Hsp70 in Fungi: Evolution, Function and Vaccine Candidate

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Abstract In fungal system, Hsp70 protein being highly conserved in nature has played a major role in various stress conditions. Genes encoding for Hsp70 proteins in fungi are highly conserved. Hsp70 protein performs chaperone dependent or independent function, essential for growth and morphogenesis of fungi. Functional distinction of Hsp70 protein conjointly depends on the prevalence of Hsp70 in numerous cellular compartments. Fungal Hsp70 protein is involved in protein aggregation, folding as well as in degradation of nascent polypeptide. Additionally, Hsp70 protein has a vital role in the formation of prions in case of yeasts. Fungi showed expression of hsp70 mRNA during interaction with plant. Also, fungal hsp70 showed expression in human during various infections, and may provide lead as a potential bio-marker for disease conditions. This chapter summarizes our present knowledge on fungal Hsp70 proteins and their role in morphogenesis, stress responses and a potential candidate for vaccine.

Keywords Fungi · HSP70 · Morphogenesis · Stress responses · Vaccine candidate

Abbreviations

AmB	Amphotericin B
CFTR	cystic fibrosis transmembrane conductance regulator
Hsp	heat shock protein
kDa	kilo Dalton
NEF	nucleotide exchange factor

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Introduction

Kingdom Fungi, distributed worldwide, having a unique entity within living organisms belong to eukaryotes. These are a diverse taxonomy, classified based on their diversity, germination stages, reproduction, evolution, ability of inflicting contamination and toxins production (Guarro et al. 1999; Shankar 2013). Throughout the phylogenies, fungi have showed a great deal of variety in reproduction mode and adaptability towards their surrounding (Galagan et al. 2005). Optimum growth conditions required for fungal growth is warm (30–37 °C) (Shankar et al. 2004) and humid conditions, which if absent leads to spore formation (dormant stage), whereas extreme increase in temperature will cause fungal death (Dix 2012). Under certain conditions or stress, life cycle and cellular processes of fungi is affected. In stress conditions such as modulation of temperature, protein denaturation has been reported that leads to misfolding and aggregation of proteins inflicting loss of biological functions (Sharma et al. 2009a, b). A unique set of proteins are involved in stress related changes, hence involved in the fungal survival, termed as Heat Shock Proteins. These proteins could be present in mitochondria, cytosol, nucleus, endoplasmic reticulum and cell membrane (Kregel 2002). Hsp are extremely preserved biomolecules involve in activation of various intermediates of signal transduction pathway in fungi (Kregel 2002; Verghese et al. 2012). Fungal kingdom encompasses three predominant heat shock proteins, Hsp90, Hsp70, and Hsp20–40, that shows crucial role in stress adaptation and morphogenesis. The class of the 70-kDa Hsp family is considered as one of the potent immunogenic protein families involved in nascent and damaged intracellular protein refolding (Lindquist and Craig 1988; Daugaard et al. 2007; Cleare et al. 2017). Hsp70 plays a major role in folding of protein and newly synthesized oligomeric gathering, the transport of protein structures across membranes, misfolded proteins refolding to prevent aggregation and also the activation and regulation of signal of transduction proteins (Bukau and Horwich 1998; Tiwari et al. 2015). In addition, Hsp70 has a great impact in the field of medicine and has been proposed as a vaccine candidate against fungal pathogen (Wormley Jr. 2011; Blatzer et al. 2015). Thus, we reviewed Hsp70 in various stress responses and alternative fungal biological conditions. In this chapter, we focused on the role of 70-kDa heat shock protein in fungi. In addition application of Hsp70 as a vaccine candidate has also been discussed.

Genes Encoding HSP70 in Fungi

Hsp70 is evolutionary conserved protein within the eukaryotes that shares approximately 50% amino acid sequence similarity with prokaryotic Hsp70 protein (DnaK) (Gupta and Singh 1994; Daugaard et al. 2007). Fungal Hsp70 molecule contains an ATP domain at N-terminal region and, a domain specific for substrate-binding which has affinity for client proteins, present in C-terminal region and a C-terminal domain

