Acta Biochimica Polonica

Vol. 42 No. 4/1995

505–508

QUARTERLY

Text of lecture presented at the 5th International Symposium on ***Molecular Aspects of Chemotherapy***

Fungal glycoproteins and their biosynthetic pathway as potential targets for antifungal agents

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Key words: cell wall, glycoprotein, dolichylphosphate, O-glycosylation

The yeast cell wall as a good antifungal target is discussed in general. More specifically the reaction, catalyzed by Dol-P-Man:protein *O*-D-mannosyltransferase is proposed as a new potential target. Six genes responsible for this endoplasmic reticulum-localized reaction have been cloned and characterized so far. Triple disruptions of these genes are either lethal or the corresponding cells have to be osmotically stabilized to survive. No inhibitors of this reaction are as yet known.

Fungal infections leading to serious diseases in humans and often the final cause of death, have greatly increased in the last 10–20 years. This has been a consequence of an increasing incidence of immunodeficiency syndromes, including the artificial immunosuppression accompanying organ transplantations. Whereas potent antibiotics are available to prevent bacterial infections under these conditions, a comparably successful treatment of fungal infections or of the unsuppressed growth of commensal fungi is not available.

An ideal target for potential antifungal drugs would of course be biological structures and reactions which occur exclusively in fungal cells and which are essential for fungal growth and propagation. An obvious cellular structure of this kind is the fungal cell wall. In this context it may be worthwhile to remember that among the most potent antibacterial agents available are antibiotics that prevent the synthesis of a cell wall component, the peptidoglycan.

In the following the components of fungal cell walls are briefly outlined and the structure and biosynthesis of one class of cell wall molecules, the glycoproteins, will be discussed in more detail. It will be pointed out that one type of protein glycosylation reaction occurring in fungal cells, essential for growth, does not appear to exist in mammalian organisms.

RESULTS AND DISCUSSION

The composition of fungal cell walls

Fungal cell walls in general consist of glucans, mannans or mannoproteins, and chitin. The fungal cell wall investigated best is that of baker's yeast, *S. cerevisiae*. Approximately 25% of the cell dry weight is due to its cell wall. Thus these cells invest a major part of their nutrients and of their metabolism into constructing this extracellular organelle, the wall. In *Saccharomyces cerevisiae* the cell wall consists mainly of β1,3-glucan (55% of the wall dry weight) and of mannoproteins (40%), whereas β1,6-glucan and chitin (2%) are two minor components [1–3]. Recently it has been shown that chitin and β1,3-glucan are covalently linked with one another [4].

Biosynthesis of yeast cell wall components

Considering the fungal cell wall as a potential target for antifungal drugs, it seems the most straightforward to try to interfere with the formation of one of the cell wall components, which then may give rise to a labilized cell wall and to osmotic lysis of the cell. An inhibitor of either β1,3-glucan or of chitin synthesis, both reactions not occurring in mammals, could ideally fulfil this task. Intensive work to clarify the biochemistry of the biosynthetic pathways giving rise to these two wall components has been carried out [5, 6] and although progress has been considerable, the genes directly responsible for β1,3-glucan synthesis have not yet been cloned, although a number of genes affecting β1,6- and β1,3-glucan synthesis to various degrees have been characterized [1, 7]. It is clear however, that mutants defective in certain genes required for chitin synthesis are lethal [5]. Thus a specific, nontoxic compound preventing chitin synthesis *in vivo* could be an ideal antifungal drug and the same would be expected to hold for the inhibition of glucan synthesis.

How about the third cell wall component, the mannoproteins? Might these macromolecules also be a potential target for antifungal drugs? At first sight, this may look less likely since mannoproteins on the one hand are considered as cell wall matrix material covering the outside of the fibrous wall and filling the space within the fibrous material and in this way these proteins, for example, may contribute less to osmotic stability. On the other hand, the biosynthesis of fungal mannoproteins to a large extent proceeds in the same way and is catalyzed by enzymes very similar to those in mammalian cells [8]. Thus, for example, the oligosaccharyl transferase, catalyzing the transfer of the oligosaccharide $GlcNAc_2Man_9Glc_3$ from dolichoylpyrophosphate to asparagine residues of the protein, is a protein complex consisting of ribophorins and additional proteins in yeast as well as in mammalian cells [9–12]. However, as pointed out before [8], O-glycosylation of proteins seems to be a fairly unique reaction sequence in fungal cells, and since a number of yeast cell wall proteins are O-glycosylated [13, 14], it has been of major interest, in the context of the problem discussed here, whether inhibition of protein O-glycosylation is detrimental to yeast cells.

