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Short Sequence-Paper

Identification of *SPT14* / *CWH6* as the yeast homologue of hPIG-A, a gene involved in the biosynthesis of GPI anchors

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Abstract

Cwh6 is a temperature-sensitive cell wall mutant of *Saccharomyces cerevisiae*. *CWH6* was found to be identical to *SPT14*, a gene that is highly homologous to both human PIG-A and to RFAK from *Salmonella typhimurium*. PIG-A and RFAK are involved in transferring *N*-acetylglucosamine to, respectively, a GPI anchor precursor and to lipopolysaccharides. Because cell walls of *cwh6* are greatly reduced in mannose, and because some cell wall proteins are known to be incorporated into the cell wall through a GPI-anchor dependent mechanism, we propose that Spt14p/Cwh6p is involved in transferring *N*-acetylglucosamine to a precursor of GPI anchors. We further propose that the majority of cell wall proteins are incorporated into the cell wall through a GPI anchor.

Keywords: Cell wall; Mannoproteins; Calcofluor white hypersensitive; GPI

The cell wall of *Saccharomyces cerevisiae* is composed of about equal amounts of glucan and heavily mannosylated proteins, and a small amount of chitin. Glucan and chitin form an internal, skeletal layer that is interspersed with and surrounded by mannoproteins [1]. The mechanism by which proteins become incorporated in the cell wall is still largely unknown. There is strong evidence, however, for a role of glycosylphosphatidylinositol (GPI) anchors in this process [1]. First, several wall proteins such as the sexual agglutinins and flocculin are known to contain a GPI anchor addition signal [2–4]. Second, elongation of a reporter protein with the C-terminal thirty amino acids of α -agglutinin, which include the GPI anchor addition signal, is sufficient to target it to the cell wall [5]. Third, deletion of the GPI anchor addition signal from α -agglutinin results in secretion of active α -agglutinin into the medium [6]. To further study the mechanism of cell wall protein incorporation, the Calcofluor white-hypersensitive cell wall mutant *cwh6* [7] was chosen. The mannose content of the cell wall in this mutant is reduced by 54%, whereas the mannosylation of

secretory proteins is not significantly impaired. This indicates that in *cwh6* the incorporation mechanism of cell wall proteins is affected. *Cwh6* is also temperature sensitive for growth, indicating that *CWH6* is an essential gene.

CWH6 was cloned by functional complementation. A haploid *cwh6 ura3⁻* strain was transformed with a YCp50-based genomic library [8] and *ura⁺* prototrophs were selected. Plasmids were isolated that allowed growth at both the restrictive temperature and at increased Calcofluor white concentrations. Subcloning identified a 3.4-kb region that was still able to complement the *cwh6* muta-

Fig. 1. Cloning and disruption strategy of *CWH6*. A: Restriction map of the *cwh6* complementing insert. B: Partial sequencing of the *cwh6* complementing fragment. C: Gene disruption of *CWH6* / *SPT14*.

Abbreviations: GPI, Glycosylphosphatidylinositol; GlcNAc, *N*-acetyl-glucosamine; Glc, glucose; PI, phosphatidylinositol; PIG, phosphatidylinositol glycan

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tion (Fig. 1A). Partial sequence analysis (Fig. 1B) revealed 99% identity over 613 base pairs to the *SPT14* gene [9] and its 3' flanking region (data not shown). As the restriction maps of *CWH6* and *SPT14* were also identical, we concluded that we had cloned *SPT14* and that *SPT14* could complement *cwh6*.

SPT14 gene was disrupted by replacing a 1.1-kb *Bam*HI fragment with the *URA3* gene (Fig. 1C). A *ura3*[−] diploid wild type strain was transformed with the *SPT14::URA3* construct, in order to induce homologous recombination. Uracil prototrophs were selected and sporulated. A 2:2 segregation was observed between *ura*[−] wild type colonies and clumps of up to fifty cells demonstrating that *SPT14* was essential for growth.

TO show that *CWH6* and *SPT14* were identical, a *cwh6*/*CWH6* heterozygous diploid strain was transformed with the *SPT14::URA3* disruption construct. Forty percent of the uracil prototrophs were temperature sensitive, indicating that the *CWH6* allele was disrupted. The *Ts*[−] cells failed to sporulate. *Ts*⁺ transformants were sporulated and showed a 2:2 segregation between *ura*[−] wild type colonies and clumps of up to fifty cells as observed before. This indicates that in *Ts*⁺ cells the *cwh6* allele was disrupted. We conclude that *cwh6* is a mutant allele of *SPT14*.