of variable length (Mayer and Bukau 2005). The deduced amino acid sequence of the Hsp70 corresponds to 636 amino acid sequences with a molecular mass of 70.56 kDa and a pI of 6.01 which exhibited a strong sequence homology with many other eukaryotic Hsp70 family proteins. Similarly, Hsp70 of B. emersonii has been cloned and showed it consists of 650 amino acids with molecular mass of 70.8 kDa. These observations suggested that yeast and fungi have conserved hsp70 genes (Stefani and Gomes 1995). Hsp70 subfamilies have shown to be conserved in terms of function and evolution. For example, *S. cerevisiae* Ssq1, involved in iron sulfur clustering which involved sub functioning as to mimic Ssc1 (Craig and Marszalek 2011). Ssa and Ssc are Hsp70 proteins, evolved due to higher change of copy number in gene evolution, while some Hsp70 family proteins such as Ssz, Lhs, Kar have evolved from change in few copy. Seven subfamilies of *S. cerevisiae* hsp70 blast analysis revealed similarity with 53 ascomycota genome associated 491 orthologs. Also, four different strains of Basidiomycota genomes analysis for hsp70 showed thirty hsp70 genes, hence studied for fungal hsp70 evolution (Kominek et al. 2013).

S. cerevisiae is the leading organism for hsp70 genomic studies, thus served as a model organism to understand hsp70 in fungi (Morano et al. 2012). *S. cerevisiae* exhibits multigene family of hsp70, which involves eight members, including constructive as well as stress inducible ones (Craig et al. 1993). There are 14 well characterized Hsp70 proteins of *S. cerevisiae* available that categorized into seven different subfamilies: four canonical-type Hsp70 chaperones (Kar, Ssa, Ssb, and Ssc), three atypical regulatory Hsp70s (Ssz, Sse, and Lhs), actively modulating Hsp70 partners activity (Kominek et al. 2013). Fungal Hsp70 have been ubiquitously distributed in all cellular compartments majorly cytosol, mitochondria, endoplasmic reticulum, and ribosome (Boorstein et al. 1994; Easton et al. 2000).From the seven Hsp70 homologues in *S. cerevisiae*, six have been observed to be located in cytosol, namely, Ssa1, Ssa2, Ssa3, Ssa4, Ssb1, and Ssb2 (Werner-Washburne et al. 1987; Černila et al. 2003). It is observed that cytosolic proteins have diversified and overlapping functions. For example, Ssb proteins did not showed substitution for Ssa protein survival in yeast (Boorstein et al. 1994). Ssd1/Kar2 is known to present in endoplasmic reticulum (Werner-Washburne and Craig 1989). Fungi have a specialized ribosomal Hsp70, have specific binding site on ribosome 36 position. Fungi also have a specialized ribosome-associated Hsp70, Ssb, which independently associates with the 60S subunit. Binding of Ssb in ribosome depends on conserved residue, which is specific in the peptide-binding domain at valine 442 region, allows flexibility (Pfund et al. 2001). In mitochondria, three different hsp70 encoded by the genes ecm10, ssc1 and ssq1 were observed. Deletion of ssc1 was lethal under all conditions. ssc1 have similar functions as of jac1 (Kampinga and Craig 2010). ssa1 and ssa2 are involved in transport of aminopeptidase-I from the cytoplasm into the vacuole (Satyanarayana et al. 2000). Recently, a variety of eukaryotes have showed a special class of protein known as Hsp110 protein which is found in the endoplamic reticulum and cytoplasm. At molecular level, they are large and vast protein but belonging to hsp70 gene superfamily as they have same functional and structural similarities (Easton et al. 2000; Nikolaidis and Nei 2004).

Role of HSP70 in Morphogenesis

Asexual reproduction in fungi accounts for fungal morphogenesis which comprises different stages, namely, conidia in dormant stage, vegetative hyphae and mycelia (Wang and Lin 2012). Hsp70 has a vital role in morphogenesis of fungi in stress related conditions (Tiwari et al. 2015). Transcripts of hsp70 were observed during lag and log phase followed by reduction at aerial hyphae in early stage and the hsp70 transcripts were found maximum at later stages of aerial hyphae. This is because of activation of hsp70 transcription or decrease mRNA degradation rate (Häfker et al. 1998). Xavier et al, also showed that expression of the heat shock inducible hsp70 gene depend on the various development stages of fungi and found maximum inducibility in late aerial hyphae due to lower mRNA degradation rate (Xavier 1998). Studies on *N. crassa* exhibited highest hsp70 transcripts at aerial and dormant conidia stage which fluctuate on later stages of germination (Fracella et al. 1997). Proteome profile of germinating conidia of *A. flavus* showed the expression of Hsp70, suggesting their role during transition from conidia to hyphae (Tiwari et al. 2016). With the increase in temperature (from room temperature to higher), Paracoccidioides converts from mycelia to yeast form, a pathogenic form. *P. brasiliensis* and *P. lutzii* showed high hsp70 transcripts in yeast form (Monteiro et al. 2009; Shankar et al. 2011a, b). Hyphal stage in *C. albicans* showed upregulated Ssa (Hsp70), suggesting the key role of Hsp70 protein in morphogenesis of fungi (Brand 2011). *C. albicans* showed higher accumulation of Hsp70 protein in its cell wall which was found to be expressed in all conditions (Lopez-Ribot et al. 1996). *A. flavus* (toxigenic and atoxigenic strains) showed expression of hsp70 transcripts at 30 °C, however, downregulated expression was observed in toxigenic strain in comparison to atoxigenic strain, thus suggesting lower expression of hsp70 transcripts favor aflatoxin biosynthesis (Thakur et al. 2016). Hsp70 induction and overexpression has been observed in the compensation of cwh41 gene, which codes for glucosidase-I in *A. fumigatus*, important for cell wall synthesis and morphology of fungi (Jin 2011).

