LIPID MEDIATED GLYCOSYLATION IN YEAST NUCLEAR MEMBRANES

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1. Introduction

The presence of glycosyltransferases in nuclei from slime mould *Dictyostelium* [1] and from rat liver cells [2] was reported previously. The present paper describes the existence of glycosyl-transfer reactions in nuclear membranes from *Saccharomyces cerevisiae* dependent on a lipid intermediate. In previously described examples of nuclear glycosylation the involvement of lipid intermediates was not reported.

The identification of glycosyltransferases in the nucleus and the presence in the nuclear membrane of lipid intermediates operating in glycosyl transfer, may point at a common character of lipid-dependent mechanism of transglycosylation and may raise new questions concerning the nature of the acceptors that are glycosylated via the lipid-mediated mechanism.

2. Materials and methods

GDP-[U-^{14}C]mannose (74 Ci/mol) and UDP-*N*-acetyl-[U-^{14}C]glucosamine (270 Ci/mol) were from the Radiochemical Centre, Amersham, England. Snail digestive juice was supplied by Industrie Biologique Française, Marseille, France. C_{55}Dolichyl phosphate was made by Dr Mańkowski [3]. Cultivation of *S. cerevisiae* was described previously [4].

To separate the nuclear fraction the procedure described by Clark-Walker et al. [5] was followed. The addition of cesium chloride to the sucrose-gradient was omitted due to the inhibitory effect on glycosyl-transfer reactions (G. Palamarczyk, unpublished). After centrifugation in the sucrose-gradient for 75 min at a 26 000 × *g* in a SW-27 rotor, the lower layer with approximate density 1.22 was collected as the nuclear fraction and examined in a phase contrast microscope. To obtain nuclear membrane the nuclear fraction was diluted with 0.05 M Tris–maleate buffer (pH 7.2) to 0.25 M sucrose concentration, subjected to mechanical disruption in a Potter homogenizer, centrifuged for 1 h at 105 000 × *g*, washed with 0.4 M NaCl in the same buffer and centrifuged again as above.

The final pellet of nuclear membranes was tested for RNA, DNA and DNA/protein ratio [6] (protein estimated by the method of Lowry et al. [7] and for the activity of DNA-dependent RNA polymerase [8]. A 10-fold increase of DNA/protein ratio and 40-fold increase of specific activity for DNA-dependent RNA-polymerase compared to protoplast membranes was estimated.

The results of these assays demonstrate a high purity of the nuclear fraction according to criteria established by Bhargawa and Halvorson [9].

Protoplast membranes were obtained by subjecting yeast protoplasts to osmotic shock and mechanical breakage according to Rozijn and Tonino [10].

The sugar transfer into lipid and insoluble polymer was measured after incubation of a given membrane preparation (0.4–0.8 mg protein/assay) with labeled nucleoside diphosphate sugars as described previously [4].

Thin-layer chromatography, DEAE-cellulose chromatography and mild acid treatment of labeled lipids was described previously [11]. Treatment of the mannosylated insoluble polymer with mild alkali or pronase was carried out according to Babczynski and Tanner [12].

3. Results

3.1. *Formation of ^{14}C-labeled lipid and polymer*

When the nuclear membranes from yeast were incubated with UDP-*N*-acetyl [^{14}C]glucosamine or GDP-[^{14}C]mannose, radioactivity was incorporated into lipid as well as into a chloroform/methanol insoluble fraction. In table 1 are shown the amounts of mannose and *N*-acetyl glucosamine incorporated into these two fractions by nuclear membranes compared to 105 000 × *g* particles and protoplast membranes. The quantity of *N*-acetyl-[^{14}C]glucosamine transferred into lipid and insoluble polymer by nuclear membranes was similar to the level estimated for the protoplast membranes whereas the transfer of [^{14}C]mannose into the two products was significantly lower.

The kinetics of the glycosyl-transfer reactions catalysed by the nuclear membranes are presented in fig.1.

