in the CWI pathway are conserved in the genomes of Ascomycota, Basidiomycota, and Mucoromycotina fungi (Table 12.1), signal transduction pathways are speculated to be present in

Table 12.1 Presence of orthologous genes involved in the cell wall integrity (CWI) pathway in fungi

	Saccharomyces cerevisiae	*Aspergillus nidulans*	*A. oryzae*	*C. cinerea*[a]	*R. delemar*[b]
Sensor	Wsc1–3, Mid2, Mtl1	WscA, WscB, MtlA	AO090009000205, AO090020000482	?	?
GEF	Rom1, Rom2	AN4719	AO090120000205	Rgf2	RO3G_07161, RO3G_00951, RO3G_03084, RO3G_02600, RO3G_10407, RO3G_08775, RO3G_14312, RO3G_16337
GTPase	Rho1	RhoA	AO090003000071	CC1G_03166	RO3G_11556, RO3G_02938
PKC	Pkc1	PkcA	AO090120000316	CC1G_07190	RO3G_14507, RO3G_11929, RO3G_06078, RO3G_15031
MAPKKK	Bck1	BckA	AO090003000662	CC1G_10513	RO3G_13559
MAPKK	Mkk1, Mkk2	MkkA	AO090009000347	CC1G_01983	RO3G_02520
MAPK	Slt2	MpkA	MpkA	CC1G_02007, CC1G_14865	RO3G_05693, RO3G_10828
Transcription factors	Rlm1	RlmA	AO090005001424	CC1G_07160	RO3G_15773, RO3G_02782, RO3G_03857, RO3G_06615
	Swi4/Swi6	AN3154/AN6715	AO090012000763/ AO090005000424	CC1G_01306/CC1G_00846	RO3G_13109, RO3G_10059, RO3G_11837, RO3G_08554, RO3G_06039/RO3G_03281

[a] *Coprinopsis cinerea* (Basidiomycota)
[b] *Rhizopus delemar* (Mucoromycotina)

filamentous fungi. The functions of upstream components in this signaling pathway have been analyzed and are described in Chap. 10. In *A. nidulan*s, MpkA and RlmA, a MAP kinase and a transcription factor in the CWI signaling pathway, respectively, are involved in the regulation of the expression of the genes encoding alpha-glucan synthases (*agsA* and *agsB*) and a glutamine-fructose-6-phosphate amidotransferase (*gfaA*) when the cells are treated with caspofungin, a β-1,3 glucan synthase inhibitor (Fujioka et al. 2007). RlmA of *A. niger* is also involved in the expression of *agsA* and *gfaA* (Damveld et al. 2005).

When *pkcA* expression is reduced in *A. nidulans*, the growth of the *pkcA*-conditional mutant is sensitive to a β-1,3 glucan synthase inhibitor, caspofungin, the chitin-binding dyes Congo red and Calcofluor white, and a PKC inhibitor, staurosporine. Abnormalities in the cell wall organization of this mutant have been observed under the *pkcA*-downregulating conditions (Ronen et al. 2007). The overexpression of *pkcA* has also been observed to induce growth sensitivity to a β-1,3-glucan synthase inhibitor, a chitin-binding dye, and chitin synthase inhibitors (Ichinomiya et al. 2007), suggesting that PkcA has a critical role in the CWI signaling pathway. To investigate the function of PkcA in this pathway, we constructed a strain that produces a constitutively active form of PkcA and analyzed the expression patterns of the genes encoding chitin synthases and alpha- and beta-glucan synthases. The results showed that most of these genes, including *chsB*, *csmA*, and *csmB*, are upregulated. Moreover, this upregulation was completely or partially dependent on RlmA (Katayama et al. 2015). These results suggest that chitin synthesis and glucan synthesis are also regulated by the CWI signaling pathway through PKC and Rlm1 orthologues in filamentous fungi. The gene *chsB* encodes a class III chitin synthase, and its gene product, ChsB, tagged with EGFP (EGFP-ChsB), was observed to localize at hyphal tips and forming septa (Fukuda et al. 2009; Horiuchi 2009). ChsB is crucial in hyphal tip growth (Borgia et al. 1996; Ichinomiya et al. 2002). The genes *csmA* and *csmB* encode chitin synthases with myosin motor-like domains, and they are located in a head-to-head configuration in the genome. There are five putative RlmA-binding sites in the promoter region between them. Their gene products, CsmA and CsmB, colocalize at hyphal tips, forming septa, and have compensatory essential functions for tip growth (Takeshita et al. 2006). CsmA is suggested to be involved in repairing cell wall damage (Yamada et al. 2005), and both CsmA and CsmB have similar characteristics (Takeshita et al. 2005; Tsuizaki et al. 2009). It is reasonable to regulate the expression of *csmA* and *csmB* by the signaling pathway through PkcA and RlmA.

