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Key transcription factors required for outburst of rice blast disease in *Magnaporthe oryzae*

Qing Wang[1], Zhicheng Huang[1], Irshad Ali Khan[2], Yan Li[1], Jing Wang[3], Jiaoyu Wang[3], Xiao-Hong Liu[4], Fucheng Lin[3,4] and Jianping Lu[1*]

Abstract

Rice blast is a serious threat to the safe production of grain crops such as rice and wheat. Sporulation, appressorium formation, and invasive growth of *Magnaporthe oryzae* are the key stages of the development and spread of rice blast epidemics. *M. oryzae* is a hemibiotrophic fungus that undergoes changes in available carbon sources during the infection cycle. Lipid is a major storage for *M. oryzae* spores and a major carbon source used in glycerol synthesis and turgor pressure generation in appressoria. The formation of a dense cell wall melanin layer is necessary for an appressorium to produce turgor and to be pathogenic. The plant cell wall is an important carbon source during the infection stage of *M. oryzae*. Transcription factors regulate gene expression in fungi and are key intermediates between the reception of external environmental signals and the control of development and pathogenicity in *M. oryzae*. The disease cycle of *M. oryzae* is controlled by some key transcription factors, such as sporulation by Cos1 and Hox2, appressorium formation by Sfl1, Hox7, and Vrf1, invasive growth by Mst12 and Mig1, and resistance to host basal immunity by Ap1 and Atf1. This review focuses on describing the key transcription factors of *M. oryzae* that regulate sporulation, appressorium formation, invasive growth, lipid metabolism, carbohydrate metabolism, melanin synthesis, oxidative response, and host basal immunity, as well as the working mechanism of the transcription factors.

Keywords Appressorium formation, Conidiation, Invasive growth, Carbohydrate metabolism, Lipid metabolism, Melanin synthesis, Oxidative response, Rice blast fungus, Transcription factor, Virulence

*Correspondence:
Jianping Lu
jplu@zju.edu.cn
[1] State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-Products, College of Life Sciences, Zhejiang University, Hangzhou 310058, China
[2] Department of Agriculture (Plant Pathology), The University of Swabi, Anbar 23561, Khyber Pakhtunkhwa, Pakistan
[3] State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-Products, Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China
[4] Institute of Biotechnology, Zhejiang University, Hangzhou 310058, China

Background

Rice blast is a plant epidemic disease caused by *Magnaporthe oryzae* (synonym *Pyricularia oryzae*), which infects rice, wheat, and other gramineous crop plants. In severe disease epidemics, it can lead to the complete loss of crops such as rice and wheat (Dean et al. 2012). The disease is spread by asexual spores (conidia) of *M. oryzae*. The asexual spores of *M. oryzae* are spread to nearby plants by wind, and the spores germinate in water droplets and form appressoria, which penetrate the plant cell wall and invade the rice cells. In 2 days post inoculation (dpi), the fungus lives a biotrophic life with rice cells, then kills rice cells and lives a necrotrophic life. The invasive hyphae simultaneously penetrate neighboring plant

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cells and repeat the same process. About 5–7 dpi, disease lesions are formed on the plant's surface, such as leaves and stalks, and a large number of secondary spores are produced and the spores fall and spread to start a new cycle of spread and disease. Sporulation (conidiation), appressorium formation, and invasive hyphal growth are the three key nodal points in the pathogenesis of *M. oryzae* (Dean et al. 2012; Valent 2021). These key nodal points are rigorously controlled by several specific transcription factors. For example, sporulation is regulated by transcription factors, such as Cos1, Hox2, and Acr1 (Lau and Hamer 1998; Kim et al. 2009; Zhou et al. 2009), appressorium formation is regulated by transcription factors, such as Sfl1, Hox7, and Vrf1 (Kim et al. 2009; Cao et al. 2016; Li et al. 2017), and invasive growth is regulated by transcription factors, such as Mst12, Ap1, and MoHtr3 (Park et al. 2002; Guo et al. 2011; Lee et al. 2023).

In its infection cycle, *M. oryzae* regulates the expression of genes in response to changes in its environment and developmental stage (Osés-Ruiz et al. 2021). The general process is that environmental signals are perceived by the receptor and transmitted through signaling pathways, activate transcription factors, promote gene expression and protein synthesis, and generate new metabolic activities that allow the cell to adapt to a new environment or to construct new cellular structures. In this process, transcription factors regulate gene expression. Melanin is one of the key components of the appressorial cell wall of *M. oryzae* and is necessary for an appressorium to maintain a huge turgor pressure (as much as 8.0 MPa) and penetrate the plant cell wall (Howard et al. 1991). Melanin synthesis is regulated by the transcription factors, such as Pig1, Cnf1, and Vrf1 (Tsuji et al. 2000; Lu et al. 2014; Cao et al. 2016). Glycerol is the main solute for an appressorium to generate turgor pressure, and lipid is one of the main carbon and energy sources for spore storage (Wang et al. 2005). Lipid degradation is regulated by transcription factors such as Crf1, Gpf1, and CreA (Lu et al. 2014; Cao et al. 2016, 2018). At different developmental stages, *M. oryzae* faces changes in the types of carbon sources available, and shifts between carbon metabolisms are necessary for appressorium turgor production and invasive hyphal growth (Fernandez et al. 2012). Crf1 and CreA are involved in the regulation of the transitions among carbon metabolisms (Cao et al. 2018; Huang et al. 2023a).

There has been a lot of research on transcription factors in *M. oryzae* (Table 1), including large-scale knockout studies (Lu et al. 2014; Tang et al. 2015; Cao et al. 2016), which have identified many transcription factors regulating important developmental and metabolic processes (Motaung et al. 2017; Tan et al. 2023), and there have been some reviews dedicated to transcription factors of pathogenic fungi (John et al. 2021); however there is a lack of systematic discussion on the biological functions of the key transcription factors involved in the development and spread of rice blast disease and the functional relationships among these transcription factors. As a number of transcription factors have been implicated in the pathogenicity of *M. oryzae* (Table 1), this review mainly discusses the roles and possible mechanisms of key transcription factors in sporulation, appressorium formation, invasive growth, carbon metabolism, melanin synthesis, oxidative response, and host basal immunity.

Key transcription factors regulating asexual reproduction in *M. oryzae*

Rice blast is spread by airborne asexual spores. The asexual reproduction of *M. oryzae* is influenced by environmental factors, such as light, humidity, and nutrients (Lee et al. 2006). These environmental factors are transmitted to transcription factors through intracellular signal sensing and transduction systems, which regulate the expression of sporulation-related genes and affect conidiogenesis, spore morphogenesis, and spore production in fungi. Fungi have several signaling pathways to transmit environmental signals to spore-producing transcription factors, such as transcription factors Som1 and Cdtf1 that are regulated by the cAMP-PKA signaling pathway (Yan et al. 2011), MoSwi6 and Mig1 that are regulated by the Mps1-MAPK signaling pathway (Mehrabi et al. 2008; Qi et al. 2012), Crz1 that is regulated by the Ca^{2+} signaling pathway (Choi et al. 2009; Zhang et al. 2009; Cao et al. 2016), and Sfl1 that is regulated by the cAMP-PKA and Pmk1-MAPK signaling pathways (Li et al. 2011, 2017). Changes in intracellular metabolic processes in *M. oryzae* also frequently affect spore production. These metabolic processes include nutrient metabolisms such as autophagy, carbon metabolism, and nitrogen metabolism (Li et al. 2020; Cai et al. 2022; Huang et al. 2023a), cell wall integrity (Soanes et al. 2002), and cell polarity growth (Chen et al. 2008). In gene function analyses of *M. oryzae*, knockout of many genes was found to affect spore production of the mutants to a greater or lesser extent (Tan et al. 2023). This information suggests that the pathways by which transcription factors affect spore production are rather complex, and therefore, only a few key transcription factors that affect the sporulation process are discussed below.

The sporulation process of *M. oryzae* involves the differentiation of substrate hyphae into aerial hyphae, the differentiation of aerial hyphae into conidiophores, the apical differentiation of conidiophores to produce newborn spores consisting of one cell, and the development of newborn spores into mature spores consisting of three cells (Fig. 1). The increase in the expression of hyphal

Table 1 Transcription factors studied by gene knockout in *M. oryzae*

Locus	Gene	Function*	References
MGG_00021	*FZC62*	C, G, S	Lu et al. (2014)
MGG_00080	*ZFP7*	N	Cao et al. (2016)
MGG_00096	*FZC63*	G, S	Lu et al. (2014)
MGG_00184	*HOX2, HTF1*	C	Kim et al. (2009), Liu et al. (2010) and Huang et al. (2022b)
MGG_00320	*FZC64*	N	Lu et al. (2014)
MGG_00329	*FZC27*	A, S	Lu et al. (2014)
MGG_00342	*BZIP1*	C	Kong et al. (2015) and Tang et al. (2015)
MGG_00354	*HAPB*	C, G	Nakajima et al. (2010)
MGG_00373	*GCP1*	C, G, V	Cao et al. (2016)
MGG_00417	*FZC65*	S	Lu et al. (2014)
MGG_00494	*PRO1*	P	Lu et al. (2014), Uchida et al. (2023)
MGG_00501	*MSN2, TDG2*	A, C, G, I, S, V	Breth et al. (2013) and Zhang et al. (2014)
MGG_00504	*NSDC*	C, G	Cao et al. (2016)
MGG_00587	*BZIP2*	C, G	Kong et al. (2015) and Tang et al. (2015)
MGG_00617	*VOSA*	N	Kim et al. (2014)
MGG_00622	*NDT80*	Cm, V	Bhatt et al. (2020)
MGG_00660	*ZFP1*	V	Cao et al. (2016)
MGG_00672	*LEU3*	C, G, V	Wei et al. (2019) and Que et al. (2020)
MGG_00692	*MSTU1*	A, C, V	Nishimura et al. (2009)
MGG_08753	*MoEITF1*	I, V	Cao et al. (2022)
MGG_08850	*GTI1*	A, C, G, I, S, V	Li et al. (2016)
MGG_01017	*CONX3*	A, C, V	Cao et al. (2016)
MGG_01057	*LDB1*	C, G, P, V	Li et al. (2010)
MGG_01127	*CONX7*	C	Cao et al. (2016)
MGG_01204	*MIG1*	C, G, I, S, V	Mehrabi et al. (2008)
MGG_01215	*COM1*	A, C, I, V	Yang et al. (2010)
MGG_01285	*TPC1*	A, C, G, S	Galhano et al. (2017)
MGG_01414	*XLR1*	Cm	Battaglia et al. (2013)
MGG_01486	*FZC2*	N	Lu et al. (2014)
MGG_01518	*NIT4, NIR1*	C, Nm, S	Wilson et al. (2010) and Lu et al. (2014)
MGG_01620	*VELB*	C, G, S	Kim et al. (2014)
MGG_01624	*FZC3*	N	Lu et al. (2014)
MGG_01632	*MoNTE1*	A, C, G, I, V	Chen et al. (2023)
MGG_01730	*HOX3*	N	Kim et al. (2009)
MGG_01734	*FZC4*	S	Lu et al. (2014)
MGG_01776	*VRF2*	C, V	Cao et al. (2016)
MGG_01779	*FZC29*	S	Lu et al. (2014)
MGG_01833	*FZC5*	C, S	Lu et al. (2014)
MGG_01836	*FAR1*	Cm	Bin Yusof et al. (2014)
MGG_01853	*FOX1*	N	Park et al. (2014)
MGG_01887	*FZC30*	S	Lu et al. (2014)
MGG_01990	*MoIDI4*	N	Kong et al. (2015) and Tang et al. (2015)
MGG_02006	*BZIP3*	A, C, Cm, G, V	Kong et al. (2015), Tang et al. (2015) and Liu et al. (2022)
MGG_02036	*ZFP8*	V	Cao et al. (2016)
MGG_02055	*CONX4*	C	Cao et al. (2016)
MGG_02089	*FZC66*	S	Lu et al. (2014)
MGG_02289	*FZC67*	G, S	Lu et al. (2014)
MGG_02377	*FZC6*	N	Lu et al. (2014)
MGG_02474	*GPF2*	G, V	Cao et al. (2016)