During stress response, fungus such as *C. albicans, P. lutzii*, *H. capsulatum* etc existing in mycelia form converts to yeast form and vice versa, this phenomenon is termed as dimorphism (Brand 2011). *Penicillium marneffei* showed temperature dependent upregulation of hsp70 transcripts during the mycelium to yeast phase transition (increase at 39 °C followed by decrease at 42 °C) (Kummasook et al. 2007). Similar studies have also shown in *H. capsulatum* with higher expression of hsp70 from mycelia to yeast transition (Caruso et al. 1987; Shearer Jr. et al. 1987). This finding showed that hsp70 plays a very essential role in heat stress dependent fungal dimorphism.Hsp90 plays a major role in the fungal morphogenesis which requires the co-operation of Hsp70. Hsp70 acts as co-chaperon for Hsp90 and requires Sti1/Hop1 as an adapter protein for Hsp90 mediated polypeptide release (Rohl et al. 2015). Hsp70 also works in combination with Hsp30 and Hsp80 to interact with unfolded polypeptide, hence mimicking Hsp90 activity in fungal morphogenesis. Hsp70 interaction with Hsp30-Hsp80 complex has been observed in various yeasts such as *S. cerevisiae, N. crassa* and *C. albicans* in their dormant conidial stage (Ouimet and Kapoor 1999; Girvitz et al. 2000).

Role of HSP70 Co-factors

Hsp70 of fungi are the ubiquitous chaperonin family protein found in cellular compartments, and involved in various cellular functions such as protein synthesis, translocation and degradation of protein and also in protein folding (Frydman 2001). To perform the vital functions Hsp70 chaperone requires a specific binding to substrate protein which is controlled by ATPase cycle. The rate of substrate binding and release from ATP bound domains of Hsp70 is high and the affinity for substrates is low. This process is reversed by the hydrolysis of ATP bound Hsp70 which is converted to ADP-bound confirmation form, hence trapping the substrate with higher affinity. This whole cycle relies on interaction of Hsp70 with some specific co-factors which are regulatory molecules (Andréasson et al. 2010). Co- factors are involved in ATP hydrolysis or exchange of ATP for ADP. Some of the major co-factors of fungal Hsp70 chaperone protein have been described in this study.

Nucleotide Exchange Factors

Substrate release from Hsp70 is mediated by Nucleotide Exchange Factors (NEFs) which performs dissociation of nucleotides and rebinding of ATP. Hsp70 requires NEFs as they have low affinity for nucleotide binding. Fungi can possess multiple NEFs which may have specific interactions with different HSp70s. For example, multiple NEFs have been reported in *S. cerevisiae* such as Sls1, Snl1, Fes1, Sil1, Lhs1, and Sse1 (Kabani et al. 2000; Tyson and Stirling 2000; Sondermann et al. 2002). However, Sse1 stimulates yeast cytosolic Hsp70s whereas endoplasmic reticulum specific Hsp70 function is enhanced by Lhs1 (Steel et al. 2004; Shaner et al. 2006; Andréasson et al. 2010). Some NEFs may have functional overlapping. Lhs1 is more potent than Sil1 whereas, Sse1 is more effective with Ssa Hsp70s than Fes1 (Steel et al. 2004; Sharma and Masison 2009). Ssa1p and Hsp110 is the cytosolic Hsp70 chaperone of *S. cerevisiae* which when present in ADP bound form associates with Fes1p (nucleotide exchange factor) and favors nucleotide release. Translation related defects have been seen due to deletion of fes1p (Kabani et al. 2002). Grp170 (Lhs1) is the NEF of endoplasmic reticulum Hsp70 in yeast.