Recently, RlmA has been shown to be involved in the regulation of *brlA* expression (Kovács et al. 2013). Because BrlA is a transcription factor that has a crucial role in asexual development (see following), the CWI signaling pathway and asexual development signaling pathway are suggested to be somewhat related.

12.2.2 Functions of PKC Under Heat Stress

12.2.2.1 Germination

As described in the former section, *pkcA* is essential for the growth of *A. nidulans*. In contrast, *bckA* encoding a MAP kinase kinase kinase of the CWI signaling pathway and *mpkA* (Table 12.1) are not essential for growth at 37 °C, an optimal growth temperature for *A. nidulans* (Katayama et al. 2012), which suggests that the PkcA has other function(s) essential for the growth of *A. nidulans*. To investigate these functions, we constructed and characterized a temperature-sensitive mutant of *pkcA*. The resulting *pkcA*-ts mutant grew as well as the wild-type strain below 30 °C, although it showed a partial growth defect and a severe conidiation defect at 37 °C. The *pkcA*-ts mutant did not form colonies at 42 °C. Furthermore, the conidiation defect at 37 °C were partially remedied by the addition of an osmotic stabilizer in the medium, whereas the growth defect at 42 °C was not remedied even in the presence of an osmotic stabilizer. Although the growth defect of *the S. cerevisiae pkc1*-ts mutant at a restrictive temperature was suppressed when Ca^{2+} was added to the medium, that of the *A. nidulans pkcA*-ts mutant was not suppressed on the addition of Ca^{2+}.

During germination, the conidia of *A. nidulans* swell isotropically for a period of time, then the growth polarity is established and germ tubes form (Fig. 12.1). The conidia of the *pkcA*-ts mutant swelled slightly and stopped growing at 42 °C. Reactive oxygen species (ROS) and DNA-strand breaks, common characteristics that are phenotypes of apoptosis, accumulated in swollen conidia, suggesting that apoptosis was induced in the *pkcA*-ts mutant at 42 °C and that PkcA suppresses the induction of apoptosis under heat stress. In contrast, induction of apoptosis was not observed in the *pkcA*-ts mutant grown at 37 °C. The *bckA* and *mpkA* deletion mutants did not form colonies, and the conidia of these mutants swelled and stopped growing at 42 °C. The accumulation of ROS and DNA-strand breaks were also observed in the swollen conidia of both mutants. These results suggest that suppression of apoptosis induction is required for the function of BckA and MpkA. On the other hand, the *rlmA* deletion mutant grew well at 42 °C, suggesting that RlmA is not required for suppression. Taken together, the suppression of apoptosis induction under heat stress conditions would occur through PKC and the downstream MAP kinase cascade but not through RlmA.

