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Saccharomyces cerevisiae Apl2p, a homologue of the mammalian clathrin AP β subunit, plays a role in clathrin-dependent Golgi functions

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SUMMARY

Clathrin-coated vesicles mediate selective intracellular protein traffic from the plasma membrane and the *trans*-Golgi network. At these sites, clathrin-associated protein (AP) complexes have been implicated in both clathrin coat assembly and collection of cargo into nascent vesicles. We have found a gene on yeast chromosome XI that encodes a homologue of the mammalian AP β subunits. Disruptions of this gene, *APL2*, and a previously identified β homologue, *APL1*, have been engineered in cells expressing wild-type (*CHC1*) or temperature sensitive (*chc1-ts*) alleles of the clathrin heavy chain gene. *APL1* or *APL2* disruptions (*apl1Δ* or *apl2Δ*) yield no discernable phenotypes in *CHC1* strains, indicating that the Apl proteins are not essential for clathrin function. However, the *apl2Δ*, but not the *apl1Δ*, allele enhances the growth and α-factor pheromone maturation defects of *chc1-ts* cells. Disruption of *APL2* also partially suppresses the vacuolar sorting defect that occurs in *chc1-ts* cells immediately after imposition of the non-permissive temperature. These Golgi-specific effects of *apl2Δ* in *chc1-ts* cells provide evidence that Apl2p is a component of an AP complex that interacts with clathrin at the Golgi apparatus.

Key words: clathrin, clathrin-associated protein, adaptin, Golgi apparatus, yeast

INTRODUCTION

Protein traffic between membrane-bounded compartments of eukaryotic cells is mediated by vesicular carriers. The genesis of transport vesicles involves the assembly of a proteinaceous coat on the cytoplasmic surface of the donor organelle membrane, followed by membrane invagination and release of a coated vesicle (for recent reviews see Pryer et al., 1992; Rothman and Orci, 1992). A number of distinct coats have been characterized. Clathrin coats participate in selective protein transport at the plasma membrane and the Golgi apparatus. Clathrin-mediated processes include receptor-mediated endocytosis, sorting of lysosomal (vacuolar) precursors from the Golgi complex to vesicles directed to endosomes, and retention of resident Golgi membrane proteins (reviewed in Brodsky, 1988; Pearse and Robinson, 1990; Wilsbach and Payne, 1993a). Distinct coats composed of the multimeric COPI or COPII complexes play roles in vesicular traffic between the endoplasmic reticulum (ER) and the Golgi complex and between Golgi subcompartments (Malhotra et al., 1989; Orci et al., 1989; Barlowe et al., 1994).

Clathrin coats are assembled into polyhedral lattices built principally with trimers of clathrin heavy (Chc) and light chains (Clc), and heterotetramers of clathrin-associated proteins (APs) (reviewed by Morris et al., 1989; Keen, 1990; Robinson, 1992; Kirchhausen, 1993). The clathrin lattice enfolds a layer of APs, positioned to bridge the lattice and the vesicle membrane. Two major AP complexes have been characterized: AP-1 is a component of *trans*-Golgi network (TGN) clathrin coats; AP-2 is a component of plasma membrane clathrin coats. Both APs can drive the in vitro assembly of free clathrin trimers into coat-like cages suggesting a role in coat assembly. Consistent with this proposal, AP-2 stimulates the formation and invagination of clathrin-coated pits in an in vitro assay for endocytic vesicle production (Smythe et al., 1992). AP-1 is thought to similarly participate in the formation of clathrin-coated vesicles at the TGN but in vitro assays for AP-1 function at the Golgi complex have not yet been developed. APs also exhibit weak but selective affinity for the cytoplasmic domains of transmembrane cargo proteins. AP-2 but not AP-1 specifically interacts with the cytoplasmic tails of plasma membrane proteins (Pearse, 1988; Chang et al., 1993). AP-1 will bind to the tails of the cation-independent and cation-dependent mannose-6-phosphate receptors, transmembrane

proteins which are associated with both Golgi and plasma membrane clathrin coats (Glickman et al., 1989; Sosa et al., 1993). Together these findings have led to the view that APs play a dual role in clathrin coat formation by nucleating assembly of clathrin trimers and by selectively collecting cargo through interactions with the cytoplasmic tails of transmembrane proteins.