Hsp40 Co-chaperones

Hsp70 interacts with a client proteins known as J-proteins (also called Hsp40s) (Kampinga and Craig 2010). Hsp40s are critical companion of all Hsp70s in the regulation of Hsp70 activity (Steel et al. 2004). Hsp40s are present in multiple isoforms and regulate ATP dependent polypeptide binding of Hsp70 protein (Shen et al. 2002; Craig et al. 2006). Hsp40 is divided in three classes viz. class I, class II

and class III which differs in their structural patterns (Cheetham and Caplan 1998; Hennessy et al. 2000). Yeast Hsp70 Sis1, a class II Hsp40 has structure similarity with Ydj1 which is a class I Hsp70 of yeast (Li et al. 2003a, b). *S. cerevisiae* Hsp70, that is, Ssa1 and Ssa2 interacts with Djp1, Ydj1, Sis, Hlj1 and Swa2, the binding/interaction of which is based on cellular location (Hettema et al. 1998; Sharma and Masison 2009). For example, Djp1 interacts with Ssa1 and 2 in peroxisomes biogenesis, Ydj1 in endoplasmic reticulum membrane, Sis1 in ribosome (Brodsky et al. 1998; Hettema et al. 1998; Horton et al. 2001). Another class of yeast Hsp40 co-chaperone Zou1 has shown interactions with Ssz1 (yeast Hsp70) (Craig et al. 2003).

Hsp70 Functions in Fungi

Hsp70 has been involved in various cellular processes. The following sections discuss the examples Hsp70 functions.

Protein Folding and Prevention of Aggregation

Hsp70 plays an important role in maintaining native conformations of folded proteins from their synthesis until their degradation. *S. cerevisiae* cytosolic Hsp70, Ssa and Ssb are highly identical (Craig et al. 1995). Ssb interacts at early stage of synthesis of polypeptide thus facilitates nascent polypeptide chains elongation whereas Ssa interacts with the secondary structure developed from nascent chain and helps in translocation of protein (Sharma and Masison 2009). Translation in fungi is sometimes hurdled by aggregation of peptides due to non-native (exposed) form of hydrophobic residues, so requires native contacts of all amino acids emerged from ribosome, which is mediated by Hsp70 (Sharma and Masison 2009).

Role of Hsp70 in Protein Degradation

Hsp70 that assists protein folding, also involved in misfolded protein degradation, in association with Hsp40. Genetic studies have shown that Ssa1, which is a yeast Hsp70 chaperone and yeast Hsp40 chaperone Sis1, together play an important role in degradation of misfolded substrate which hampers the quality of proteins (Shiber et al. 2013). Function of Sis1 is ubiquitinization of substrate that is carry forwarded by Ssa1 which functions by forwarding the ubiquitinilated substrate to proteosome (Shiber et al. 2013; Shiber and Ravid 2014). Hsp70 subfamilies are highly dependent on substrates hence, showing specificity towards protein degradation. For example, normal yeast strains containing Ssa1 showed cystic fibrosis

transmembrane conductance regulator (CFTR) degradation whereas, 60% CFTR degradation was observed by ssa1 mutant yeast cells. Also, yeast Kar2 (BiP), which is known to have a regulatory domain which responded to unfolded proteins in endoplasmic reticulum, when mutated showed no effect on CFTR degradation. This suggests that CFTR degradation in yeast is dependent on Hsp70 chaperone, Ssa, and not on Kar2 (Molinari et al. 2002). Ssb and Ssa have also been involved in cytosolic protein degradation in yeast. Proteosomal protein degradation in yeast is mediated by Ssb1 (Luders et al. 2000).