Table 1 (continued)

Locus	Gene	Function*	References
MGG_02595	*FZC7*	A	Lu et al. (2014)
MGG_02632	*MoFCR3*	N	Kong et al. (2015) and Tang et al. (2015)
MGG_02755	*NUT1, AREA*	Nc	Froeliger and Carpenter (1996) and Wilson et al. (2010)
MGG_02773	*MCM1*	A, P, S, V	Zhou et al. (2011)
MGG_02775	*CONX2*	C, V	Cao et al. (2016)
MGG_02865	*BZIP4*	N	Kong et al. (2015) and Tang et al. (2015)
MGG_02866	*FZC31*	G, S	Lu et al. (2014)
MGG_02879	*FZC8*	S	Lu et al. (2014)
MGG_02880	*FZC9*	N	Lu et al. (2014)
MGG_02962	*CNF1*	C, M, V	Lu et al. (2014) and Huang et al. (2022b)
MGG_03030	*CONX11*	C	Cao et al. (2016)
MGG_03133	*CONX6*	C	Cao et al. (2016)
MGG_03183	*CNF4*	C, S	Lu et al. (2014)
MGG_03288	*BZIP5*	A, C	Kong et al. (2015) and Tang et al. (2015)
MGG_03451	*GCF6*	C, G, V	Cao et al. (2016)
MGG_03463	*FZC10*	S	Lu et al. (2014)
MGG_03538	*WC1*	C, V	Kim et al. (2011)
MGG_03581	*ZFP9*	G	Cao et al. (2016)
MGG_03669	*FZC68*	S	Lu et al. (2014)
MGG_03711	*FZC87*	S	Lu et al. (2014)
MGG_03977	*COS1*	C	Zhou et al. (2009), Cao et al. (2016) and Huang et al. (2022b)
MGG_04000	*RFX1*	A, C, G, S, V	Sun et al. (2017)
MGG_04009	*BZIP6*	N	Kong et al. (2015) and Tang et al. (2015)
MGG_04108	*TAS1*	S	Lu et al. (2014)
MGG_04141	*FZC69*	A, G, S	Lu et al. (2014)
MGG_04326	*FZC70*	G, S	Lu et al. (2014)
MGG_04328	*ZFP10*	G, V	Cao et al. (2016)
MGG_04360	*FZC71*	S	Lu et al. (2014)
MGG_04387	*FZC1*	S	Lu et al. (2014)
MGG_04456	*ZAP1*	C	Fernandez et al. (2012) and Cao et al. (2016)
MGG_04489	*MNH6*	A, C, G, V	Lu et al. (2007)
MGG_04580	*MoHTR3*	I, V	Lee et al. (2023)
MGG_04674	*FZC32*	S	Lu et al. (2014)
MGG_04699	*FLB3, MoFLBC*	A, C, V	Cao et al. (2016) and Matheis et al. (2017)
MGG_04708	*SOM1*	C, G, M, P, V	Yan et al. (2011)
MGG_04758	*BZIP12*	C	Kong et al. (2015) and Tang et al. (2015)
MGG_04843	*C19TRF1, FZC11, IRR*	Cm	Lu et al. (2014), Jacob et al. (2017) and Wang et al. (2018)
MGG_04853	*HOX1, C19TRF2*	Cm	Kim et al. (2009) and Jacob et al. (2017)
MGG_04865	*ZFP2*	G	Cao et al. (2016)
MGG_04933	*FZC72*	S	Lu et al. (2014)
MGG_05033	*FZC33*	G, S	Lu et al. (2014)
MGG_05133	*CRZ1*	C, G, V	Choi et al. (2009), Zhang et al. (2009) and Cao et al. (2016)
MGG_05153	*FZC73*	G, S	Lu et al. (2014)
MGG_05287	*CON7*	A, C, V	Odenbach et al. (2007) and Cao et al. (2016)
MGG_05306	*MoMEAB*	C, G, Nm	Kong et al. (2015) and Tang et al. (2015)
MGG_05343	*COD1*	C, G, S, V	Chung et al. (2013) and Lu et al. (2014)
MGG_05501	*ZFP11*	V	Cao et al. (2016)
MGG_05659	*CCA1*	A, C, G, S, V	Lu et al. (2014)
MGG_05709	*CRF1*	A, C, Cm, G, V	Cao et al. (2018) and Huang et al. (2023a)

Table 1 (continued)

Locus	Gene	Function*	References
MGG_05714	*ZFP3*	G	Cao et al. (2016)
MGG_05724	*FZC74*	S	Lu et al. (2014)
MGG_05829	*FZC12*	S	Lu et al. (2014)
MGG_05845	*FZC75*	A, S	Lu et al. (2014)
MGG_05891	*FZC13*	C, G, S	Lu et al. (2014)
MGG_05959	*MoHAPX*	N	Kong et al. (2015) and Tang et al. (2015)
MGG_06072	*SVF1*	C, G	Fu et al. (2023)
MGG_06131	*BZIP10*	A, C, G, I, V	Kong et al. (2015) and Tang et al. (2015)
MGG_06243	*FZC76*	S	Lu et al. (2014)
MGG_06258	*MoFKH1*	C, G, V, S	Park et al. (2014)
MGG_06279	*FZC34*	S	Lu et al. (2014)
MGG_06285	*HOX4*	C	Kim et al. (2009)
MGG_06312	*FZC35*	N	Lu et al. (2014)
MGG_06328	*GCF4*	C, G	Cao et al. (2016)
MGG_06355	*FZC14*	C, S	Lu et al. (2014
MGG_06364	*GCF7*	C, G	Cao et al. (2016)
MGG_06416	*FZC36*	N	Lu et al. (2014
MGG_06422	*HCM1*	C, G, S, V	Park et al. (2014
MGG_06455	*FZC77*	S	Lu et al. (2014)
MGG_06575	*CONX9*	C	Cao et al. (2016)
MGG_06626	*FZC37*	C, S	Lu et al. (2014)
MGG_06778	*FZC38*	N	Lu et al. (2014)
MGG_06832	*TDG3, FZC39*	N	Breth et al. (2013) and Lu et al. (2014)
MGG_06848	*CONX8*	C	Cao et al. (2016)
MGG_06898	*FLB4, MoMYB1*	C, G, S, V	Dong et al. (2015) and Matheis et al. (2017)
MGG_06954	*ARA1*	Cm	Klaubauf et al. (2016)
MGG_06971	*SFL1*	A, C, I, V	Li et al. (2011) and Li et al. (2017)
MGG_07011	*GPF3*	G, V	Cao et al. (2016)
MGG_07013	*ZFP12*	N	Cao et al. (2016)
MGG_07063	*GCC1*	C, G, S	Lu et al. (2014) and Huang et al. (2022b)
MGG_07149	*GTA1*	G, C, S, V	Lu et al. (2014)
MGG_07215	*PIG1*	M, S	Tsuji et al. (2000), Lu et al. (2014) and Huang et al. (2022b)
MGG_07218	*HTFG*	N	Oh et al. (2008)
MGG_07269	*ZFP13*	N	Cao et al. (2016)
MGG_07305	*BZIP7*	N	Kong et al. (2015) and Tang et al. (2015)
MGG_07339	*CONX5*	C, V	Cao et al. (2016)
MGG_07437	*HOX5*	N	Kim et al. (2009)
MGG_07450	-	N	Minh et al. (2023)
MGG_07458	*FZC78*	N	Lu et al. (2014)
MGG_07534	*FZC41*	S	Lu et al. (2014)
MGG_07549	*FZC42*	G, S	Lu et al. (2014)
MGG_07636	*FZC79*	G, S	Lu et al. (2014)
MGG_07681	*FZC28*	G, S	Lu et al. (2014)
MGG_07777	*FZC43*	C, S	Lu et al. (2014)
MGG_07800	*FZC15, TAS2*	Sm	Lu et al. (2014) and Yun et al. (2017)
MGG_07845	*FZC44*	G	Lu et al. (2014)
MGG_07925	*BZIP11*	C	Kong et al. (2015) and Tang et al. (2015)
MGG_08058	*MoACEII*	G, S	Lu et al. (2014)
MGG_08094	*FZC16*	S	Lu et al. (2014)

Table 1 (continued)

Locus	Gene	Function*	References
MGG_08130	*FZC17*	S	Lu et al. (2014)
MGG_08185	*FZC45*	C, G, S	Lu et al. (2014)
MGG_08199	*FAR2*	Cm	Bin Yusof et al. (2014)
MGG_08203	*MBF1*	G, S, V	Fan et al. (2017)
MGG_08212	*ATF1*	C, S, V	Guo et al. (2010), Kong et al. (2015) and Tang et al. (2015)
MGG_08314	*FZC46*	G	Lu et al. (2014)
MGG_08361	*FZC80*	S	Lu et al. (2014)
MGG_08493	*CONX10*	C	Cao et al. (2016)
MGG_08556	*VEA*	A, C, V	Kim et al. (2014)
MGG_08777	*FZC18*	S	Lu et al. (2014)
MGG_08784	*FZC47*	S	Lu et al. (2014)
MGG_08974	*FZC81*	S	Lu et al. (2014)
MGG_09010	*HAC1*	C, G, V	Kong et al. (2015) and Tang et al. (2015)
MGG_09027	*FZC49*	S	Lu et al. (2014)
MGG_09031	-	N	Oh et al. (2008)
MGG_09200	*TDG1*	C, V	Breth et al. (2013) and Cao et al. (2016)
MGG_09263	*COD2*	C, V	Chung et al. (2013)
MGG_09273	*FZC50*	G, S	Lu et al. (2014)
MGG_09276	*CRG1*	N	Oh et al. (2008)
MGG_09312	*FZC51*	S	Lu et al. (2014)
MGG_09676	*FZC52*	S	Lu et al. (2014)
MGG_09780	*ZFP4*	N	Cao et al. (2016)
MGG_09829	*FZC53*	S	Lu et al. (2014)
MGG_09847	*ACR1*	C	Lau and Hamer (1998) and Nishimura et al. (2000)
MGG_09869	*MoSWI6*	C, G, M, S, V	Qi et al. (2012)
MGG_09950	*FZC54*	G, S	Lu et al. (2014)
MGG_10150	*PACC*	C, S, V	Landraud et al. (2013)
MGG_10197	*TRA1*	A, V, S	Breth et al. (2013) and Lu et al. (2014)
MGG_10276	*MoHTR1*	I, V	Kim et al. (2020)
MGG_10280	*MoHTR2*	I, V	Kim et al. (2020)
MGG_10595	*GCF2*	C, G, V	Cao et al. (2016)
MGG_10660	*BZIP8*	N	Kong et al. (2015) and Tang et al. (2015)
MGG_11116	*FZC19*	S	Lu et al. (2014)
MGG_11201	*CREA*	C, Cm, V	Cao et al. (2016), Cao et al. (2018), Hong et al. (2021) and Huang et al. (2023a)
MGG_11252	*GCF3*	C, G, V	Cao et al. (2016) and Huang et al. (2022b)
MGG_11346	*CDTF1*	C, G, M, P, V	Yan et al. (2011)
MGG_11712	*HOX6*	C, G	Kim et al. (2009)
MGG_11764	*FZC55*	A, S	Lu et al. (2014)
MGG_11925	*ZFP14*	A	Cao et al. (2016)
MGG_12037	*FZC20*	S	Lu et al. (2014)
MGG_12339	*FZC82*	A, S	Lu et al. (2014)
MGG_12349	*CONX1*	C, S, V	Lu et al. (2014)
MGG_12424	*FZC21*	G, S	Lu et al. (2014)
MGG_12560	*MoEITF2, BZIP9*	C, I, V	Kong et al. (2015), Tang et al. (2015) and Cao et al. (2022)
MGG_12776	*FZC56*	C, S	Lu et al. (2014)
MGG_12814	*AP1*	C, S, V	Guo et al. (2011), Kong et al. (2015), Tang et al. (2015) and Guo et al. (2019)
MGG_12865	*HOX7*	A, M, V	Kim et al. (2009), Osés-Ruiz et al. (2021) and Huang et al. (2022a)
MGG_12958	*MST12, HOX8*	A, I, V	Park et al. (2002), Park et al. (2004), Kim et al. (2009), Cao et al. (2016) and Osés-Ruiz et al. (2021)
MGG_13350	*FZC57*	C, S	Lu et al. (2014)