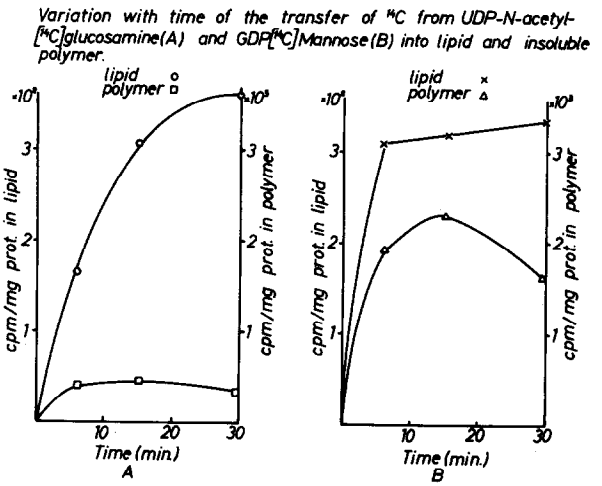

Fig.1. Variation with time of the transfer of ^{14}C from UDP-*N*-acetyl-[^{14}C]glucosamine (A) and GDP-[^{14}C]mannose (B) into lipid and insoluble polymer.

The effect of exogenous C_{55}dolichyl-phosphate under the standard incubation conditions on the transfer of mannose and *N*-acetylglucosamine into lipid was negligible, but the amount of radioactivity transferred into polymer increased 2-fold (table 2). However, when the amount of enzymic protein in the incubation mixture was decreased from 0.8–0.18 mg, a 20-fold stimulation of radioactivity of GDP-[^{14}C]-mannose into the lipid was observed upon addition of exogenous C_{55}dolichyl-phosphate.

3.2. *Characterization of ^{14}C-labeled, lipid-linked glycosyl*

On studying the ^{14}C-labeled lipid-linked sugars by thinlayer chromatography on silica-gel in chloroform/methanol/water/ 65:25:4 by vol., two mannosylated lipids were detected (R_F 0.41 and 0.48) and two containing *N*-acetylglucosamine lipids (R_F 0.24 and 0.28). The mannosylated lipid of R_F 0.41 and *N*-acetylglucosamine containing lipid R_F 0.28 were eluted from DEAE-cellulose by 50 mM and 100 mM ammonium acetate respectively as reported for yeast prenyl-phosphate mannose and prenyl-diphosphate *N*-acetylglucosamine [4] and behaved as the latter compounds on mild acid treatment [4]. The content of prenyl-phosphate mannose in the total ^{14}C-labeled lipids was 80% in all three

Table 1
The incorporation of mannose and *N*-acetylglucosamine from the ^{14}C-labeled nucleoside diphosphate sugars into lipid and insoluble polymer in yeast subcellular fractions

Fraction	Radioactivity (cpm/mg protein)			
	GDP-man.[a]		UDP-gluc.Nac.[b]	
	Lipid	Polymer	Lipid	Polymer
Nuclear membranes	386	6286	1423	1155
105 000 × *g* particles	2765	9461	726	400
Protoplast membranes	5432	21 643	1365	1050

[a] GDP-man. = GDP-[^{14}C]mannose
[b] UDP-gluc.Nac. = UDP-*N*-acetyl-[^{14}C]glucosamine

Table 2
The effect of exogenous C_{55}dolichyl-phosphate on the amount of [^{14}C]sugars transferred into lipid and insoluble polymer by yeast nuclear membranes (Standard incubation conditions[c])

NDPS added[a]	Radioactivity (cpm/mg protein)	
	Lipid	Polymer
GDP-man.[b]	978 (692)	7943 (4151)
UDP-gluc.Nac.[b]	1423 (1423)	651 (309)

[a] NDPS – Nucleoside diphosphate sugar
[b] See footnotes [a,b] to table 1
[c] Standard incubation conditions: 15 min incubation at 30°C. Incubation mixture contained in a total vol. 250 μl: 2 μM $MnCl_2$, 2 μM $MgCl_2$, 0.25 μM EDTA, 1 μM 2-mercapto-ethanol 8 μM NaF, 0.1 μCi of NDP [^{14}C]S. 0.5 mg enzymatic protein.