The *bckA* and *mpkA* deletion mutants formed colonies at 42 °C when osmotic stabilizer was added to the medium, although the *pkcA*-ts mutant did not form colonies under the same condition, suggesting that PkcA has another function in the growth of *A. nidulans* under heat stress conditions. To clarify the function of PkcA, we analyzed the terminal phenotype of the *pkcA*-ts mutant in the presence of the osmotic stabilizer at 42 °C. Under this condition, apoptosis was not induced in the *pkcA*-ts mutant. However, the mutant formed extremely swollen conidia and did not form germ tubes 8 h after incubation at 42 °C. The DNA content in the swollen conidia of the mutant increased as well as that of the wild-type strain, and polar localization of actin filaments was not observed in the mutant. These results suggest

that the cell cycle progressed regularly, but polarity was not established in the mutant. In contrast, the *bckA* deletion mutant established cell polarity and formed germ tubes under the same condition (Katayama et al. 2012), suggesting that PkcA is required to establish polarity in the swollen conidia, although BckA and downstream factors of the CWI signaling pathway are not involved.

12.2.2.2 Hyphal Growth

The role of PkcA in hyphal tip growth under heat stress conditions was investigated. When the *pkcA*-ts mutant was cultivated for 16 h to induce hyphal growth at a permissive temperature (30 °C) and shifted to a restrictive temperature (42 °C), the growth of the *pkcA*-ts mutant stopped and its hyphal tips swelled. These observations suggest that the hyphal tips were depolarized. Actin filaments are usually observed at the hyphal tips in *A. nidulans*. When hyphae were exposed to heat stress, actin filaments at the hyphal tips rapidly disappeared in both the *pkcA*-ts mutant and the wild-type strain. In the wild-type strain, actin filaments at the hyphal tips reappeared within 60 min after the shift to 42 °C, whereas they did not reappear 120 min after the sift in the *pkcA*-ts mutant. The reappearance of actin filaments at the tips was observed in the *bckA* deletion mutant as well as the wild-type strain (Katayama et al. 2012), suggesting that the repolarization of hyphae does not depend on the function of BckA. As already mentioned, EGFP-ChsB localizes at the hyphal tips. After the shift to 42 °C, EGFP-ChsB disappeared from hyphal tips in the *pkcA*-ts mutant and the wild-type strain. EGFP-ChsB was observed again at the hyphal tips a few hours after the shift in the wild-type strain, whereas it was not observed at the hyphal tips 5 h after the shift in the *pkcA*-ts strain. The localization of microtubules was not affected in the *pkcA*-ts mutant and wild-type strain when exposed to heat stress (Takai et al., unpublished results). These results suggest that the redistribution of EGFP-ChsB to hyphal tips depends on the actin filament repolarization through the function of PkcA. A model for the PKC functions during germination and hyphal tip growth is shown in Fig. 12.4.

12.2.2.3 Asexual Development

As described in the former section, induction of the expression of *brlA* is an essential step for conidiation in *Aspergillus nidulans. brlA* encodes a C_2H_2 transcription factor and controls the expression of genes involved in regulating the early stages of conidiation. The expression of *brlA* was not induced in the *pkcA*-ts mutant at 40 °C, suggesting that PkcA is required to induce the expression of *brlA* (Katayama and Horiuchi, unpublished results). The expression of *brlA* is induced by the upstream activators FluG, FlbA, FlbB, FlbC, FlbD, and FlbE (Fig. 12.5). FluG is a key activator of the initiation of conidiation and induces conidiation via two independent processes (Etxebeste et al. 2010; Park and Yu 2012). First, FluG inhibits the vegetative growth via the activation of FlbA, which is a key regulator of the G-protein