Table 1 (continued)

Locus	Gene	Function*	References
MGG_13360	*CNF3*	C	Lu et al. (2014)
MGG_13385	*FZC58*	S	Lu et al. (2014)
MGG_13629	*FZC59*	S	Lu et al. (2014)
MGG_13927	*FZC83*	G, S	Lu et al. (2014)
MGG_14175	*FZC60*	S	Lu et al. (2014)
MGG_14561	*METR*	G, C, Nm, V	Kong et al. (2015) and Tang et al. (2015)
MGG_14719	*VELC*	C, I, S, V	Kim et al. (2014)
MGG_14728	*FZC48*	N	Lu et al. (2014)
MGG_14806	*ZFP5*	G, V	Cao et al. (2016)
MGG_14816	*FZC84*	S	Lu et al. (2014)
MGG_14852	*FZC61*	C, S	Lu et al. (2014)
MGG_14931	*VRF1, ZNF1*	A, M, V	Cao et al. (2016), Yue et al. (2016), Huang et al. (2022a) and Wang et al. (2022)
MGG_15023	*CNF2*	C, S, V	Lu et al. (2014)
MGG_15139	*FZC85*	S	Lu et al. (2014)
MGG_15508	*ZFP6*	V	Cao et al. (2016)
MGG_15991	*ZFP15*	N	Cao et al. (2016)
MGG_16444	*FZC22*	C, S	Lu et al. (2014)
MGG_16756	*FZC23*	N	Lu et al. (2014)
MGG_17012	*FZC86*	S	Lu et al. (2014)
MGG_17060	*FZC24*	S	Lu et al. (2014)
MGG_17623	*PCF1*	C, S, V	Lu et al. (2014)
MGG_17669	*FZC25*	C	Lu et al. (2014)
MGG_17821	*FZC26*	C, S	Lu et al. (2014)
MGG_17841	*GPF1*	A, Cm, G, S, V	Lu et al. (2014)
MGG_17953	*GCF5*	C, G	Cao et al. (2016)

*A, Appressorium formation; C, Conidiation; Cm, Carbohydrate metabolism; G, Growth; I, Invasive growth; L, Lipid metabolism; M, Melanin synthesis; N, Non-essential; Nm, Nitrogen metabolism; P, Perithecium formation; S, Stress response; Sm, Secondary metabolism; V, Virulence

hydrophobic proteins plays a key role in the differentiation of the substrate hyphae into aerial hyphae of *M. oryzae*. The hydrophobic protein Mpg1 is distributed on the surface of hyphae, spores, and appressoria, and the *MPG1* deletion mutant Δ*mpg1* has a reduced hydrophobicity of aerial hyphae and reduced spore production (Beckerman and Ebbole 1996; Talbot et al. 1996). Deletion of another hydrophobic protein gene, *MHP1*, reduces surface hydrophobicity and spore production (Kim et al. 2005). Flb3 (=MoFlbC) and Flb4 (=MoMyb1) are two transcription factors containing Cys_2His_6 (C2H2) and Myb-like domains. The expression of *MPG1* is down-regulated by 133-fold in Δ*flb3* and 45-fold in Δ*flb4*. Δ*flb3* has few aerial hyphae and produces few spores, but the colonies are darker (Cao et al. 2016; Matheis et al. 2017). Δ*flb4* has normal aerial hyphae but does not produce spores and conidiophores and has white colonies (Dong et al. 2015; Matheis et al. 2017). Thus, Flb3 regulates the differentiation of substrate hyphae into aerial hyphae, whereas Flb4 regulates the differentiation of aerial hyphae to conidiophores (Matheis et al. 2017) (Fig. 1a). Cos1 is a C2H2 domain transcription factor. The aerial hyphae of Δ*cos1* cannot differentiate into conidiophores and produce no spores. Cos1 regulates the differentiation of aerial hyphae into conidiophores (Zhou et al. 2009) (Fig. 1a). Hox2 (=Htf1) is a homeobox transcription factor. The aerial hyphae of Δ*hox2* (=Δ*htf1*) can form conidiophores, but the tips of conidiophores cannot differentiate to form spores. Therefore, Hox2 regulates conidiophore differentiation (Kim et al. 2009; Liu et al. 2010) (Fig. 1a). The Δ*msn2* mutant has decreased expression of *COS1* and *HOX2* and produces few elongated spores. Msn2 binds to the AGGGG motif of the *COS1* promoter, suggesting that the transcription factor Msn2 acts upstream of Cos1 (Zhang et al. 2014). Alternatively, Msn2 also affects mitochondrial morphology and the growth of invasive hyphae by regulating the expression of *MoAUH1* encoding a putative mitochondrial 3-methylglutaconyl-CoA hydratase (Xiao et al. 2021).

Generally, *M. oryzae* spores are sympodially borne from an aerial conidiophore (Fig. 1a). Three transcription factors (Acr1, Cca1, and Con7) are associated with spore-bearing mode on a conidiophore, but the functions of the three genes are distinctly different from

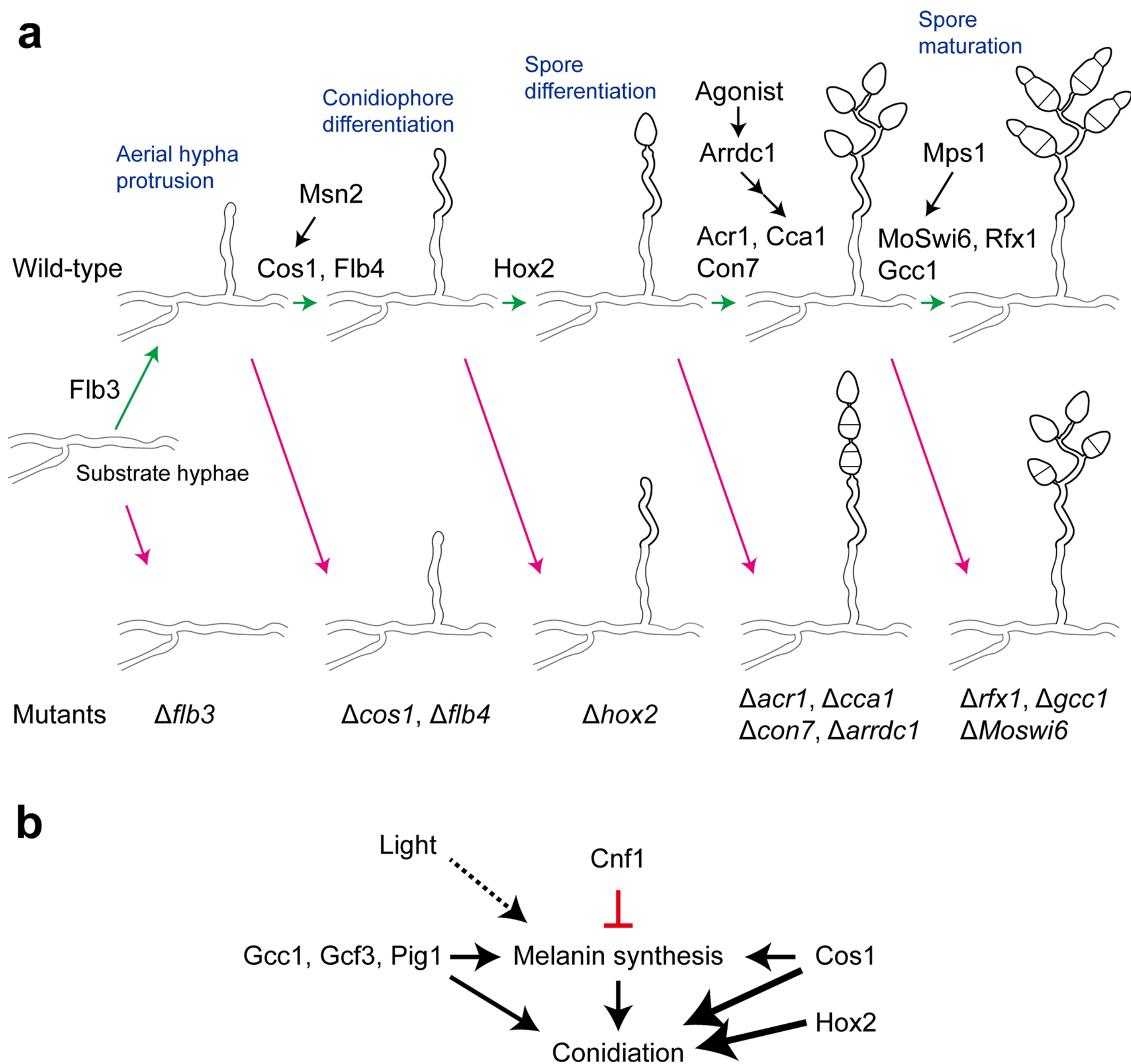


Fig. 1 Transcription factors regulating asexual reproduction in *M. oryzae*. **a** Functions of transcription factors during conidiation. The time points at which transcription factors work (Flb3, Cos1, Flb4, Hox2, Acr1, Cca1, Con7, Rfx1, Gcc1, and MoSwi6) and the phenotypes of their mutants. Arrdc1 is an α-arrestin protein that interacts with G protein-coupled receptors. **b** Effect of transcription factors (Gcc1, Gcf3, Pig1, Cnf1, Cos1, and Hox2) on conidiation through melanin synthesis

each other. Spores of the random insertion mutant *acr1* form in a head-to-tail (acropetal) array on a conidiophore (Lau and Hamer 1998; Nishimura et al. 2000). Further functional analysis of Acr1 in *M. oryzae* is lacking, but Acr1 homologous proteins Ren1, MedA, and Med1 in *Fusarium oxysporum*, *Aspergillus fumigatus*, and *F. graminearum* are localized in nuclei (Ohara et al. 2004; Al Abdallah et al. 2012; Fan et al. 2019), suggesting that Acr1 is a transcriptional regulator. After deletion of *CCA1*, a Zn(II)$_2$Cys$_6$ transcription factor gene, the spores are produced from conidiophores in head-to-tail arrays, and the spores also become longer (Lu et al. 2014). In the C2H2 transcription factor *CON7*-deletion mutant (Δ*con7*), spores also differentiate in an acropetal manner at the top of conidiophores; however, the formed spores are easily dried out, and conidial cell walls are severely damaged (Cao et al. 2016). In the *con7* mutant, in which an exogenous DNA was inserted into the promoter region of *CON7*, the morphology of spores and appressoria is abnormal in the chitin layer structure of

cell walls, but the spores are not shrivelled (Odenbach et al. 2007). Thus, spore desiccation in Δ*con7* is caused by the loss of water from the spores due to the abnormal structure of the cell wall chitin layer. Arrestins are adaptor proteins involved in the regulation of G protein-coupled receptor (GPCR) signaling and trafficking, including endocytosis, protein degradation, and exocytosis (Puca and Brou 2014). In *M. oryzae*, Δ*arrdc1* spores differentiate in an acropetal manner from conidiophores, and the expression of *CCA1* and *COS1* is significantly down-regulated in the aerial mycelia of Δ*arrdc1* (Dong et al. 2016). Δ*arrdc1* produces few spores consisting of 1–4 cells, with an elongated morphology, of which about 20% have more than 3 cells and about 40% have 1–2 cells (Dong et al. 2016). It suggests that the arrestin signaling pathway is involved in the regulation of spore patterning on conidiophores and conidial cell division, which may be carried out through Cca1 and Cos1 (Fig. 1a).