Role of Hsp70s in Formation and Propagation of Yeast Prions

Prions are isoform of proteins which self-engendering and transmissible. Prions have been reported in several yeasts which exists in soluble form (normal state) or amyloid form (transmitting state) (Liebman and Chernoff 2012). To maintain themselves in fungal/yeast population, prions relies on Hsp70 function, which may depend on the abundance of Hsp70 for prion propagation (Jones et al. 2004). Extensively studied prions in *S. cerevisiae* are [PSI+] and [URE3], which are derived from Sup35 and Ure2 proteins in their amyloid form responsible to cause infection (Jung et al. 2000; Tibor Roberts et al. 2004). Hsp70 chaperons such as Hsp40s and Hsp104 have impact on propagation of prions, as studies showed that conjugated role of Ssa1 and Ydj1 is involved in inhibition of polymerization of Sup35 (Song and Masison 2005).In yeast, mutant strain of Ssa1 (Hsp70 chaperone) has shown impair mitotic stability leading to partial inhibition of [PSI+] mediated allosuppresion (Jung et al. 2000; Song et al. 2005). This inhibition of prion is also dependent on the variety of Hsp70 chaperons present, as if mutated Ssa1 is only present in cytosol, then it have more pronounced effect on prions weakening. Mutation in Ssa1 also weakens [URE3] activity whereas mutation in Ssa2 weakens both [PSI+] and [URE3] activity (Sharma et al. 2009a, b). In absence of Ssa1 polymers have shown to be self associated and self aggregated, so it can be said that Ssa1 may prevent higher polymer aggregation hence preventing aggregation of prions (Song et al. 2005). Apart from similar homology of Ssa and Ssb, both vary in yeast prion propagation, as Ssb increases [PSI+] formation and Ssb inhibits propagation of [PSI+] (Allen et al. 2005). Prions propagation is also influenced by Hsp70 specific co-factors. For example, Hsp40 over expression, TPR, or NEFs (Fes1 or Sse1) deletion, is involved in the impairment of propagation of [PSI+] (Jones et al. 2004; Qiu et al. 2006). Long term interaction of Hsp70 with Sup35 present in improper folded form may cause inhibition of conversion to prion state. So it is required for Hsp70 to perform proper activity alone or in co-operation with co-factors to prevent aggregation of polymers or prion regeneration (Sharma and Masison 2009).

Role of Hsp70 in Stress

Hsp are induced by two mechanisms in fungi, specific mechanism and general mechanism. Specific mechanism generally encompasses temperature related stress whereas, generalized mechanism by other stresses such as pH, oxidative stress, osmotic stress, starvation, or antifungal stress (Tereshina 2005). Thus, the roles of Hsp70 involved in stress responses have been categorized below.

Hsp70 Response Against Heat Stress/Thermotolerance

Hsp70 is a high molecular weight chaperone, which exists in fungi without any heat stress, but as the heat stress is given to fungi they starts increasing in number (Plesofsky-Vig and Brambl 1985a). The enhancement of fungal hsp70 mRNA due to different temperature gradients revealed a strong stress induced response. In response to heat stress, Hsp70 in association with Hsp40 and Hsp104 has been involved in unfolding of denatured protein, mediating thermotolerance in fungi (Sanchez et al. 1993). A temperature dependent increase of hsp70 expression was found in *A. fumigatus*, *A. terreus*, *C. cladosporioides* and *T. mentagrophytes*, but *P. chrysogenum* and *S. apiospermum* showed no stress induced response. At different incubation temperatures, *A. fumigatus* showed an induction of the hsp70 expression level. *A. fumigatus* showed upregulation of gene expression from 25 to 35 °C, while further increase in temperature resulted in lowering the expression of hsp70. Similarly, *A. terreus* and *C. cladosporioides* showed higher hsp70 expression at 40 °C. Hence it suggests that expression of hsp70 is most at temperature range between 35 and 40 °C. Other fungi such as *T. mentagrophytes* showed hsp70 expression at lower temperature of 25 °C. However, expression of hsp70 in these fungi (*P. chrysogenum* and *S. apiospermum*) is temperature independent (Salzer 2008). *Achlya ambisemalis*, an oomycete, in response to heat shock of 30–35 °C for 10–30 min, showed higher expression of hsp70 (Gwynne and Brandhorst 1982). Similar results were also seen during heat shock response in *A. nidulans*, at 37–43 °C for 1 h (Newbury and Peberdy 1996). *N. crassa* is a filamentous fungus widely studied for stress responses. Heat stress mediated response of *N. crassa* showed accumulation of nuclear associated 78 kDa heat shock protein (Kapoor 1983). Later, *N. crassa* conidia in response to heat stress at 45 °C, showed assembly of 70 kDa Hsp (Plesofsky-Vig and Brambl 1985b). Different morphological stages (mycelia or yeast) have shown no effect on the expression of hsp70 in few fungi, in response to stress (Kummasook et al. 2007). For example, *P. marneffei*, dimorphic fungi have shown same expression level of hsp70 when exposed to 39 °C (Kummasook et al. 2007). Also, *N. crassa* converts from mycelia to hyphae in response to heat stress (25–37 °C), with the higher expression of hsp70 in response to temperature but not during the transition state (Perlman and Feldman 1982). In in-vivo condition, expression of hsp70 plays a very important role, as it mediates resistance of fungi in host in response to high body temperature. Further, suggesting Hsp70 as a virulent

factor in fungal mediated infection. To uphold this statement, studies on *N. crassa* treatment at 48 °C for 14 h, showed upregulation of hsp70 expression (Kapoor et al. 1995). Although, studies on *P. brasiliensis* showed that temperature above 42 °C have decreased the hsp70 mRNA expression (Da Silva et al. 1999). So, hsp70 mediates thermo-tolerance in fungi is thought to be species dependent.

