S5-3

Functional Characterization of Thiol-Specific Antioxidant (TSA) of *Candida albicans*

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Living aerobic organisms are constantly exposed to reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), alkyl-hydroperoxides, and superoxide anion that are produced during metabolism or in response to external stimuli (Jamieson D.J., 1998). ROS has been implicated in various physiological and pathological processes including metabolism, immunity, inflammation, cell signaling, transcriptional regulation, and apoptosis (Jamieson D.J., 1998, Nathan C., and Shiloh M.U., 2000, Marshall H.E., *et al.*, 2000). The cellular defense against oxidative stress is important for homeostasis and survival. Antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase are important components of the cellular defense system against ROS (Hallywell B., and Gutteridge J.M.C., 1989). Peroxiredoxin (Prx) is a family of peroxidases with molecular size of 20-30 kDa that are present in organisms from all kingdoms.

The thiol-specific antioxidant enzyme (TSA), also known as thioredoxin peroxidase, is an new antioxidant enzyme originally isolated from yeast and later found in mammalian tissues. This protein has the ability to protect biomolecules from oxidative damage if a thiol reductant such as dithiothreitol (DTT) or thioredoxin is present (Kim K., *et al.*, 1988, Chae H.Z., *et al.*, 1994). *S. cerevisiae* Tsa1p, cytosolic thioredoxin peroxidase I (cTPx I), was the first peroxiredoxin isolated form an eukaryotic cell (Kim K., *et al.*, 1988). The transcription of the *TSA1*, cTPx I encoded gene, is activated in different situations where cells were exposed to high H_2O_2 concentration. Moreover a comparison between a *TSA1* deletion mutant and its corresponding wild-type isogenic strain indicated that this deletion renders cells more sensitive to H_2O_2 (Demasi A.P., *et al.*, 2000).

Candida albicans is the most prevalent opportunistic fungal pathogen in humans. It causes various forms of candidiasis from superficial mucosal infections to life-threatening systemic diseases, especially in immunocompromised patients, such as people with AIDS, cancer or organ transplantation (Fridikin S.K., and Jarvis W.R., 1996). Several virulence factors of *C. albicans* are known including dimorphism, which is the ability to grow in either a yeast or a hyphae form in response to different environmental factor (Odds F.C., 1988). Given the nature of the immune defense against pathogens, consisting of (at least in part) the production of ROS, pathogens will be exposed to oxidative stress when phagocytosed by phagocytes such as neutrophils of macrophages. Recent studies have implicated a role of adaptive response to oxidants in pathogenesis (Jamieson D.J., *et al.*, 1996).

In a previous study, we identified several proteins that are differentially expressed in pathogenic hyphae form by comparing protein profiles between yeast and hyphae of *C. albicans* (Choi, Y. *et al.*, 2003). One of them, thiol-specific antioxidant (TSA) was attracted because it may play some roles to surviving the oxidative environment of macrophages, in the most prominent immune cell responsible for elimination of invading fungi. We have found that TSA protein converts to oxidized form in large quantity during transition to hyphae. In this study, following isolation of the CaTSA1 gene and characterization of functional 4 copies, its null mutant was constructed to study cellular functions. Reduction of H_2O_2 was considerably decreased in the mutant compared to wild type in the presence of oxidative stress, indicating that TSA protien (CaTsa1p) of *C. albicans* is an enzyme with antioxidizing activity. We also produced antibody against CaTSA in mouse and revealed by Western analysis that CaTsa1p is induced by exogenous reactive oxygen species(ROS). We also found that CaTsa1p forms homodimer by disulfide bond linkage when SH group of cysteine residue is oxidized by ROS under the non-reducing condition. When intracellular localization during morphogenic transition was examined, considerable amount of CaTsa1p that is exclusively present in the cytoplasm of yeast was translocated to the nucleus in hyphae. Biological significance of this CaTsa1p translocation will be further studied in term of differentiation and pathogenicity.