The maturation of spores, which is the process of spore division from 1 to 3 cells, is regulated by the cell cycle. Approximately 80% of spores in the wild-type strain 70-15 consist of 3 cells (Sun et al. 2017; Huang et al. 2022b). Many proteins that regulate the cell cycle (mitosis or cytokinesis) and apical growth are involved in regulating the spore cell division process. For example, deletion of the *CDC15* gene, encoding a kinase that regulates the mitotic process, leads to the production of conidia consisting of 1–5 cells in *M. oryzae* (Goh et al. 2011); deletion of the *TEA1* or *TEA4* genes, which regulate apical growth, leads to the production of conidia predominantly consisting of 2 cells (Patkar et al. 2010; Qu et al. 2022). Rfx1 is a regulatory factor X (RFX) domain transcription factor that regulates cell division. Δ*rfx1* only forms spores consisting of 1 or 2 cells and fails to produce spores with 3 cells (Sun et al. 2017) (Fig. 1a). In the yeast *Saccharomyces cerevisiae,* Swi6 is a transcription factor that regulates cell division. In *M. oryzae*, about 40% of the Δ*Moswi6* spores consist of 2 cells (Qi et al. 2012). Δ*gcc1* produces very few spores, and about 80% of the spores consist of 1 or 2 cells; however, the molecular mechanism of Gcc1, a C2H2 transcription factor, has not yet been identified (Huang et al. 2022b) (Fig. 1a). The spore morphology of Δ*cca1* is also abnormal, mainly consisting of 1 or 2 cells, and some spores are very long (Lu et al. 2014) (Fig. 1a). The $Zn(II)_2Cys_6$ transcription factor Tpc1 regulates polar cell growth, and 1% of Δ*tpc1* spores consist of 4 cells (Galhano et al. 2017). When *MoFKH1*, encoding a forkhead-box (FOX) transcription factor, is deleted, more than 10% of spores in the mutant consist of 4–6 cells (Park et al. 2014). In addition, some transcription factors preferentially affect the spore morphology (the ratio of conidial length to width, etc.). For example, spores of knockout mutants of the transcription factor genes (*COM1*, *MNH6*, *AP1*, and *TPC1*) are elongated compared to the wild-type spores (Lu et al. 2007; Yang et al. 2010; Guo et al. 2011; Galhano et al. 2017).

More transcription factors have been identified to affect spore production primarily. For example, deletions of transcription factor genes *CONX1* (Lu et al. 2014), *COD1* and *COD2* (Chung et al. 2013; Lu et al. 2014), *CONX2* (Cao et al. 2016), *MSTU1* (Nishimura et al. 2009), *MCM1* (Zhou et al. 2011), *LDB1* (Li et al. 2010), *MIG1* (Mehrabi et al. 2008), *MoNTE1* (Chen et al. 2023), bZIP transcription factors (*HAC1*, *METR*, and *BZIP10*) (Kong et al. 2015; Tang et al. 2015), velvet genes (*VEA*, *VELB*, and *VELC*) (Kim et al. 2014), and the pH-regulation-related transcription factor *PACC* (Landraud et al. 2013) result in reduced spore production in *M. oryzae*.

Among different *M. oryzae* wild-type strains, many transcription factors have different effects on sporulation. One of the reasons for this difference is related to the fact that the melanin content in aerial mycelia varies considerably among strains. During genetic manipulations such as knockouts in *M. oryzae*, transformants with lighter colony colors often imply a reduction in spore production. In both 70-15 and Guy11 strains, deletion of *CNF1* significantly increases melanin synthesis, but their spore production change in the opposite direction (Huang et al. 2022b). In the strain 70-15, which has a low background melanin content, increasing the fungal melanin content promotes spore production significantly, e.g., Δ$cnf1_{70\text{-}15}$ produces up to 40-fold more spores than the wild-type (Lu et al. 2014). In contrast, the deletion of *CNF1* in Guy11, which already has a high background melanin content, leads to significantly fewer spores than the wild-type (Huang et al. 2022b). Δ$gcc1_{70\text{-}15}$ and Δ$gcf3_{70\text{-}15}$ of 70-15 strain have whiter colony colors and produce considerably fewer spores than the wild-type, whereas the double knockout mutants Δ*cnf1*Δ$gcc1_{70\text{-}15}$ and Δ*cnf1*Δ$gcf3_{70\text{-}15}$ (deletion of *CNF1* in Δ$gcc1_{70\text{-}15}$ or Δ$gcf3_{70\text{-}15}$) show significantly increased spore production, which is 2.2- or 4.4-fold higher than that of the wild-type strain, respectively. Moreover, the aerial mycelia of Δ*cnf1*Δ$gcc1_{70\text{-}15}$ and Δ*cnf1*Δ$gcf3_{70\text{-}15}$ are darker in color (Huang et al. 2022b). The aerial mycelium of Δ*cos1* is whiter in color and cannot form conidiophores, whereas the conidiophores of Δ*hox2* cannot differentiate and produce spores (Kim et al. 2009; Zhou et al. 2009; Liu et al. 2010; Li et al. 2013). After deletion of *CNF1* in Δ$cos1_{70\text{-}15}$ and Δ$hox2_{70\text{-}15}$, the aerial mycelium of Δ*cnf1*Δ$cos1_{70\text{-}15}$ is denser and whiter in color, and the aerial mycelium of Δ*cnf1*Δ$hox2_{70\text{-}15}$ is denser and darker. Still, both mutants fail to produce any spores (Huang et al. 2022b), suggesting (1) Cos1 and Hox2 initiate conidiophore differentiation and spore differentiation, respectively. Cos1 and Hox2 are essential for sporulation; (2) Melanin does not

initiate conidiophore or spore differentiation but can facilitate these differentiation processes. Thus, many transcription factors only affect, but do not determine, conidiogenesis, and this effect on spore production is achieved in part by affecting the melanin synthesis of *M. oryzae* (Fig. 1b).

Light promotes the formation of aerial mycelium and spore production in *M. oryzae* (Lee et al. 2006). The blue light receptor WC1 regulates the spore release from conidiophores, but the pathway through which light regulates spore production in *M. oryzae* has not been revealed (Kim et al. 2011). Light also promotes melanin synthesis in the aerial mycelium of *M. oryzae*. Thus, one of the mechanisms by which light promotes spore production may be achieved by increasing melanin synthesis (Fig. 1b).

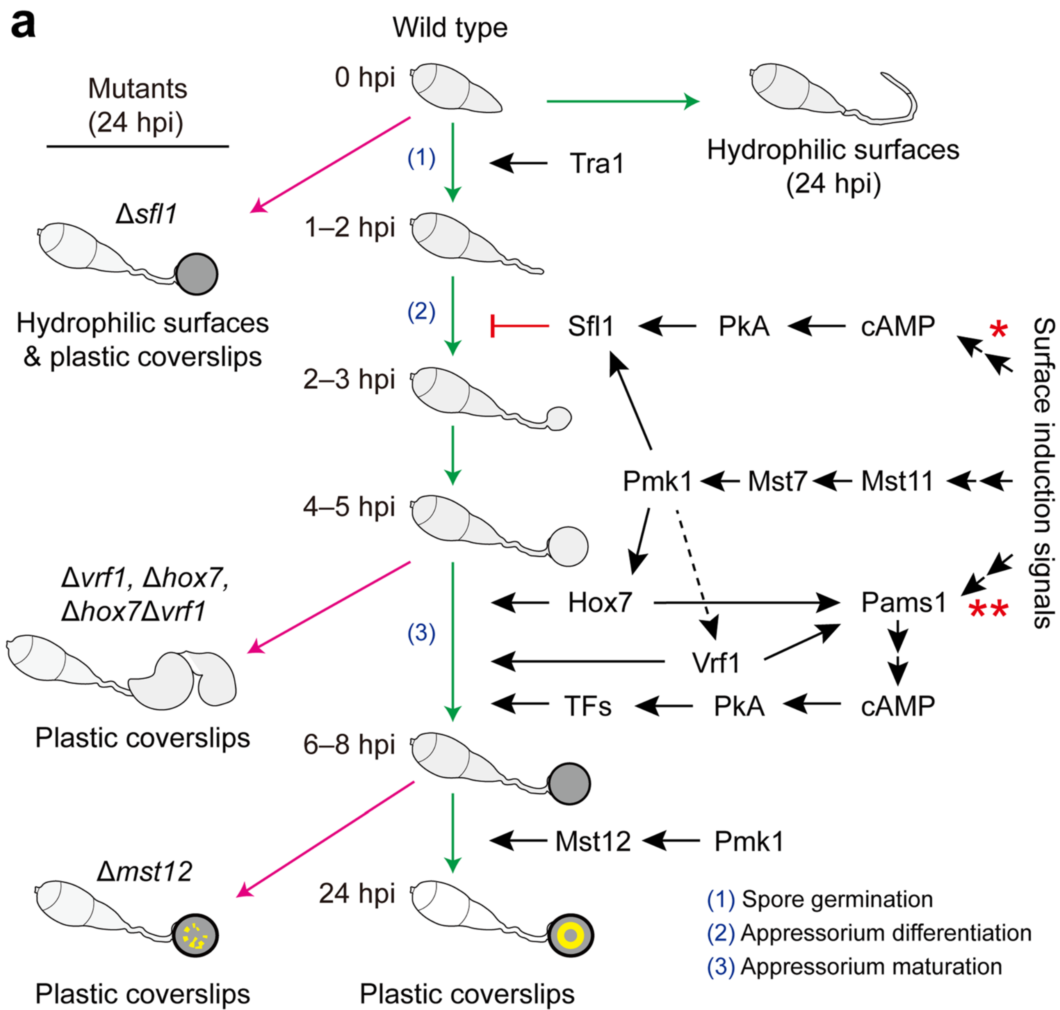
Key transcription factors regulating appressorium differentiation and maturation in *M. oryzae*

M. oryzae invades graminaceous plants through the mediation of an appressorium. An appressorium is a hemispherical cell with a dense melanin layer in the cell wall and a huge intracellular turgor pressure (Howard et al. 1991). The bottom of an appressorium has an appressorium pore, the periphery of which is firmly attached to the plant surface, and a septin ring surrounds the appressorium pore (Momany and Talbot 2017). *M. oryzae* spores germinate in water droplets, attach to the cuticle of graminaceous plants, differentiate at the tip of germ tubes to form appressoria, and the appressoria penetrate the cuticle and invade the plant cell (Dean et al. 2012). The transcription factor Tra1 regulates spore germination by modulating the secretion of hydrophobic proteins (Breth et al. 2013). The appressorium formation process can be divided into two stages: (1) during the appressorium differentiation stage, a germ tube stops growth, and its tip curves and expands to form an incipient appressorium; (2) during the appressorium maturation stage, the cell wall of an incipient appressorium is melanized, intracellular structures such as the septin ring of a melanized appressorium are formed, and a huge turgor is generated, resulting in the formation of a functional mature appressorium (Fig. 2a). The differentiation from a germ tube to an appressorium is influenced by environmental factors on the plant surface, including surface hydrophobicity, cutin and waxy components, substrate hardness, and nutrients, and pH in water droplets (Liu et al. 2011; Ryder et al. 2022). The cAMP-PKA and Pmk1-MAPK signaling pathways regulate the appressorium formation of *M. oryzae* (Jiang et al. 2018; Ryder et al. 2022). Surface hydrophobicity and cutin monomers promote the appressorium formation of *M. oryzae* spores by activating the cAMP-PKA and Pmk1-MAPK signaling pathways. The wild-type spores can form appressoria on hydrophobic surfaces but not on hydrophilic surfaces. However, with the addition of exogenous cAMP, the wild-type spores can form appressoria on hydrophilic membranes (Jiang et al. 2018; Qu et al. 2021). Activation of appressorium differentiation or maturation requires a certain amount of cAMP content, and this cAMP threshold has not been determined (Wang et al. 2022). During appressorium formation, the cAMP-PKA signaling pathway is required to be activated twice. The first cAMP-PKA signaling activation promotes incipient appressorium formation, and the second cAMP-PKA signaling activation promotes the cell wall melanization and maturation of appressoria (Wang et al. 2022) (Fig. 2a). The second cAMP-PKA signaling activation (but not the first) is regulated by a cell membrane and endosomal membrane protein Pams1. *PAMS1* is specifically expressed under the control of the transcription factors Vrf1 and Hox7 at the appressorium stage (Wang et al. 2022). Δ*pams1* spores can differentiate normally on the plant cuticle into incipient appressoria with non-melanized cell walls, but about 35% of incipient appressoria do not continue to form mature appressoria with melanized cell walls. These non-melanized appressoria can continue to develop into melanized mature appressoria if exogenous cAMP is added (Wang et al. 2022).