Role of Hsp70 in Acid/Alkaline Stress

pH signaling is important for various cellular processes in fungi such as, gene expression regulation, virulence of fungi, secretion of nutritional enzymes, metabolic processes, etc. (Caddick et al. 1986; Ferreira-Nozawa et al. 2006). PacA/PacC pathway is involved in the pH mediated response in fungi (Penalva and Arst 2002). *A. nidulans* is known fungal model used for the study of pH response (Silva et al. 2008). Nucleotide sequence studies of hsp70 of *A. nidulans* revealed that it contains a binding site for PacC in their upstream region. It suggests that in normal conditions (acidic, optimum temperature) PacC with Hsp70 is responsible to maintain normal conditions in fungi. The transcription of hsp70 is mediated by palA (transcriptional factor) in normal growth condition (acidic environment), which is encoded by pacC (Tilburn et al. 1995). But, if the heat stress is given to fungi for 1–2 h in the absence of palA, the hsp70 response becomes independent of both acid and alkaline pH, so PalA in that condition does not play any role in hsp70 expression. *A. nidulans* showed high expression of hsp70 at pH 8 and the activation of palA (Freitas et al. 2011). Studies on pH mediated hsp70 response in *N. crassa* showed that hsp70 was highly expressed at acidic pH and optimum temperature (30 °C) but did not show any upregulation at alkaline pH, depending on PacC mediated signaling pathway. Also, Squina et al, showed that pH mediated expression of hsp70 vary depending upon culture conditions and heat stress. Some hsp70 genes respond to these conditions in addition to pH response (For example; hsp70-1 and hsp70-2 of *N. crassa*) while some hsp70 only depends on culture conditions or heat stress but not on pH (For example; hsp70-3 of *N. crassa*) (Squina et al. 2010). Another fungus, *Candida glabrata*, also showed same expression of hsp70 towards alkaline pH. Hsp70 (cytosolic Ssa1) protein was found to be downregulated at pH 7.4 and 8, which suggests that in fungi hsp70 expression is mediated by acidic pH (Schmidt et al. 2008).

Oxidative Stress and Osmotic Pressure Response

Oxidative stress and response towards osmotic pressure is a functional challenge for fungal cells that generally all fungus experience. Fungus overcomes the effect of reactive oxygen species by mediating activation of transcriptional processes and by producing stress response proteins. Very little information is available for the role of Hsp70 in oxidative stress response in fungi. Skn7 is a oxidative stress response mediated transcription factor which in co-ordination with heat shock transcriptional

factor hsf1 induces heat shock genes (Raitt et al. 2000). Loss in Skn7 in *S. cerevisiae* showed inhibition of Hsp70 (Ssa1) (Morano et al. 2012). This finding might also related Hsp70 role in oxidative stress response. In response to H_2O_2 stress in Paracoccidioides yeast cells, hsp70 was found to be expressed which showed the important part of oxidative stress response fungal machinery (de Arruda Grossklaus et al. 2013). Kunal et al, showed that in response to oxidative stress in fungi, *Ascochyta rabiei*, hsp70 was found to be upregulated (Singh et al. 2012). Hypoxia is a state of deprived oxygen, causing oxidative stress. In fungi, the hypoxia conditions has led to heat shock responses, for example, C. *neoformans, Drosophila melanogaster, C. albicans* and *B. emersonii* in hypoxia condition, showed higher expression of hsp70 and the survivability of fungi was found to be increased significantly (Setiadi et al. 2006; Chun et al. 2007; de Castro Georg and Gomes 2007; Azad et al. 2009). *Metarhizium anisopliae*, an entomopathogenic fungus, is responsible for infection in insects. Osmotic stress response in this fungus is mediated by mos1 gene. When mos1 was knock down, showed the downregulated expression of hsp70 transcripts, possibly leading to delay in osmotic stress response (Wang et al. 2008).