Protein-O-glycosylation in *S. cerevisiae*

The pathway of protein-O-glycosylation is summarized in Fig. 1. In contrast to the situation in mammalian cells, in yeast the first sugar attached to the protein is a mannosyl residue which is transferred in the ER from dolichoylphosphate mannose (Dol-P-Man) to

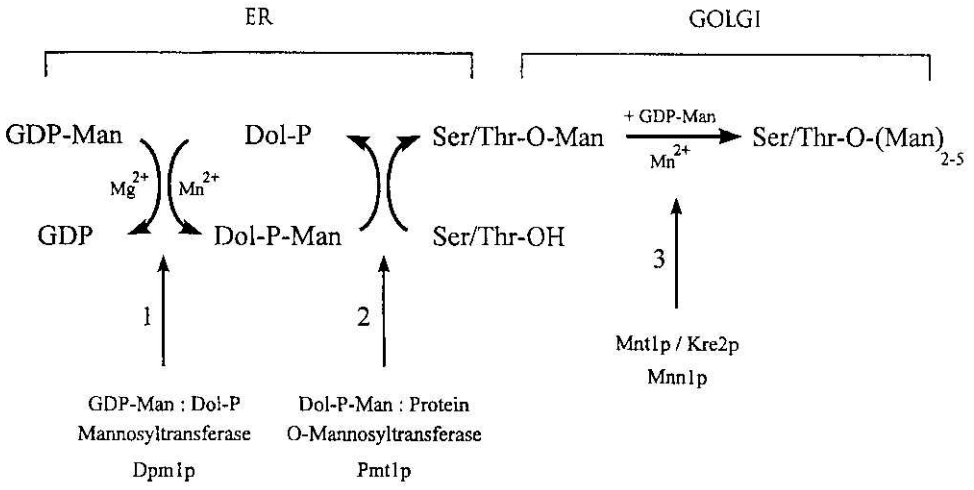

Fig. 1. O-Glycosylation in yeast.
Reaction 1 and 2 take place in the endoplasmic reticulum (ER). The synthesis of the lipid intermediate Dol-P-Man is highly conserved in all eukaryotic organisms (reaction 1). Reaction 2 has only been shown in fungal cells so far. In this step mannose is transferred from Dol-P-Man to serine or threonine residues of protein entering the secretory pathway. The transferases catalyzing the elongation of the mannose chain (reaction 3) are localized at the Golgi apparatus. The α1,2-mannosyltransferase Mnt1p (= Kre2p) adds the third mannose [20], the α1,3-mannosyltransferase Mnn1p the fourth mannose [21] of the O-linked carbohydrate chain.

seryl/threonyl residues of secretory proteins [8]. In O-glycosylation, in mammalian cells the first sugar generally is an *N*-acetyl galactosamine and is transferred to secretory proteins from UDP-GalNAc in the Golgi [15]. Although mannosyl residues have been reported to be present on exceptional mammalian proteins, no evidence for Dol-P-Man as the sugar donor has been obtained [16].

To test whether protein-O-glycosylation is an essential reaction for yeast growth and propagation, it was decided to purify the Dol-P-Man:protein *O*-mannosyltransferase (Pmt1p in Fig. 1), to clone the gene and to subsequently construct a corresponding null mutant. The protein has been purified and an antibody raised against it [17]. The gene (*PMT1*) has been cloned; the null mutant, however, was not lethal [14]. Further analysis showed that a second gene (*PMT2*) is located on yeast chromosome I [18], a third one (*PMT3*) on chromosome XV and a fourth one (*PMT4*) on chromosome X [19]. All these genes are expressed. Lately also a *PMT5* (Dommaschk, unpublished) and *PMT6* gene have been detected (the latter on chromosome VII in the yeast genome project, Guerreiro *et al.*, unpublished).

It was not surprising, therefore, that the disruption of only the *PMT1* gene did not give rise to a lethal phenotype. In the meantime multiple gene disruptions have been constructed (M. Gentzsch, unpublished) and from these it is clear that the triple disruptants pmt2pmt3pmt4 and pmt1pmt2pmt4 are lethal and that the disruptant pmt1pmt2pmt3 can not grow if it is not osmotically stabilized by 1 M sorbitol.

In summary, therefore, protein-O-glycosylation is an essential process for baker's yeast; the identical pathway for protein O-glycosylation has been shown to exist in all fungal cells tested so far [8]. Since the fungal pathway seems to be unique, or at least not detectable in mammalian cells, a drug interfering with this reaction may not be harmful to humans. The enzyme Dol-P-Man:protein *O*-D-mannosyltransferase, catalyzing the transfer of mannosyl residues to proteins, should be considered, therefore, as a potential target for antifungal drugs.

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