Each AP complex consists of two large (≈100 kDa), one medium (≈50 kDa) and one small (≈20 kDa) subunit (Ahle et al., 1988; Virshup and Bennett, 1988; Matsui and Kirchhausen, 1990). The AP-1 large subunits are designated γ and β1 (formerly β′) and their cognates in AP-2 are designated α and β2 formerly (β). The μ1 and μ2 chains are the medium subunits, and σ1 and σ2 are the small subunits, of AP-1 and AP-2, respectively. Analyses of the large subunits, also termed adaptins, suggest that the α and γ chains may contribute to the differential localization of the AP complexes (Chang et al., 1993; Robinson, 1993). The β chains are involved in clathrin assembly (Gallusser and Kirchhausen, 1993), while the functions of the μ and σ subunits are still unknown.

A number of genes encoding potential AP subunit homologues have been identified in the yeast *Saccharomyces cerevisiae* (Kirchhausen, 1990; Nakayama et al., 1991; Kirchhausen et al., 1991; Nakai et al., 1993; Phan et al., 1994). These homologues offer the possibility of a genetic approach to determining AP subunit function. Initial studies focused on two putative σ subunits encoded by *APS* genes (Phan et al., 1994). These proteins were shown to be associated with clathrin-coated vesicles and to be components of distinct, large complexes. Disruptions of the *APS* genes (*aps1Δ*) in otherwise wild-type cells produce no detectable phenotypes suggesting that the yeast AP complexes do not require the σ subunits for normal function. However, *aps1Δ* but not *aps2Δ* exacerbates the growth and α-factor pheromone maturation defects in cells carrying a temperature-sensitive allele of clathrin heavy chain (*chc1-ts*). Two other clathrin-dependent processes, vacuolar protein sorting and pheromone receptor endocytosis, were unaffected in *chc1-ts* cells by either *aps1Δ* or *aps2Δ*. These results provide evidence that Aps1p is involved in a clathrin-dependent function at the Golgi complex.

Here we describe a new putative β homologue gene (*APL2*) in *S. cerevisiae* and present phenotypic characterizations of strains carrying disruptions of this gene or a previously identified β-type gene (*APL1*). The results show that, like disruptions of the *APS* genes, neither *apl1Δ* or *apl2Δ* affect otherwise wild-type cells. In *chc1-ts* cells, *apl2Δ* but not *apl1Δ* yields growth and α-factor maturation defects that are more pronounced than *aps1Δ*, and also alters a vacuolar protein sorting defect. Our results provide genetic evidence for clathrin-dependent function of Apl2p at the Golgi complex and suggest that Apl2p may be part of an AP complex containing Aps1p.

MATERIALS AND METHODS

Plasmids and nucleic acid techniques

Plasmid constructions were carried out using standard molecular biology techniques (Sambrook et al., 1989). The sequence of the *APL2* locus was obtained during the course of the European Community Yeast Genome Sequencing Project of *Saccharomyces cerevisiae* chromosome XI (Dujon et al., 1994). The 5 kb *Eco*RI fragment containing *APL2* was subcloned into pBluescript KS^+ (Stratagene, La Jolla, CA). Nested deletions were generated using a kit (Pharmacia) and specific, fluorescently-labelled oligonucleotide primers were used with T7 DNA polymerase to obtain overlapping sequence from both strands by the dideoxynucleotide chain termination method (Sanger et al., 1977). Sequencing reactions were analyzed on a Pharmacia ALF DNA sequencer.

To generate a plasmid for *APL2* disruption, a plasmid consisting of the pT7T3 U19 vector (Pharmacia) carrying the *Eco*RI-*Sna*BI *APL2* fragment was modified by replacing the 0.76 kb *Hpa*I-*Spe*I fragment with the 1.16 kbp *Sma*I-*Spe*I fragment of *URA3* (Rose et al., 1984). This construct results in the replacement of amino acids 102-357 in the Apl2p sequence. For single step gene replacement the resulting plasmid was digested with *Bst*XI.

To generate a disrupted *APL1* allele, first the 6.1 kb *Hin*dIII fragment from pH27 (Roy et al., 1991) was transfered to pUC118 (Vieira and Messing, 1087) to make pAPL1-100. The 1 kb *Ssp*I-*Stu*I *TRP1* fragment from YRp17 (Tschumper and Carbon, 1980) was used to replace the *APL1 Bgl*II-*Cla*I fragment in pAPL1-100 to make papl1-Δ3. This construct lacks amino acids 19-590 of Apl1p. For disruptions papl1-Δ3 was cleaved with *Bam*HI and *Sal*I. An Apl1p overproducing plasmid was constructed by inserting a 4 kb *Bam*HI-*Hin*dIII *APL1* fragment into the multicopy vector pRS424 (Christianson et al., 1992). Overexpression was confirmed by immunoblotting.