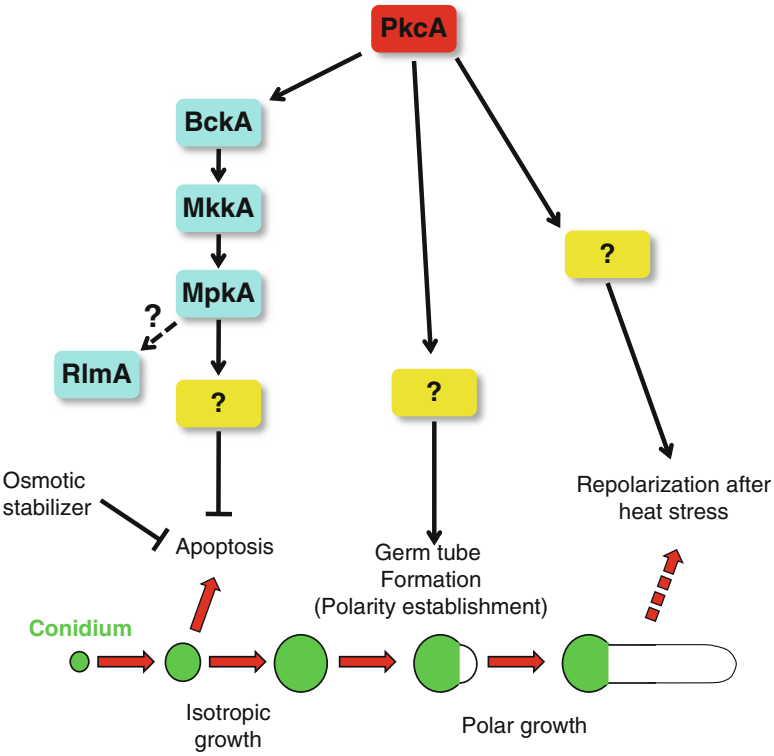

Fig. 12.4 A model for the function of PkcA under heat stress. "?" in *yellow panels* means the possible presence of unknown factors. (Modified from fig. 10 in a previous paper by Katayama et al. 2012

signaling pathway that regulates the balance between hyphal proliferation and conidiophore differentiation. Second, FluG induces the expression of *brlA* by activating the two separate pathways. One pathway contains FlbC, which is a C_2H_2 transcription factor and induces the expression of *brlA* by directly binding to the promoter of *brlA*. Another pathway consists of FlbE, FlbB, and FlbD. In this pathway, FlbB functions as a bZIP-type transcription factor and forms a complex with FlbE. This complex induces the expression of *flbD*, which encodes a cMyc-type transcription factor. FlbE interacts and colocalizes with FlbB at the hyphal tips, suggesting that FlbE ensures the proper localization and function of FlbB (Garzia et al. 2009). Both FlbB and FlbD induce the expression of *brlA* by directly binding to the promoter of *brlA*. The expression levels of *brlA* significantly decrease in mutants of the genes encoding these activators, and the mutants show the fluffy phenotype (Wieser et al. 1994). Negative regulators of conidiation have also been identified. SfgA is a putative transcription factor with a Gal4-type $Zn(II)_2Cys_6$ binuclear cluster DNA-binding domain. The deletion of *sfgA* suppressed the fluffy phenotype of the *fluG* deletion mutant but not the deletion mutants of *flbA*, *flbB*, *flbC*, and *flbD*, indicating that SfgA acts as a negative regulator of conidiation by functioning