SPT14 was initially identified as a suppressor of Ty insertion in the *HIS4* promoter region and proposed to function as a trans-acting transcription factor [9]. However, the Spt14-protein sequence showed no similarity to any known transcription factors. In contrast, 46% of 429 amino acids were identical and 86% were found to be conserved between Spt14p and the human PIG-A protein (Fig. 2A) [10]. PIG-A is one of three genes that are necessary for the transfer of *N*-acetyl-glucosamine (GlcNAc) to phosphatidylinositol (PI → PIG), an essential step in the formation of GPI anchors [11]. Spt14p also has 21% identical and 70% conserved residues compared to 214 amino acids from *Salmonella typhimurium* RFAK protein (Fig. 2A) [12–15]. RFAK is involved in the transfer of GlcNAc to glucose in lipopolysaccharides. The high degree of conservation between the predicted protein sequences of PIG-A, RFAK, and SPT14 strongly indicates that these proteins have a similar function. Based on this and on the phenotypic characteristics of *cwh6* (see above), it seems likely that Spt14p/Cwh6p, like human PIG-A, is involved in transferring GlcNAc to phosphatidylinositol to form a GPI anchor precursor. Interestingly, smaller conserved regions (from amino acid 194–239 and 283–428 in Spt14p) were found in six glycogen synthases and seventeen sucrose synthases (Fig. 2B). As both types of enzymes use UDP-Glc as a glucose donor, whereas a similar compound, UDP-GlcNAc, functions as a donor of *N*-acetylglucosamine to form phosphatidylinositol-GlcNAc, this further supports our conclusion that Spt14p/Cwh6p is involved in the transfer of GlcNAc to phosphatidylinositol. Finally, since the mannose content of *cwh6* cell walls is less than