Total RNA was extracted from exponentially growing cultures (Sherman et al. 1986). Poly-adenylated RNA was selected by oligo(dT)-cellulose chromatography. 2 μg of poly$(A)^+$ RNA was fractionated on a formaldehyde-1% agarose gel and then blotted and fixed on a Hybond-N nylon membrane (Amersham). DNA fragments used as probes were purified from agarose gels and labelled with a random priming DNA labelling kit according to the instructions supplied in the kit (Boehringer Mannheim).

Strains, genetic methods and media

Yeast strains used in this study are listed in Table 1. Yeast mating, sporulation and tetrad analyses were conducted as described by Sherman et al. (1986). DNA transformations were performed by the lithium acetate procedure of Ito et al. (1983). Gene disruptions were engineered using single-step gene replacement (Rothstein, 1991). All gene replacements were verified by Southern blot analysis.

YP medium is 1% Bacto-Yeast extract and 2% Bactopeptone (Difco Laboratories, Inc., Detroit, MI). YP medium was supplemented with 2% dextrose, 0.1% dextrose, 2% ethanol and 2% glycerol, or 1% acetate. SD medium is 0.67% yeast nitrogen base (Difco Laboratories, Inc., Detroit, MI) without amino acids and 2% dextrose. Supplemented SD is SD with 20 mg/ml histidine, uracil, and tryptophan and 30 mg/ml leucine, adenine and lysine. SD CAA medium is SD with 5 mg/ml vitamin assay casamino acid mix (Difco Laboratories, Inc.) with 15 mg/ml adenine, and 10 mg/ml methionine, histidine, uracil, and tryptophan. SDYE is SD with 0.2% yeast extract. Cell densities in liquid culture were measured in a 1 cm plastic cuvette using DU62 Beckman spectrophotometer. One A_{500} unit is equivalent to 2.3×10^7 cells per ml.

Radiolabelling and immunoprecipitations

For metabolic labelling of α-factor, cells were grown to mid-logarithmic phase in SDYE at 24°C. Cultures were labelled at 24°C or shifted to 37°C for 2 hours prior to labelling. Labelling and immunoprecipitation was performed as described by Seeger and Payne (1992a) except that labelling was for 45 minutes instead of 10 minutes. Quantitation of the various forms of α-factor was carried out using an AMBIS phospho-imager.

For metabolic labelling of CPY, cells were grown to mid-logarithmic phase in SDYE at 24°C. Cultures were labelled at 24°C, or shifted to 30°C or 37°C for 5 minutes or 2 hours before labelling. Labelling and immunoprecipitation was conducted as described by Seeger and Payne (1992b).

Table 1. Yeast strains used in this study

Strain	Genotype	Source
FY1679-18B	*MATα leu2-Δ1 ura3-52 his3-Δ200 trp1-Δ63*	B. Dujon
GPY1100α	*MATα leu2-3,112 ura3-52 his4-519 trp1-289 can1*	Payne and Schekman (1989)
GPY74-15	*MATa leu2-3,112 ura3-52 his4 and/or his6 trp1-289 sst1-3*	This study
GPY398	*MATa leu2-3,112 ura3-52 his4 and/or his6 trp1-289 chc1-521(ts) sst1-3*	This study
GPY409	*MATa leu2-3,112 ura3-52 his4 and/or his6 trp1-289 chc1-521(ts) sst1-3 pep4::LEU2*	This study
GPY418	*MATα leu2-3,112 ura3-52 his4-519 trp1-289 can1 chc1-521(ts)*	Phan et al. (1994)
GPY719	*aps1-Δ1::LEU2* transformant of GPY418	Phan et al. (1994)
GPY721	*apl1-Δ3::TRP1* transformant of GPY418	This study
GPY727	*apl1-Δ3::TRP1* transformant of GPY398	This study
GPY778	*apl1-Δ3::TRP1* transformant of GPY409	This study
GPY905	*aps1-Δ1::LEU2 apl2-Δ1::URA3* transformant of GPY1100α	This study
GPY906	*apl2-Δ1::URA3* transformant of GPY418	This study
GPY907	*apl2-Δ1::URA3* transformant of GPY719	This study