The Pmk1-MAPK signaling pathway functions downstream of the cAMP-PKA pathway (Osés-Ruiz et al. 2021). Disruption of the Pmk1-MAPK signaling pathway, such as Δ*ral2*, prevents the fungus from forming appressoria (Qu et al. 2021). The transcription factor Sfl1 is activated by the cAMP-PKA and Pmk1-MAPK signaling pathways (Li et al. 2011, 2017) (Fig. 2a). The Δ*cpkA*Δ*cpk2* mutant is unable to form appressoria on hydrophobic surfaces because of the disruption of the cAMP-PKA signaling pathway, and its growth is also slowed down. Deletion of *SFL1* in the wild-type and Δ*cpkA*Δ*cpk2*

(See figure on next page.)

Fig. 2 Transcription factors regulating appressorium differentiation and maturation in *M. oryzae*. **a** Functions of transcription factors during appressorium formation. The time points at which transcription factors (Tra1, Sfl1, Hox7, Vrf1, and Mst12) work and the phenotypes of their mutants. Spores are inoculated to germinate on hydrophilic surfaces or hydrophobic plastic coverslips surfaces at 25 °C. The yellow ring or spots in an appressorium is a septin ring. One red asterisk (*) indicates first cAMP-PKA signaling activation; two red asterisks (**) indicate second cAMP-PKA signaling activation. **b** Genes regulated by Hox7 and Vrf1 during appressorium maturation



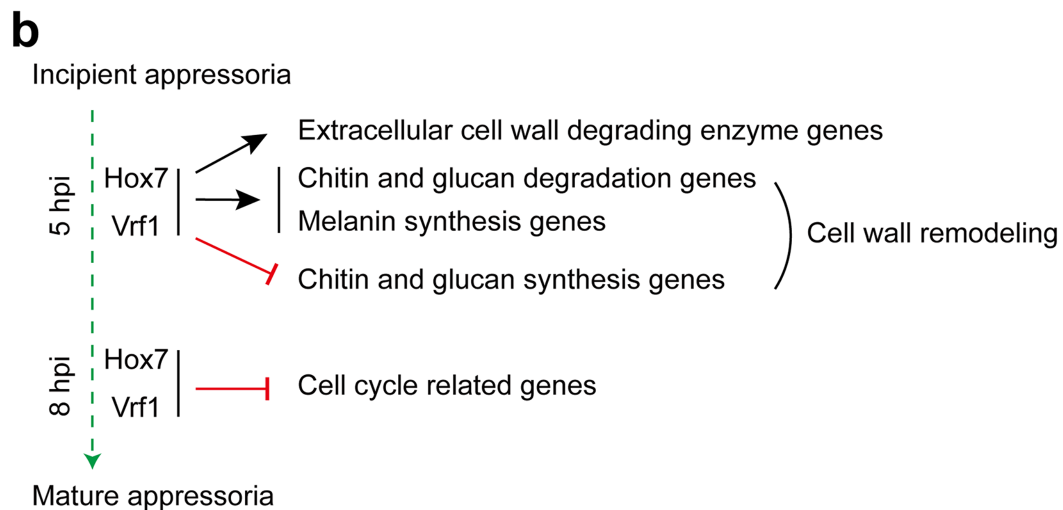


Fig. 2 (See legend on previous page.)

results in the mutants (Δ*sfl1* and Δ*cpkA*Δ*cpk2*Δ*sfl1*) being able to form appressoria on both hydrophobic and hydrophilic membranes, suggesting that Sfl1 is a negative regulator of appressorium differentiation, and it has been further confirmed that the C-terminal end of Sfl1 is required for its function (Li et al. 2017). The phosphorylation of the S211 site in Sfl1 can restore the growth of Δ*cpkA*Δ*cpk2* but not appressorium formation. Mass spectrometry analysis of the interacting proteins with Sfl1 or its C-terminus identified two transcriptional repressors, Tup1 and Cyc8, that interact with the C-terminus of Sfl1. The addition of exogenous cAMP reduced the level of interaction between Sfl1 and Cyc8. In *M. oryzae*, thus, plant surface signals activate Mac1 (an adenylate cyclase) to synthesize cAMP, cAMP activates PKA, PKA phosphorylates Sfl1, and phosphorylated Sfl1 dissociates from the Cyc8-Tup1 complex, which deregulates the repressor complex from binding to the promoter, thereby promoting gene expression and appressorium differentiation (Li et al. 2017).

After forming an incipient appressorium, the appressorium develops into a mature appressorium through cell wall melanization and synthesis of large amounts of glycerol solutes. This process is regulated by two transcription factors (Vrf1 and Hox7). Vrf1 is a C2H2 transcription factor (Cao et al. 2016), and Hox7 is a homeobox transcription factor (Kim et al. 2009), both specifically expressed at the appressorium stage (Huang et al. 2022a). In strain 70-15, colony growth, spore production, and spore germination of Δ*vrf1* and Δ*hox7* do not show obvious differences from the wild-type (Cao et al. 2016; Huang et al. 2022a). At 2–6 h post inoculation (hpi), the germ tube apexes of Δ$vrf1_{70\text{-}15}$ rapidly expanded to form incipient appressoria with no melanization of the cell wall. After 6 hpi, the cell walls of incipient appressoria of Δ$vrf1_{70\text{-}15}$ failed to form a melanization layer but instead budded to form a curved hyphal-like structure, which is bulbous, non-melanized, and filled with vesicles (Cao et al. 2016; Huang et al. 2022a). The appressorium formation process of Δ$hox7_{70\text{-}15}$ on hydrophobic surfaces is similar to that of Δ$vrf1_{70\text{-}15}$. Δ*hox7*Δ$vrf1_{70\text{-}15}$ is also phenotypically similar to both Δ$vrf1_{70\text{-}15}$ and Δ$hox7_{70\text{-}15}$, but its incipient appressorium is slightly smaller (Huang et al. 2022a). Different from the wild-type, the nuclei of Δ$vrf1_{70\text{-}15}$, Δ$hox7_{70\text{-}15}$, and Δ*hox7*Δ$vrf1_{70\text{-}15}$ could not stop division after 6 hpi. At 24 hpi, the number of nuclei within the hyphal-like structures of Δ$vrf1_{70\text{-}15}$, Δ$hox7_{70\text{-}15}$, and Δ*hox7*Δ$vrf1_{70\text{-}15}$ was 2, 4, 6, respectively (Huang et al. 2022a). At 5 hpi, melanin synthesis genes, chitinase genes, chitin deacetylase genes, endoglucanase genes, and extracellular cell wall-degrading enzyme genes were significantly down-regulated, while chitin synthase genes and glucan synthase genes were up-regulated or unchanged in Δ$vrf1_{70\text{-}15}$, Δ$hox7_{70\text{-}15}$, and Δ*hox7*Δ$vrf1_{70\text{-}15}$ (Huang et al. 2022a) (Fig. 2b). The melanin layer of an appressorium is an intermediate layer of the cell wall, slightly closer to the cell membrane (Wang et al. 2022). Calcofluor white (CFW) did not stain the chitin layer of the melanized appressorial cell wall of the wild-type but stained the cell walls of Δ$vrf1_{70\text{-}15}$, Δ$hox7_{70\text{-}15}$, and Δ*hox7*Δ$vrf1_{70\text{-}15}$, suggesting that there is a chitin layer between the cell membrane and the melanin layer (Huang et al. 2022a). During the maturation of an incipient appressorium, the cell wall undergoes a remodeling process in which glucan and chitin are partially degraded, and melanin is synthesized to form a new melanized cell wall. Decreased gene expression of chitin and glucan degrading enzymes, decreased gene expression of melanin synthases, and increased gene expression of chitin and glucan synthases hindered cell wall remodeling and melanin layer formation in the appressoria of Δ$vrf1_{70\text{-}15}$, Δ$hox7_{70\text{-}15}$, and Δ*hox7*Δ$vrf1_{70\text{-}15}$ (Huang et al. 2022a). After 8 hpi, Δ$vrf1_{70\text{-}15}$, Δ$hox7_{70\text{-}15}$, and Δ*hox7*Δ$vrf1_{70\text{-}15}$ showed a significant decrease in the expression of cell division-related genes as well as an increase in the number of nuclei, suggesting that cell division was not inhibited during appressorium maturation in the mutants. S158 of Hox7 is phosphorylated by Pmk1 at 3–4 hpi, suggesting that Hox7 is activated by the Pmk1-MAPK signaling pathway (Osés-Ruiz et al. 2021). Vrf1 (= Znf1) may also be regulated by Pmk1, but it remains to be further confirmed (Yue et al. 2016). In addition to Δ$vrf1_{70\text{-}15}$ and Δ$hox7_{70\text{-}15}$, the appressorial morphology of Δ$cca1_{70\text{-}15}$ was also abnormal, and its cell wall was not fully melanized (Lu et al. 2014).

Mst12 is a transcription factor containing C2H2 and STE domains (Park et al. 2002). Δ*mst12* forms a melanized appressorium, which cannot penetrate the plant cuticle and has no virulence. Invasive hyphae formed by wounded inoculation also cannot penetrate nearby cells and form spreading disease lesions (Park et al. 2002). Further studies have revealed that the septin ring assembled in Δ*mst12* is abnormal, suggesting that Mst12 regulates the septin ring formation of appressoria (Osés-Ruiz et al. 2021). S133 of the MAPK phosphorylation motif in Mst12 is directly phosphorylated by Pmk1 (Osés-Ruiz et al. 2021). Tpc1 is a $Zn(II)_2Cys_6$ transcription factor. At 8 hpi, two cells in 50–60% of Δ*tpc1* spores germinate, many of which form two appressoria (Galhano et al. 2017). The Nox2-NoxR complex is essential for septin-mediated reorientation of the cytoskeleton. NoxD interacts with Nox1 and Nox2. Tpc1 regulates the expression of *NOXD* by interacting with Mst12, which regulates the septin ring in an appressorium. The regulation of *NOXD*

expression by Tpc1 may be influenced by Pmk1 (Galhano et al. 2017). Moreover, the appressoria of Δ*mig1* also have a low penetration rate into plant cells (Mehrabi et al. 2008).