Figures in brackets indicate the amount of radioactivity incorporated when incubation was performed without the exogenous prenyl phosphate.

membrane fractions. The amount of prenyl-diphosphate *N*-acetylglucosamine was nuclear membranes 70%, 105 000 × *g* particles and mitochondrial membranes 35%. The other mannosylated lipid (R_F 0.48) and *N*-acetylglucosamine containing lipid (R_F 0.24) were neutral lipids not retained on DEAE-cellulose.

3.3. *Characterization of ^{14}C-labeled insoluble polymer*

Insoluble polymer mannosylated by nuclear membranes from GDP-[^{14}C]mannose was sensitive to alkaline treatment (β-elimination). According to the data presented in table 3, about 30% of radioactivity still remained non-dialyzable after this treatment. Most of the non-dialyzable radioactivity was released by treatment with pronase (90%). Alternatively when the insoluble polymer, not subjected to β-elimination was treated with pronase, about 54% of radioactivity become dialyzable. Treatments with trypsin or DNAase were without effect.

4. Discussion

The results of the present studies demonstrate the existence in nuclear membranes of glycosyl transferases stimulated by prenyl-phosphate. It should be mentioned that in yeast (C_{60}–C_{80}dolichols were found as natural constituents [13].

On mitochondrial membranes and 105 000 × *g* particles only 30% of the ^{14}C-labeled *N*-acetylglucosamine-labeled lipid had properties consistent with prenyl diphosphate *N*-acetylglucosamine whereas this compound accounted for the majority (70%) of the labeled lipid using nuclear membranes.

As presented in table 3, the mannosylated polymer formed by nuclear membranes contains a relatively large fraction (31%) that is stable to β-elimination, therefore not *O*-glycosydically linked to protein. Possibly this mild-base stable-fraction contains mannosyl units linked to polymer via *N*-acetylglucosamine residues in a manner similar to the yeast cell wall mannam–protein complex described by Nakajima and Ballou [14,15]. The relatively high amount of prenyl-diphosphate *N*-acetylglucosamine formed by nuclear membranes together with the comparatively high amount of mild-base stable-mannose in the insoluble polymer are consistent with a role for prenyl-diphosphate *N*-acetylglucosamine as an intermediate on the glycosylation of nuclear glycoproteins.

Table 3
Effect of β-elimination on mannosylated insoluble polymer

Fraction	Original radioactivity (cpm)	% Radioactivity non-dialyzable after β-elimination
Nuclear membranes	3336	31[a]
Mitochondrial membranes	16 521	8
105 000 × *g* particles	19 786	18
Protoplast membranes	2218	27

[a] Dialyzable in 90% after pronase treatment

The participation of dolichyl diphosphate oligosaccharides containing *N*-acetylglucosamine and mannose as intermediates in the glycosylation of the yeast cell-wall polymannan–protein complex was discussed previously [4,16].

The transfer of mannose and *N*-acetylglucosamine to insoluble polymer was stimulated by exogenous C_{55}prenyl-phosphate. This result provides additional evidence for the intermediatory role of prenyl-phosphate derivatives of both sugars in their transfer into chloroform/methanol insoluble polymer, possibly glycoprotein.

The presence of glycosyltransferases in the nucleus raises several questions concerning their biological role. The interesting suggestions from the work by Rogge et al. [1] was that glycosylation of nuclear proteins in nucleus might be related to genetic regulation. On the other hand, the glycosylation of proteins existing in the nucleus appears to be a normal functional purpose of the nuclear transferases [2]. The results of the present studies suggest that lipid intermediates (prenyl-phosphate sugars) may be involved in the glycosylation of these, as yet uncharacterized, glycoproteins.

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