Drug Response

To prevent the harmful effect of fungal species, various antifungal medications have been employed which have specific fungal targets for their action. Major antifungal drugs used are amphotericin, itraconazole, fluconazole, ketoconazole, clotrimazole, miconazole, terbinafine, etc. (Odds et al. 2003). Some of these antifungals have been studied for their effect on hsp70 mediated responses in fungi. In *A. terreus* hsp70 play a vital role in response to amphoteracin B (AmB). Transcriptome analysis of AmB resistance strain of *A. terreus* revealed relatively high ssa and ssb mRNA expression in comparison to AmB susceptible strains whereas mitochondrial and endoplasmic reticulum specific hsp70 members, kar and ssc, were downregulated. In addition, sse and ssz (nucleotide exchange factors) were also uplifted in response to AmB resistant *A. terreus*. Susceptibility of AmB towards A. terreus were found to increased in absence of Hsp70. AmB when combined with pifithrin (Hsp70 inhibitor) inhibited AmB resistant *A. terreus* (Blatzer et al. 2015), whereas *A. fumigatus* treated with AmB showed downregulation of mitochondrial Hsp70 protein (Gautam et al. 2008). 17β-estradiol is a female hormone that inhibits dimorphic fungus (Paracoccidioides) in their transition from mycelia to yeast, thus women are observed to be resistant to paracoccidioidomycosis (Shankar et al. 2011a, b). In Paracoccidioides, the transcriptional profile during temporal transition from mycelia to yeasts form showed differential regulation of hsp70 in response to 17β-estradiol. Expression of hsp70 was observed during 4–12 h of germination, which dropped further at 144 h, which suggested the role of hsp70 in delaying the morphological transitions from mycelium to yeast under the influence of 17β-estradiol (Shankar et al. 2011a, b). Genome wide studies of *C. albicans* treated with 17β-estradiol showed expression of hsp90, whereas the regulation of hsp70 was not onserved

(Burt et al. 2003; Banerjee et al. 2007; Clemons et al. 2010). Paromomycin is an antibiotic which is used as antifungal compound. *S. cerevisiae* when exposed to paromomycin showed induction of hsp70 mRNA translation (Grant and Tuite 1994). In other studies on *S. cerevisiae* treated with thiolutin (antifungal), induce the transcription of ssa4 (hsp70) (Adams and Gross 1991). These findings may signify that hsp70 is a novel target against pathogenic fungi.

Other Stress Responses

Apart from heat stress, Hsp70 in fungi have been found to play important role in salt, metal or ion stress etc. Weitzel et al, showed the increase in expression of hsp70 in response to dinitrophenol in *S. cerevisiae* (Weitzel et al. 1985). Also, *S. cerevisiae* has shown the increase in expression of hsp70 in response to NaCl ranging from 0.3 to 0.7 M (Antelo et al. 1992; Lewis et al. 1995). Thiolutin (RNA polymerase inhibitor) and parornomycin (antibiotic) was also showed high expression of ssa4 in *S. cerevisiae* (Grant et al. 1989; Adams and Gross 1991). In continuation, *S. cerevisiae* showed same expression level of hsp70 in response to ethanol treatment, which was not observed in case of *N. crassa* (Plesset et al. 1982; Roychowdhury and Kapoor 1988). *N. crassa* cells when treated with hydrogen peroxide showed the synthesis of Hsp70 protein (Kapoor and Lewis 1987). In several studies high concentration of sodium arsenite showed the increased transcripts of hsp70 in *N. crassa*. In addition, cadmium when inoculated with sodium arsenite, showed no effect on expression of hsp70 (Chakraborty et al. 1995). However, cadmium showed upregulation of hsp70 in *A. fumigatus*. Further, *A. fumigatus* also showed upregulation hsp70 on treatment with cadmium chloride (Damelin et al. 2000; Georg and Gomes 2007).

Role of HSP70 in Fungus-Plant Interaction

Fungus mediated plant pathogenesis and disease is responsible to cause loss of food products worldwide. So, studies are needed further to deduce the fungal targets which are essential for disease progression. In few studies on fungal-plant interaction, hsp70 has been observed to be upregulated, contributing to pathogenesis. For example, *Colletotrichum gloeosporioides*, showed higher expression of hsp70 in coffee plant in its infectious state (coffee berry disease) (Chen et al. 2003). *Magnaporthe oryzae* Hsp70 proteins (Lhs1p and Kar2p), were also found to accumulate in the endoplasmic reticulum of fungi and responsible for disease development in rice plant (Yi et al. 2009). *Rhizoctonia solani* is a plant pathogenic fungus causing various diseases in plants such as damping, root rot, collar rot, etc. (Zhu et al. 2016). Proteomic studies revealed that heat shock protein of 70 kDa was observed during cell wall degradation of soybean seedlings, thereby, assisting virulence (Lakshman et al. 2016).