In addition to the above-mentioned transcription factors that regulate the differentiation, maturation, and penetration of appressoria, there are many other transcription factors that affect the appressorial morphology, formation rate, formation time, and turgor pressure, such as *MSTU1*, *MoSWI6*, *MCM1*, *LDB1*, *SOM1*, *CDTF1*, *CON7* (Odenbach et al. 2007; Nishimura et al. 2009; Li et al. 2010; Yan et al. 2011; Zhou et al. 2011; Qi et al. 2012). These transcription factors are required for fungal pathogenicity and also regulate various developmental processes (such as sporulation and mycelial growth) in *M. oryzae*.

Transcription factors regulating invasive growth in *M. oryzae*

The appressoria of *M. oryzae* form penetration pegs, which penetrate the plant cell wall and form invasive hyphae. The growth of invasive hyphae undergoes two stages: the biotrophic stage and the necrotrophic stage. Based on the target of their action (either pathogen or host), fungal transcription factors can be categorized into 3 types. (1) Type 1 transcription factors act on the rice blast fungus itself. These pathogen-specific transcription factors regulate the expression of genes related to the growth and development of invasive hyphae, such as Mst12, Mig1, Gti1, MoEitf1, and MoEitf2 (Park et al. 2002; Mehrabi et al. 2008; Li et al. 2016; Cao et al. 2022). This type of transcription factor genes can also be expressed in multiple developmental stages (vegetative hyphae, spores, and appressoria). (2) Type 2 transcription factors are expressed in invasive hyphae, secreted, and translocated into the nuclei of rice cells, such as MoHtr1, MoHtr2, and MoHtr3 (Kim et al. 2020; Lee et al. 2023). These host-specific transcription factors regulate the expression of rice genes related to disease resistance and alter the resistance response of rice cells. (3) Type 3 transcription factors can act both on the pathogen itself and the host plant, such as MoNte1 (Chen et al. 2023). They regulate fungal invasive growth and rice gene expression (Fig. 3).

The type 1 pathogen-specific transcription factors (Mst12, Mig1, Gti1, MoEitf1, and MoEitf2) regulate appressorium penetration, invasive growth and other fungal development in *M. oryzae*. Mst12 is involved in regulating invasive growth and appressorial septin ring formation (Park et al. 2002; Osés- Ruiz et al. 2021). When inoculated through wounds, Δ*mst12* fails to form spreading lesions (Park et al. 2002). During plant tissue invasion, Mst12 regulates the expression of genes for septin-dependent cytoskeletal re-organization, polarized exocytosis, and effectors in invasive hyphae (Osés-Ruiz et al. 2021). The MADS-Box transcription factor Mig1 has been implicated in the invasive growth of *M. oryzae* and affects sporulation and appressorium formation (Mehrabi et al. 2008). The efficiency of appressorium penetration into rice leaves was low in Δ*mig1*. Appressoria formed by Δ*mig1* develop penetration pegs and primary infectious hyphae, but cannot further differentiate into the secondary infectious hyphae in live plant cells. Δ*mig1* can form a structure similar to the infectious hyphae in heat-killed plant cells. In transformants expressing the Mig1-GFP fusion protein directed by *Mig1* promoter, no

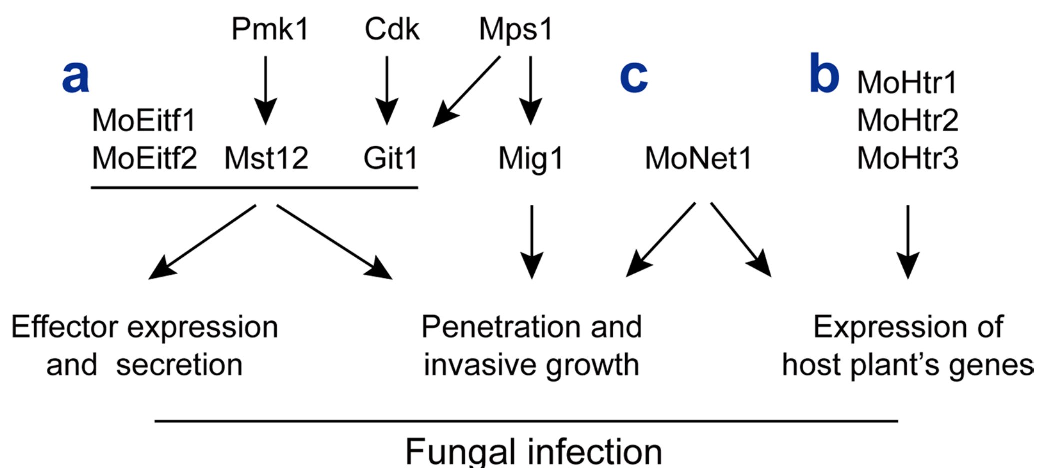


Fig. 3 Transcription factors regulating appressorial penetration and invasive growth in *M. oryzae*. Functions of transcription factors (MoEitf1, MoEitf2, Git1, Mig1, MoNet1, MoHtr1, MoHtr2, and MoHtr3) during infection. **a** Type 1 pathogen-specific transcription factors, **b** Type 2 host-specific transcription factors, **c** Type 3 transcription factors that function inside both pathogen and host

green fluorescent protein (GFP) signal was detected in the vegetative hyphae and conidiophores, but GFP signals were detected in the nucleus of conidia, appressoria, and the invasive hyphae. Mig1 may be required for *M. oryzae* in living plant cells to overcome the plant defense response and differentiate secondary infectious hyphae (Mehrabi et al. 2008).

The kinase Mps1 controls cell wall integrity, sporulation, and infection by indirectly regulating the expression of the transcription factor *GTI1* in *M. oryzae* (Li et al. 2016). Δ*gti1* has relatively normal appressorium formation and appressorial turgor pressure, whereas it is defective in appressorium penetration and growth of invasive hyphae. Gti1 regulates the expression of effector genes. In Δ*gti1*, the expression of effector genes *BAS1*, *BAS4*, *BAS107*, *AvrPita*, and *PWL2* were down-regulated, and the expression of effector genes *BAS2* and *SLP1* were up-regulated (Li et al. 2016). The serine at site 77 of Gti1 (S77) is located at a conserved phosphorylation site (S^{*77}PSR) of cyclin-dependent kinase (CDK) and is required for invasive growth but not for sporulation and appressorium formation (Li et al. 2016). The effector gene *PWL2* is specifically and highly expressed upon fungal invasion of living plant cells, and this expression is also activated by three tandem DNA repeats (48–49 bp each) containing cis-regulatory sequences in the *PWL2* promoter. However, the signaling molecule(s) from living plant cells and fungal transcription factor(s) that bind this *PWL2* cis-regulatory sequence are still unidentified (Zhu et al. 2021a, b).

MoEitf1 is a Zn_2Cys_6 domain transcription factor, whereas MoEitf2 (=Bzip9) is a bZIP domain transcription factor. *MoEITF1* and *MoEITF2* are highly expressed at 8–24 hpi (Cao et al. 2022), which corresponds to the time period between melanization of the appressorium cell wall and the early stage of invasion (Fig. 2). Δ*Moeitf1* and Δ*Moeitf2* had normal sporulation, spore germination, appressorium formation, and appressorial turgor, but delayed appressorium penetration and invasive hyphal growth, and reduced virulence to rice. Rice cells infected with Δ*Moeitf1* and Δ*Moeitf2* showed an increase in reactive oxygen species (ROS). Δ*Moeitf1* and Δ*Moeitf2* had a decrease in the expression of effector genes, such as *T1REP* and *T2REP*. T1rep and T2rep have signal peptides and are localized in the plant apoplast and the biotrophic interfacial complex (BIC). Δ*t2rep* has reduced virulence but normal colony growth, sporulation, spore germination, and appressorium formation (Cao et al. 2022).

The type 2 host-specific transcription factors (MoHtr1, MoHtr2, and MoHtr3) regulate the expression of resistance response-related genes in rice cells (Kim et al. 2020; Lee et al. 2023). MoHtr1, MoHtr2, and MoHtr3 are three nuclear effectors of *M. oryzae* with C2H2 domains, which are secreted and translocated to the nuclei of rice cells through BIC (Kim et al. 2020; Lee et al. 2023). Compared with the wild-type strain, Δ*Mohtr1* or Δ*Mohtr2* infection induced higher levels of expression of the plant immunity-related gene *OsMYB4* or *OsHPL2* and *OsWRKY45*, respectively. Δ*Mohtr1* and Δ*Mohtr2* showed no alteration in colony growth, sporulation, and appressorium formation but had reduced virulence in rice and barley. Heterogeneous expression of *MoHTR1* or *MoHTR2* in rice inhibited the expression of *OsMYB4* or *OsHPL2* and *OsWRKY45*, respectively, and increased the susceptibility of rice to *M. oryzae* and the hemibiotrophic bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Kim et al. 2020). Δ*Mohtr3* showed no change in virulence in rice, but *M. oryzae* strains overexpressing *MoHTR3* showed decreased virulence, reduced invasive hyphal growth, and reduced accumulation of reactive oxygen species (ROS) inside rice cells (Lee et al. 2023). The expression of the lipid-derived phytohormone jasmonate (JA) synthesis genes *AOS2*, *LOX2*, and *OPR2*, and the JA signaling pathway genes *JAMYB* and *JMT1* was down-regulated in rice that was infected by Δ*Mohtr3* but up-regulated in rice that was infected by a *M. oryzae* strain overexpressing *MoHTR3* (*MoHTR3ox*). Transcript levels of ethylene, salicylic acid, and defense-related genes were also affected in rice infected with Δ*Mohtr3* or *MoHTR3ox* (Lee et al. 2023).

MoNte1 is an atypical secreted protein localized to the nuclei of hyphae, spores, and appressoria of *M. oryzae*, as well as to the nuclei of rice cells during infection (Chen et al. 2023). Thus, MoNte1 can potentially regulate gene expression in *M. oryzae* and rice and belongs to the type 3 transcription factors that regulate both the pathogen and the host plant. In Δ*Monte1*, mycelial growth was slowed down, spore production was significantly reduced, appressorium formation was delayed, appressorium turgor pressure was reduced, invasive hyphal growth was slowed down, and virulence to rice was reduced (Chen et al. 2023).

Key transcription factors regulating lipid and non-preferred carbon source utilization in *M. oryzae*

Fungi preferentially utilize sugars such as glucose, and when faced with a switch in carbon sources available in the environment, they regulate carbon metabolic pathways as well as the expression of the corresponding genes and the degradation of proteins through carbon catabolite repression (CCR) and derepression (CCDR) (Huang et al. 2023a). The infection cycle of *M. oryzae* is complex and includes two nutritional (biotrophic and necrotrophic) lifestyles. *M. oryzae* utilizes different carbon sources in two nutritional stages: they could obtain

soluble sugars, amino acids, and lipids from plant cells during biotrophic growth; they could obtain starch and lipids in the plant cytoplasm and cellulose and xylan in the plant cell wall during necrotrophic growth (Battaglia et al. 2013).