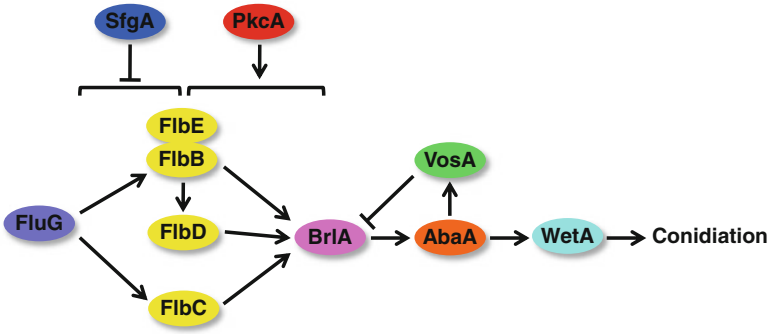

Fig. 12.5 Involvement of PkcA in conidiation. Details discussed in text

downstream of FluG but upstream of FlbA, FlbB, FlbC, and FlbD (Seo et al. 2006). The deletion of *sfgA* did not suppress the decrease in conidiation efficiency of the *pkcA*-ts mutant at 37 °C, suggesting that PkcA regulates the expression of *brlA* downstream of SfgA (Katayama and Horiuchi, unpublished results). The *velvet* protein VosA is suggested to repress conidiation during vegetative growth (Bayram and Braus 2012; Park et al. 2012). In addition, VosA binds to the promoter of *brlA* and represses the expression of *brlA* (Park et al. 2012). The expression of *vosA* is induced by AbaA, which is a TEA/ATTS transcription factor that functions downstream of BrlA, suggesting that VosA plays a role in the negative feedback regulation of *brlA* (Park and Yu 2012). The deletion of *vosA* did not suppress the decrease in the conidiation efficiency of the *pkcA*-ts mutant at 37 °C, suggesting that VosA is not involved in the PkcA regulation of *brlA* expression (Katayama and Horiuchi, unpublished results). These results are summarized in Fig. 12.5.

Because conidiophores of *Aspergillus* have distinctive structural features and those are not conserved in the filamentous fungi other than Ascomycota, there are no orthologues of the genes involved in the formation of conidiophores and conidia in filamentous fungi belonging to Basidiomycota and Mucoromycotina. In Ascomycota, only the orthologous genes of *brlA* are conserved in the genomes belonging to the genus *Aspergillus* or *Penicillium*. Future studies are required for understanding the role of PKC in asexual development of filamentous fungi of other genera.

12.2.3 Other Functions of PKC in Filamentous Fungi

PKCs in filamentous fungi have also been reported to have other functions, as described next.

Unfolded protein response (UPR) is a stress response caused by endoplasmic reticulum stresses. Farnesol induces apoptosis in filamentous fungi, and farnesol treatment of *Aspergillus nidulans* has been reported to induce UPR via the PKC signaling pathway, suggesting that PkcA is involved in the induction of UPR (Colabardini et al. 2010). In *Neurospora crassa*, PKC is known to regulate light

responses via phosphorylating the WC-1 protein, which is a blue light photoreceptor (Franchi et al. 2005). In *Aspergillus oryzae*, the assembly of a structural protein of Woronin body, AoHex1, has been suggested to be regulated by PKC activity (Juvvadi et al. 2007) (see Chap. 11). Because the orthologues of the genes encoding WC-1 and AoHex1 are present in the genome of filamentous fungi belonging to Ascomycota, PKCs in these fungi likely have similar functions.

Recently, transcriptome analysis of *A. niger* was performed under carbon starvation conditions. Carbon starvation induces hyphal morphological changes and asexual development. The expression of a PKC-encoding gene was slightly upregulated under this starvation condition (Nitsche et al. 2012). This observation may suggest a novel function of PKC for morphogenesis in filamentous fungi.

In this chapter, we described the functions of PKC in the morphogenesis of filamentous fungi by focusing on the *A. nidulans* PkcA under cell wall stress or heat stress. The factors that function upstream of PKC are currently unknown under these conditions, except for MtlA of *A. nidulans* (Futagami et al. 2014) (see Chap. 10).

Although PKC signaling pathways in *S. cerevisiae* are activated by many environmental stresses, such as cell wall stress, heat stress, oxidative stress, hypoosmotic stress, and endoplasmic reticulum stress (Levin 2005, 2011), these functions remain largely uncharacterized in filamentous fungi, partly because of the difficulties of handling filamentous fungi at the molecular level. Because filamentous fungi encounter various stresses in the culture conditions used in industry, it is very important to understand their responses to these stresses. This information enables us to improve the culture conditions and how to breed strains at the molecular level. PKC is probably pivotal in many aspects of these conditions. Because molecular biological techniques have much improved recently in filamentous fungi, other functions of PKCs related to the stresses affecting morphogenesis and conidiation will be elucidated in the near future.

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