HSP70 in Pathogenesis and Potential Diagnostic or a Vaccine Candidate

Fungal diseases, in recent years, have been taken into account due to their dramatic increase and aggressiveness that demands better medical diagnosis and therapies. Fungal diseases/contamination has made its impact on immune-compromised patients (Thakur et al. 2015) as well as on several food crops (Chauhan et al. 2016). Fungal resistance over major antifungal drugs has raised a need to develop new and efficient drug targets for pathogenic fungi and vaccine candidate. Fungal pathogen after entering into the host body suffers temperature shifts. Hence, to overcome temperature shift stress, fungus produces heat shock proteins that may play role in fungal survival/growth in the host. In various transcriptomic and proteomic studies, it has been shown that Hsp of fungi are up-regulated during infection. Hsp70 is a major chaperone that has been widely studied due to their role in infection/contamination in animals/plants. For example, Hsp70 in *C. albicans* has been reported in systemic murine candidiasis, which has been identified as an antigen by host immune system (Bromuro et al. 1998). Proteomic studies of *A. flavus* cultured on corn flour showed expression Hsp70 proteins (Tiwari and Shankar 2018). Thus, it suggests that fungal Hsp70 is important for both human and plant. Allergens from *C. cladosporioides* also showed expression of 72 kDa protein (Li et al. 2008). Systemic mycosis is prevalently caused by *P. brasiliensis* in yeast phase showed higher expression of hsp70 mRNA during infection (McEwen et al. 2000; Goldman et al. 2003; Cleare et al. 2017). *Cryptococcus neoformans* being a pathogenic fungus, is involved in impairing immunity of patients (HIV, defective T cells) causing severe infections. Studies on murine pulmonary cryptococcosis caused by *C. neoformans* have shown hsp70 as a target molecule for immune response (Kakeya et al. 1999). Hsp70 mediated immune response e.g., IL-12 and IFN-γ has been observed in immunological studies of *H. capsulatum* (Allendoerfer et al. 1996; Deepe Jr. and Gibbons 2002; Cutler et al. 2007). Immune response against Hsp70 also accounts production of tumor necrotic factors and interleukin 6, which have role in the prevention yeast infection (Bromuro et al. 1998). AntEroles et al., showed that heat stress induces the formation of *C. albicans* spores. These spores when reacted with polyclonal antibody of rabbit, exhibited hsp70 expression having homology with *S. cerevisiae* Ssa1 gene (Eroles et al. 1995). Studies from Li et al, showed that histatin 5 (a salivary protein) has antifungal properties and possess affinity towards Ssa1 and Ssa2 (Li et al. 2003a, b). Previously hsp70 of *Penicillium citrinum* has also been screened from asthmatic patients (Shen et al. 1997). Moreover, in other bacteria such as *P. carinii*, *P. rattus* and *P. jirovecii* also showed the expression of hsp70 during their pathogenic phase (Stedman et al. 1998; Latouche et al. 2001; Burnie et al. 2006). These literatures suggest that fungal Hsp70 plays a major role in pathogenesis and act as a potent antigen for mediating host immune response. Thus, Hsp70 has potential as a diagnostic candidate and may be a promising protein to study as a vaccine candidate (Cleare et al. 2017).

Conclusions

Fungi are the diverse genera produce Hsp70 in response to stress conditions for accomplishing various biological functions. Hsp70 has been observed to be highly conserved in the evolution of fungal kingdom. *S. cerevisiae* had been a best studied model for nucleotides and amino acid sequences of hsp70, which correlates with other fungal species. Majorly, Hsp70 protein has been reported in protein folding, protein aggregation and disaggregation, regulating transcription and post-transcription, morphogenesis and thermotolerance. Also, it has also showed role in other stress response such as osmotic, oxidative or response towards pH and stress response towards meal ion, antibiotics, alcohols etc. Hsp70 has emerged as a major heat shock protein expressed in fungi during the stress after Hsp90. Expression of fungal Hsp70 in the host system added a molecule to study for the development of vaccine candidate using Hsp70 protein/epitopes against various fungal infections.

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