A		
PIG-A	37	MVSDFFYPNM GG--VESHIY QLSQCLIERG HKVIIVTHAY GNRKGI RYLT
RFAK	9	--TIFSIIPPR GAAAVETWYI QVAKRLSIPN ATACIKNAGY PEYNTI---N
Spt14	1	MLCDFFFYPQL GG--VEFHIY HLSQKLDLGL HSYVIITHAY KDRVGV RHLT
PIG-A	85	SGLKVYYLPL KVMYNQSTAT -TLFHS LPL- LRYIFVRERV TIIHSHSSES
RFAK	56	DNCDIHYIGF SKVYKRLFQK WTRLDLPLYS QRILNIRDQV T-TQDSVSV
Spt14	49	NGLKVYHVPF FVIFRETFEP -TVSFTEPI- IRNILLRQV QIVHSHGASV
PIG-A	133	AMAHDALEF-H AKTMGLQTVF TDHSLFGFAD VSSVLTKLL TVSLCDTNHI
RFAK	105	IHNSMKLYRQ IRERNPNAKL VMHMHNAF-- -EPELPNDNA KIIV--PSQF
Spt14	97	TFAHEGIL-H ANTMGLRTVF TDHSLYGFNN LTSIWNKLL TFTLTNIDRV
PIG-A	182	ICVSYTSKEN TVLRAALNPE IVSVIPNAVD PTDETP-DP- -----FRRH
RFAK	150	LKAFYEER-- --LPAA---- AVSIVPNPFC AETTK-RNPQ DNLROQLNIA
Spt14	146	ICVSNTECKN MIVRTELSPD IISVIPNAVY SEDFKPRDP- TGTGKRKQSR
PIG-A	224	DSITIVV-VS RLVIYRKGDIL LSGIPELQCY KYPDNLFIIG GE-----GP
RFAK	242	EDATVLLYAG RISPDKGLTL LLQAKQLRVT LRSNKLKLVV GQPYASRKGE
Spt14	295	DKIVIVV-IG RLFPNKGSDI LTRIPKVCYS SHEDVEFTVA GD-----GP
PIG-A	267	KRIILEEVRE RY-QLHDRV RLGALEHKKV RNVL-VQGH I FLNLSL TEAF
RFAK	242	KAQYQKKYLD AAKEIGTDCI MAGGQSPDQM HNFYHIALDV IVPQVEEAF
Spt14	238	KFIDFQQMIE SH-RLQKRVQ LLGSVPHEKV RDVL-CQGD I YELHASL TEAF
PIG-A	315	CMAIVEAASC GLQVSVTRVG GIPEVLNENL II--LCEP-S VKSLCEGLEK
RFAK	292	CMVAVEAMAA GKAVLASKKG GISEFVL DGI TGYHLAEPMS SSSINDINR
Spt14	286	GTILVEAASC NLLIVTTQVG GIPEVLNENM TVY--AEQTS VSDVLQATNK
PIG-A	362	AIFQLKSGTL PAPIENHNV KTFYTRNVA ERTEKVYDRV -SVEAVLPMD
RFAK	342	ALAD--KERH QIAEKAISLV FSKYSWENVA QRFE-----QMKN
Spt14	334	AIIIRISKAL DTS-SFHDSV SKMYDMMOVA KRTVEITYNTI SSTSSADDD
PIG-A	411	---KRLRLI SHCGPVTGYI FALLAVFNFL FLIFLRWMTPT DSIIDVAIDA
RFAK	379	WFDK.....
Spt14	383	WM-KMVANLY KRDGIWAKHL YLLCGIV EYM LFFLLEWLYP RDEIDLA---
PIG-A	458	TGPRGAWTNN YSHSKRGGEN NEISETR.....
RFAK	429	---PK--WPKK ----TVSNET KEARET.....
Spt14		
B		
PIG-A	224	DSI--TIVVV SRLVYRKGDIL LLSG-IIPEL CQKYPDLNFI IGGE-----
RFAK	192	DAT--VLLYA GRISPDKGL LLLQ-AFKQL RTLRSNIKLV VVGDPYASRK
Spt14	195	DKE--VIVVI GRIFPNKGS D LTR-ITPKV CSSHEDVEFT VAGD-----
UGST	390	DRKVPVLVAFI GRLEEQKQPD VMIA-AIPEI -LKEEDVOIE LLGT-----
SUS1	577	DR---VKNMT GLVEMYGKNA HLRDLA--NL -----VI VCGDHGQSK
PIG-A	265	GPKRIILEEV RERY-QLHDR VRLIGALEHK D---VRNVLV QGHTEI NT--
RFAK	239	GEKAEYQKKV LDAKEIGTD CIMAGGGS-- --PDQMHN FYHTAD---
Spt14	236	GPKRIDFQQM IESH-RLQKR VQLLGSPVHE K---VRDVEC QGDYILHA--
UGST	432	GKKKFEK--- -LLKSMEEK FPGKVRVVR FNAPLAHQMM
SUS1	614	DREEQAEFFK MYGLTDQYKL KGHTRWISAQ MN-RVRNGEL YRYICDTK--
PIG-A	309	-----S LTEAFCMVAV EAASCGLOVV STRVGGIPEV LPENLII--L
RFAK	279	---LVIVPSQ VEEAFCMVAV EAMAAGKAVL ASKKGGESEF VLDGITGYHL
Spt14	280	-----S LTEAFETILV EAASCNLLIV TTQVGGIPEV LPNEMTYAE
UGST	467	AGADLLAVTS RFEPGGLIQL QAMRYGTPCV CASTGGLVDT IVEGKTGFHM
SUS1	661	---GVFVQPA FYEAEGLTVI EMATCGLPIT ATCHGGPAEI IVDGVSGLHT
PIG-A	348	CEPSV-KSLC EGLEKAIFQL KSGTLAPEN IHNIVKTFYT WRNVAERTEK
RFAK	326	AEPMSDSDII NDINRALAD- KERHQA-EK AKSLVFSKYS WENVAERTEE
Spt14	321	QTSVSD-LV QATNKATNII RSKALDT-SS FHDSVSKMYD WMDVAKRTVE
UGST	517	GRLSVDCNVV EPADVKKVAI TLKRAVKVVG TPAYQEMVKN CMQDLSWKG
SUS1	708	-DPYHSDKAA DILVNFEECK KQDSTYWDNI SQGGLQRIYE KYTNKLYSER

Fig. 2. Sequence alignments of Spt14p. Identical amino acids are in grey boxes. A: Sequence alignment of human PIG-A protein, RFAK protein from *Salmonella typhimurium*, and Spt14 protein from yeast. B: Sequence alignment of a conserved domain in the protein sequences of PIG-A, RFAK, *SPT14*, UGST, a glycogen synthase precursor from *Hordeum vulgare* [13], and SUS1, a sucrose synthase from *Oryza sativa* [14]. Calculation of the percentage of conservation between proteins was performed according to Lipman and Pearson [15], using the MacDNASIS Pro DNA and Protein Sequence Analysis System.

half of the wild type level, this indicates that a majority of cell wall proteins makes use of a GPI anchor-dependent incorporation mechanism.

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