Lipids are important carbon and energy sources in plants and fungi. Lipid utilization is regulated at multiple levels by at least six transcription factors in *M. oryzae*, and impaired function of a single transcription factor does not result in a complete loss of lipid utilization capacity (Fig. 4). The transcription factors Far1 and Far2, as well as their homologous proteins, regulate the utilization of lipids in the culture medium in a variety of fungi, such as *Aspergillus nidulans* and *Candida albicans* (Hynes et al. 2006; Ramírez and Lorenz 2009). In *M. oryzae*, growth of Δ*far1*, Δ*far2*, and Δ*far1*Δ*far2* are severely slowed down on long-chain lipids media (such as olive oil and triolein), and Δ*far2* and Δ*far1*Δ*far2* fail to grow on short-chain fatty acids media (such as acetic acid, propionate, and butyrate), suggesting that Far1 and Far2 are necessary for the utilization of lipids in the medium (Bin Yusof et al. 2014). Far1 and Far2 regulate the expression of genes involved in lipid metabolic activities such as fatty acid β-oxidation, acetyl-CoA translocation, peroxisomal biogenesis, and the glyoxylate cycle. However, Δ*far1*, Δ*far2*, and Δ*far1*Δ*far2* mutants have normal lipid droplet mobilization during appressorium formation and have normal pathogenicity, suggesting that these transcriptional regulators control lipid substrate utilization by the fungus but not the mobilization of intracellular lipid reserves during appressorium formation (Bin Yusof et al. 2014). Gpf1, Vrf2, and Crf1 are $Zn(II)_2Cys_6$, C2H2, and basic helix-loop-helix (bHLH) transcription factors, respectively. The growth of Δ*gpf1* and Δ*vrf2* on olive oil medium is slowed by 30% and 15%, respectively, compared to the wild-type (Lu et al. 2014; Cao et al. 2016). Δ*crf1* grows slower in the media using olive oil, triolein, ethanol, glycerol, L-arabinose, sodium acetate, aspartic acid, glutamine, and leucine as the sole carbon source (Cao et al. 2018). Δ*gpf1* and Δ*crf1* are not pathogenic to rice and barley, and Δ*vrf2* is reduced in virulence (Lu et al. 2014; Cao et al. 2016, 2018). Crf1 promotes the expression of genes encoding lipases, β-oxidation enzymes, peroxisomal proteins, isocitrate lyase Icl1, 2-methylisocitrate lyase Mcl1 (=Icl2), ethanol utilization enzymes, and enzymes in the pentose catabolic pathway and glycerol metabolic pathway (Cao et al. 2018). However, the mechanism by which Gpf1 regulates lipid metabolism is still unclear (Lu et al. 2014).

Plant cell walls are another important source of carbon and energy. Xylan content in cereal crops can be as high as 50% of the cell wall biomass. Cereal xylan (glucuronoarabinoxylan) mainly comprises D-xylose and L-arabinose (Scheller and Ulvskov 2010). Xylan utilization is synergistically regulated by several transcription factors (Fig. 4). In addition to Crf1, which promotes the expression of L-arabinose utilization genes (Cao et al. 2018), the transcription factors Ara1 regulates the utilization of L-arabinose and Xlr1 regulates the utilization of D-xylan (Battaglia et al. 2013; Klaubauf et al. 2016). Δ*ara1* is

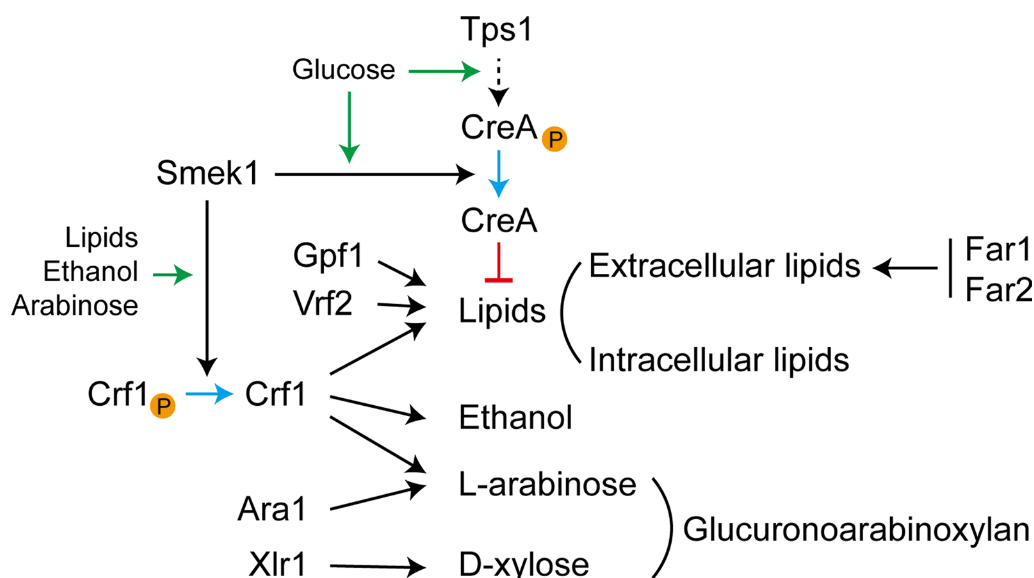


Fig. 4 Transcription factors regulating lipid and carbohydrate metabolism in *M. oryzae*. Functions of transcription factors (Far1, Far2, CreA, Gpf1, Vrf2, Crf1, Ara1, and Xlr1) in lipids, ethanol, arabinose, and xylose utilization. Smek1 is a phosphatase regulatory subunit, and Tps1 is a trehalose-6-phosphate synthase

unable to utilize L-arabinose but can utilize D-xylose, and Δ*ara1* has reduced activities of the enzymes Prd1, Lad1, and Lxr1 (Klaubauf et al. 2016). Δ*xlr1* can utilize L-arabinose but has a reduced ability to utilize D-xylose. In the xylose medium, Δ*xlr1* has decreased expression of genes such as *LAD1*, *PRD1*, *XYR1*, and *XDH1* (Battaglia et al. 2013). Δ*ara1* (=Δ*fzc40*) and Δ*xlr1* have normal virulence similar to the wild-type (Battaglia et al. 2013; Lu et al. 2014; Klaubauf et al. 2016).

The C_2H_2 transcription factor CreA is a ubiquitous repressor of carbon metabolism in fungi. In the presence of glucose, it represses the expression of non-preferred carbon source utilization genes (Adnan et al. 2017). Δ*creA* has an increased capacity to utilize lipids and L-arabinose compared to the *M. oryzae* wild-type strain (Cao et al. 2016, 2018; Huang et al. 2023a). CreA also regulates the utilization of glucose, xylose, sucrose, starch, carboxymethyl cellulose, glycerol, and ethanol in *M. oryzae* (Hong et al. 2021). CreA and Crf1 synergistically regulate the degradation of lipids, ethanol, and arabinose via carbon catabolite repression and derepression in *M. oryzae* (Cao et al. 2018; Huang et al. 2023a).

The synergistic regulation of carbon catabolite repression and derepression by CreA and Crf1 is controlled by Smek1 (Huang et al. 2023a) (Fig. 4). Smek1 is a conserved phosphatase regulatory subunit and involved in diverse physiological and metabolic activities, such as DNA repair, immune suppression and inflammation regulation, neuronal differentiation, miRNA biogenesis, and glucose homeostasis in yeast, plants, or mammals (Huang et al. 2023a). In *M. oryzae*, Smek1 regulates the repression and derepression of carbon catabolites and coordinates the utilization of glucose, glycerol, ethanol, arabinose, and lipids by the fungus. When glucose is used as a carbon source, Smek1 inhibits the expression of genes involved in lipolysis, fatty acid activation, fatty acid transport, peroxisomal β-oxidation, the glyoxylate cycle, and the methylcitrate cycle by dephosphorylating and then activating the transcriptional repressor CreA. In contrast, when lipids, ethanol, and arabinose are used as carbon sources, Smek1 dephosphorylates and then activates the transcription activator Crf1, which promotes the expression of genes involved in fatty acid activation, fatty acid transport, and peroxisomal β-oxidation, as well as the expression of *ICL1* and *MCL1* (Huang et al. 2023a). Isocitrate lyase Icl1 and 2-methylisocitrate lyase Mcl1 are key enzymes of the glyoxylate cycle and methylcitrate cycle, respectively, which are required for lipid utilization, gluconeogenesis, and virulence of pathogenic fungi (Huang et al. 2023b). In addition, a trehalose-6-phosphate synthase (Tps1) senses changes in intracellular glucose-6-phosphate levels and regulates the carbon catabolite repression in *M. oryzae* (Wilson et al. 2007; Fernandez et al. 2012). Thus, Tps1 may also function upstream of CreA (Fig. 4).

Key transcription factors regulating melanin synthesis in *M. oryzae*

The 1,8-dihydroxynaphthalene (DHN) melanin distributed in the cell wall is a fungal barrier against external extreme environmental conditions, such as radioactivity, extreme temperature, oxidative stress, and osmotic pressure, and is also necessary for sporulation, maintenance of huge appressorial turgor, and pathogenicity in *M. oryzae* (Zhu et al. 2021a, b). The cell walls of hyphae, conidiophores, spores, and appressoria in *M. oryzae* contain a melanin layer. Pig1 is the first transcription factor found to promote the expression of melanin synthesis genes in *M. oryzae* (Tsuji et al. 2000; Huang et al. 2022b). In other fungi, Pig1 regulates hyphal and spore melanin synthesis (Tsuji et al. 2000; Guegan et al. 2023). In *M. oryzae*, however, Δ*pig1* can form melanized conidiophores, spores, and appressoria, suggesting that Pig1 mainly regulates hyphal melanin gene expression and melanin synthesis but is not a key regulator of melanin synthesis in conidiophores, spores, and appressoria (Huang et al. 2022b) (Fig. 5). Vrf1 and Hox7 are two transcription factors that promote appressorial melanin synthesis gene expression and melanin synthesis, but do not affect melanin synthesis in hyphae, conidiophores, and spores (Cao et al. 2016;

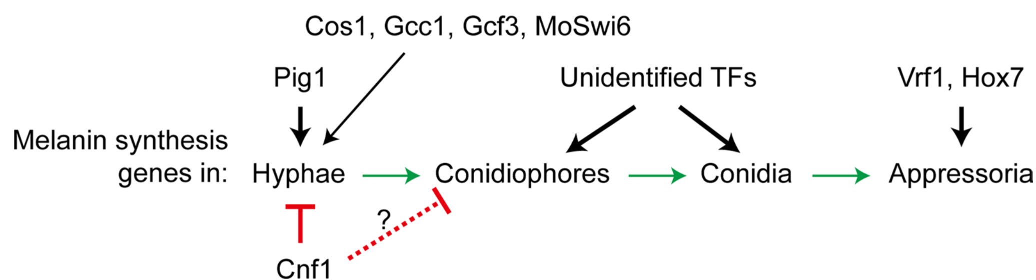


Fig. 5 Transcription factors regulating melanin synthesis in *M. oryzae*. Transcription factors (Pig1, Cos1, Gcc1, Gcf3, MoSwi6, Vrf1, Hox7, and Cnf1) function on melanin synthesis at different fungal developmental stages

Huang et al. 2022a). Cos1, a transcription factor that regulates conidiophore differentiation, also promotes the expression of melanin synthesis genes and melanin synthesis in aerial hyphae (Li et al. 2013). Cnf1 represses melanin synthesis gene expression and melanin synthesis in *M. oryzae*. Δ*cnf1* increased melanin synthesis in aerial mycelium (Huang et al. 2022b). MoSwi6 also affects the expression of mycelial melanin synthesis genes, a 1,3,8-trihydroxynaphthalene (1,3,8-THN) reductase gene *BUF1* and a scytalone dehydratase gene *RSY1* (Qi et al. 2012), and a transcription factor Bc2 promotes the expression of a polyketide synthase gene *ALB1* and activates hinnulin A synthesis (Hantke et al. 2019). In addition to the above-mentioned transcription factors, Ap1 affects mycelial melanin synthesis by altering the level of intracellular reactive oxygen species. Δ*ap1* showed increased reactive oxygen species levels and decreased laccase activity, leading to a reduction in melanin monomer polymerization (Guo et al. 2011).