Summary

1. TSA1 in C.albicans exists as two functional genes of four alleles (Fig. 1) .
2. The null mutants of TSA are all viable, indicating that TSA is not essential for normal growth (Fig. 2).
3. The null mutants of TSA show significantly higher H2O2 generation than wild (Fig. 3).
4. TSA protein is induced by oxidative stress (Fig. 4).
5. TSA protein is translocalized from the cytoplasm to the nucleus following to morphological differentiation of C.albicans (Fig. 5).

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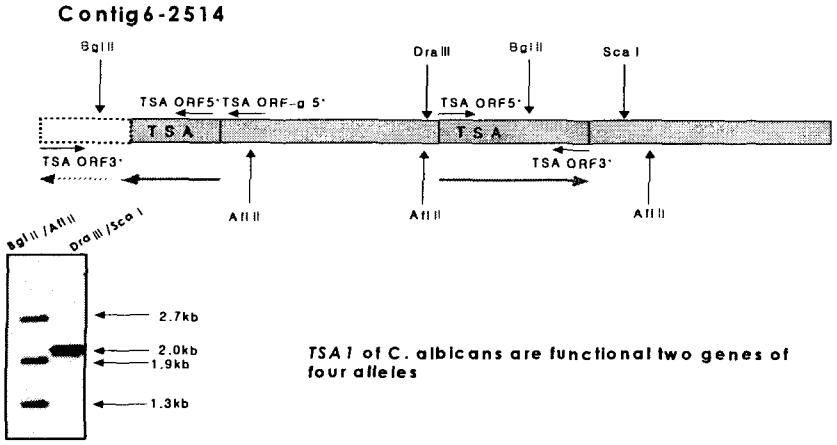


Fig. 1

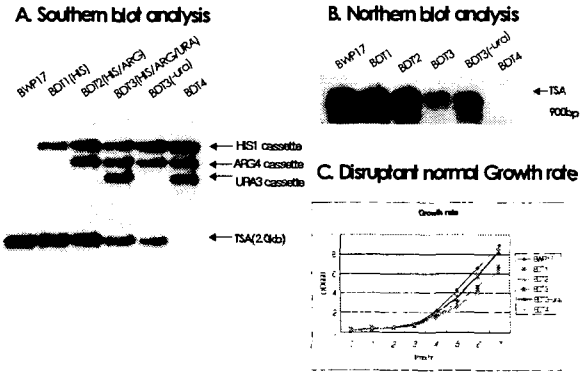
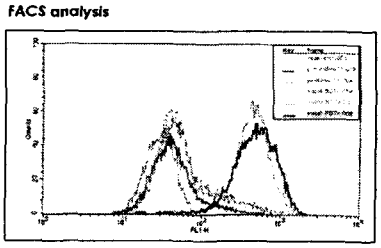
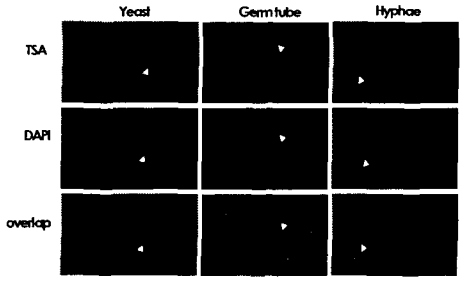


Fig. 2



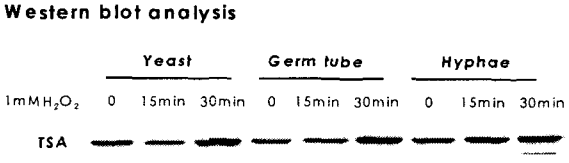
The strain BDT3(*tsa*/*tsa*, TSA/*tsa*), and BDT4(*tsa*/*tsa*, *tsa*/*tsa*) showed significantly higher H_2O_2 generation than the BWP1(W/T), BDT1(TSA/*tsa*, TSA/TSA), and BDT2(*tsa*/*tsa*, TSA/TSA) strains.

Fig. 3



TSA protein was shown to be differentially localized to the nuclear in C. albicans depending on the hyphal transition.

Fig. 4



TSA protein expression is induced by oxidative stress.

Fig. 5