In *A. fumigatus*, a bHLH transcription factor DevR and a MADS-box transcription factor RlmA regulate conidial melanin synthesis (Valiante et al. 2016). In *Pestalotiopsis microspora*, spores of the *PMR1*-deleted mutant are pale in color (Zhou et al. 2021). Knockout of *PfMAF* in *Pestalotiopsis fici* affects spore development and spore melanin synthesis but not spore production (Zhang et al. 2019). Knockout of *PIG1* in *Scedosporium apiospermum* affects the synthesis of the melanin layer of spores (Guegan et al. 2023). The *M. oryzae* homologous proteins of DevR, RlmA, Pmr1, PfmaF, and Pig1 are Bhlh8 (MGG_10837), Mig1, Pig1 (Cmr1), Htfg, and Pig1, respectively. These transcription factor genes have been knocked out in *M. oryzae*, but impaired melanin synthesis in conidiophores and spores has not been reported (Mehrabi et al. 2008; Oh et al. 2008; Cao et al. 2018; Huang et al. 2022b). Consequently, the functions and mechanisms of transcription factors vary in different fungi and, in some cases, widely. To date, no transcription factor has been identified that regulates melanin synthesis in the conidiophores and spores of *M. oryzae*.

Transcription factors regulating oxidative response and host basal immunity in *M. oryzae*

The outburst of reactive oxygen species is a fundamental immune defense response of plants against pathogen attack (Guo et al. 2010; Guo et al. 2011). Reactive oxygen species activate the expression of plant defense genes and are required for the pathogen-associated molecular pattern (PAMP) triggered immunity (PTI) response. Reactive oxygen species are also an environmental and intracellular stress faced by *M. oryzae* during growth, conidiation, and appressorium formation. Detoxification of reactive oxygen species is required for fungal development and infection. The *Schizosaccharomyces pombe* transcription factor Atf1/CreB and the *S. cerevisiae* transcription factor Yap1 regulate fungal oxidative response (Guo et al. 2010; Guo et al. 2011). In *M. oryzae*, Atf1, a homologous transcription factor of SpAtf1, regulates the response to oxidative stress (Fig. 6a). Knockout of *ATF1* resulted in slower colony growth, increased sensitivity to oxygen stress, and reduced virulence (Guo et al. 2010). The Δ*atf1* mutant had reduced expression levels of extracellular enzymes, laccases, and peroxidases (Fig. 6a). The infectious hyphal growth of Δ*atf1* was restricted to primary infected cells, and this hyphal growth restriction was caused by the large amount of reactive oxygen species produced by the strong activated defense response in the plant. Inhibition of NADPH oxidase activity and reactive oxygen species accumulation in plant cells by diphenyleneiodonium can allow the infectious hyphae of Δ*atf1* to grow normally in plant cells (Guo et al. 2010). Further results revealed that the infectious hyphae of *M. oryzae* establish a self-balancing circuit that regulates the response to plant ROS and fungal virulence through

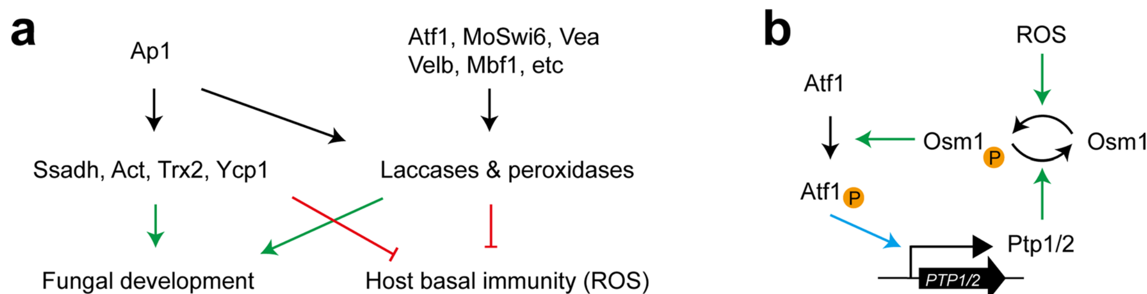


Fig. 6 Transcription factors regulating oxidative response (ROS) or host basal immunity in *M. oryzae*. **a** Transcription factors (Ap1, Atf1, MoSwi6, Vea, Velb, and Mbf1) function on ROS, host basal immunity, and fungal development. **b** A self-balancing circuit that regulates the response to plant ROS by the infectious hyphae of *M. oryzae*. ROS induce phosphorylation of the kinase Osm1; Osm1 phosphorylates Atf1; Atf1 initiates expression of the protein phosphatase Ptp1/2; and lastly, Ptp1/ 2 dephosphorylates Osm1

the activation of Atf1. During infection, ROS induce phosphorylation of the kinase Osm1 and its nuclear translocation. In the nuclei, Osm1 phosphorylates the transcription factor Atf1, which dissociates the Atf1-Tup1 complex, allowing Atf1 to initiate expression of the protein phosphatase Ptp1/2. In turn, Ptp1/ 2 dephosphorylates Osm1, restoring the circuit to its initial state (Liu et al. 2020) (Fig. 6b).

AP1, a homolog of *YAP1* in *M. oryzae*, regulates the expression of peroxidase- and laccase-related genes. The Δ*ap1* mutant is severely defective in aerial hyphal growth, conidiation, virulence, infectious hyphal growth, melanin synthesis, and response to oxygen stress (Guo et al. 2011) (Fig. 6a). The downstream genes of Ap1, *SSADH* (encoding a succinate-semialdehyde dehydrogenase), and *ACT* (encoding aacetyltransferase) are also required for fungal development, resistance to oxygen stress, and infectious hyphal growth (Guo et al. 2011). *AP1* also regulates the expression of the thioredoxin Trx2 and the flavodoxin-like protein Ycp4. Δ*trx2* exhibits severe defects in sulfite assimilation, asexual and sexual reproduction, infectious hyphal growth, and virulence (Wang et al. 2017). Δ*ycp4* shows defects in growth, conidiation, and virulence (Chen et al. 2017).

Silencing of *AP1* in *M. oryzae* by feeding artificial siRNAs (asiRNAs) targeting *AP1* can inhibit fungal growth, spore production, and pathogenicity. In contrast, asiRNAs targeting downstream genes of *AP1* (*SSADH* and *ACT*) do not affect fungal growth. Transgenic rice plants expressing RNA hairpins targeting *AP1* show greater resistance to infection by *M. oryzae* strains. Thus, in vitro asiRNA and in vivo host-induced gene silencing (HIGS) can enhance rice resistance to rice blast and are promising and valuable approaches to controll rice blast (Guo et al. 2019).

In addition to Ap1 and Atf1, several transcription factors (e.g., Vea, Velb, MoSwi6, and Mbf1) regulate the expression of laccases, peroxidases, and extracellular wall-degrading enzymes and are involved in antioxidant and cell wall stress responses (Qi et al. 2012; Kim et al. 2014; Fan et al. 2017) (Fig. 6a). The transcription factors required for invasive growth, such as Mig1, MoEitf1, MoEitf2, and MoHtr3 are also involved in oxidative response and resistance to host basal immunity (Mehrabi et al. 2008; Cao et al. 2022; Lee et al. 2023) (Fig. 3).

Functional diversity of transcription factors among *M. oryzae* strains

M. oryzae has many field strains that infect different gramineous plants or rice cultivars. The functions of the transcription factors are usually the same between different wild-type strains, but sometimes there are significant differences. The transcription factor Cnf1 inhibits melanin synthesis in strains Guy11 and 70-15 similarly, but there are significant functional differences in the regulation of sporulation by Cnf1 in the two strains (Huang et al. 2022b). Deletion of *CNF1* in the 70-15 strain promotes spore production, whereas deletion of *CNF1* in the Guy11 strain inhibits spore production. Functional differences in Cnf1 between the two strains are related to the different background melanin content of the strains (Huang et al. 2022b). The carbon metabolism repressor CreA functions to coordinate glucose and non-preferred carbon source utilization in both Guy11 and 70-15 strains, but deletion of *CREA* in Guy11 resulted in reduced virulence (Hong et al. 2021), whereas deletion of *CREA* in 70-15 did not affect virulence (Cao et al. 2016). The transcription factors Hox7 and Vrf1 regulate appressorium formation, but some functional differences exist among strains 70-15, Guy11, and KJ201 (Huang et al. 2022a). Deletion of *VRF1* in Guy11 promoted spore production (Yue et al. 2016), but spore production did not change after the deletion of *VRF1* in 70-15 (Cao et al. 2016). In Guy11, the addition of exogenous cAMP promoted the construction of Δ$vrf1_{\mathrm{Guy11}}$ or Δ$hox7_{\mathrm{Guy11}}$ incipient appressorium morphology, maintained the incipient appressorium morphology for a longer period of time, and delayed appressorium re-germination (Yue et al. 2016; Osés-Ruiz et al. 2021). In 70-15, no exogenous cAMP is required for Δ$vrf1_{\mathrm{70\text{-}15}}$ and Δ$hox7_{\mathrm{70\text{-}15}}$ to maintain their incipient appressorium morphology on hydrophobic surfaces before 6 hpi (Huang et al. 2022a). In strain KJ201, Δ$hox7_{\mathrm{KJ201}}$ forms only a curved and swelled hyphal-like structure (Kim et al. 2009). In strain Y34, Bzip3 is involved in regulating carbon metabolism associated with the generation of appressorium turgor, and Δ*bzip3* has reduced virulence (Liu et al. 2022). However, in strains Guy11 and KJ201, the virulence of Δ*bzip3* is normal (Tang et al. 2015; Kong et al. 2015).

The diversity of transcription factor functions among fungal strains may be related to their different genetic backgrounds, such as the total number of genes in the genomes, the arrangement of genes on the chromosomes, and the polymorphism of genes. Phenotypically, differences in mycelial color and spore production are common between strains. These functional differences may lead to the formation of physiological races. Therefore, it is necessary to compare the functional differences of key transcription factors or other important functional genes in different strains to understand better their molecular mechanisms.

Conclusions and future perspectives

M. oryzae causes an epidemic disease on gramineous crops. Transcription factors are essential in causing rice blast disease by *M. oryzae*. The function of

transcription factors in *M. oryzae* has been extensively studied over the past decades, and many transcription factors involved in pathogenicity have been identified. This review summarizes our understanding of the functions of key transcription factors and their mechanisms in sporulation, appressorium formation, invasive growth, oxidative response, lipid and carbohydrate metabolism, and melanin synthesis. However, many unanswered questions remain about the functions of transcription factors in the fungal infection cycle and against host basal immunity in *M. oryzae*: (1) The working mechanisms of many transcription factors have not been thoroughly studied. For example, the reasons for the loss of pathogenicity of Δ*gpf1* and Δ*vrf2*, and the diminished pathogenicity of Δ*mnh6*, a deletion mutant of an HMG non-histone gene *MNH6*, are not clear. (2) Key transcription factors that regulate gene expression in certain important metabolic pathways, such as autophagy, have not been identified. (3) The external environment influences and regulates transcription factors' activity. There is insufficient knowledge about *M. oryzae* receptors that sense external signals and signaling pathway proteins that directly function on transcription factors. (4) Many downstream genes regulated by transcription factors remain unknown, especially those expressed during the appressorium formation and invasive growth stages. (5) Compared with a single structural protein, a single key transcription factor regulates fungal development and virulence to a broader extent and scope. Therefore, transcription factors are effective targets (such as *AP1*) for breeding rice against rice blast; their applications have yet to be developed. Elucidating the molecular mechanisms of *M. oryzae* pathogenicity-related transcription factors during infection is indispensable for understanding the mechanisms of plant-fungus interactions, the mechanisms of fungus-host mutual evolution, and the design of scientific strategies against rice blast disease.

Abbreviations

asiRNAs	Artificial small interference RNAs
C2H2	Cys_2His_6
cAMP	Cyclic adenosine 3′,5′-monophosphate
CFW	Calcofluor white
dpi	Days post inoculation
hpi	Hours post inoculation
HIGS	Host-induced gene silencing
JA	Phytohormone jasmonate
MPa	Megapascal
MAPK	Mitogen-activated protein kinases
PKA	Protein kinase A
PAMP	Pathogen associated molecular pattern
PTI	PAMP triggered immunity response
ROS	Reactive oxygen species

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