Endocytosis assays

Endocytosis assays were carried out as described by Dulic et al. (1991) and Tan et al. (1993). Briefly, cells were grown to mid-log phase in SD CAA medium at 24°C, then collected by centrifugation and resuspended at 1-2×10^9 cells/ml in ice cold KPO_4 buffer (50 mM KPO_4, pH 6, containing 1% bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 10 mM *p*-tosyl-L-arginine methyl ester (TAME)). ^{35}S-labelled α-factor (prepared as by Tan et al., 1993) was added at 1-2×10^5 cpm/10^9 cells and allowed to bind to cells on ice for 30 minutes. Following the incubation, the cells were sedimented by centrifugation and the supernatant was aspirated to remove unbound α-factor. The cell pellet was resuspended in an equal volume of ice cold KPO_4 buffer and 100 μl aliquots were then incubated at 24°C, 30°C or 37°C for 5 minutes. Under these conditions α-factor remains bound to the cells but is not internalized (Chvatchko et al., 1986; P. Tan, unpublished). Glucose was then added to 2% to stimulate internalization, and the incubation continued at 24°C, 30°C or 37°C for 5 or 20 minutes. At these points, cells were diluted in ice cold 50 mM sodium citrate, pH 1.1, and incubated for 15 minutes to remove surface bound α-factor. The low pH-treated cells were collected by vacuum filtration on a Whatman GF/A filter disc. The filters were washed with 2× 5 ml ice cold 50 mM KPO_4, pH 6, and internalized α-factor was measured by scintillation counting of the filters. Total bound α-factor was assessed by washing and filtering cells in ice cold 50 mM KPO_4, pH 6 after the binding step.

RESULTS

Identification of *APL2*

As part of the European Yeast Genome Sequencing Project's sequencing of chromosome XI, an open reading frame (ORF YKL135c) was identified that encodes a predicted protein with significant similarity to mammalian AP β chains (Dujon et al., 1994). Fig. 1 presents the nucleotide sequence and the amino acid translation of the YKL135c ORF which lies approximately 187 kb from the left telomere of chromosome XI. Starting with the first available methionine, this ORF predicts a 726 amino acid protein of 81.8 kDa.

Sequence alignment reveals that the YKL135c ORF displays 39% identity (64% similarity) with rat β2 and 24% identity (50% similarity) with yeast Apl1p (Fig. 2). Based on this identity we have designated YKL135c as *APL2* and the putative protein product Apl2p. Since the mammalian β1 and β2 chains are highly related (84% identity; Kirchhausen et al., 1989) it is not possible to predict whether Apl2p will be part of an AP-1 or AP-2 complex based on sequence comparisons. Mammalian adaptins are divided into two domains separated by a protease-sensitive hinge region rich in glycines and prolines (Kirchhausen et al., 1989; Matsui and Kirchhausen, 1990; Kirchhausen, 1993). The amino-terminal domains are part of the core of the AP complex along with the σ and μ subunits, whereas the carboxy-terminal domains extend from the core as appendages (Kirchhausen et al., 1989). Both yeast Apl proteins are shorter than mammalian β subunits. As was shown earlier for Apl1p (Kirchhausen, 1990), the sequence alignment of Apl2p displays relatedness to the amino-terminal domain (≈600 amino acids) of the mammalian β chains but not the C-terminal appendage domains (Fig. 2).

Probes derived from the 5′ and 3′ ends of *APL2* (Fig. 3) were used to probe poly(A)$^+$ RNA from wild-type yeast (Fig. 4A) grown in media with different carbon sources. The blots were also hybridized to an actin probe (*ACT1*) to control for variations in the amount of RNA loaded in each lane. An RNA species of 2.5 kb was detected by both *APL2* probes indicating that it represents the *APL2* transcript. Compared to actin RNA levels, *APL2* transcript levels do not significantly change in cells grown in the different carbon sources (compare Fig. 4A, 5′ and 3′ *APL2* panels to *ACT1* panel), or under conditions of low sulfate or phosphate levels (data not shown). A 0.63 kb transcript was detected with the 3′ but not the 5′ probe (Fig. 4A, 3′ *APL2* panel). This RNA most likely corresponds to a transcript of ORF YKL136w (132 amino acids) which overlaps with the 3′ end of the *APL2* and is oriented in the opposite direction (Dujon et al., 1994). On chromosome XI there are 43 examples of partially overlapping ORFs and in many cases one of the ORFs is much shorter, leading to the speculation that the shorter ORF may not be expressed (Dujon et al., 1994). The presence of a YKL136w RNA suggests that the ORF is expressed. A 2.4 kb poly(A)$^+$ RNA was detected with an *APL1* probe indicating that this gene is also expressed (data not shown).

Disruption of *APL2* accentuates the growth defect of *chc1-ts* cells

Deleted versions of *APL1* and *APL2* were engineered for targeted gene disruptions (Fig. 3). The region of *APL2* that was removed in the disruption does not overlap YKL136w. Northern blots prepared from *apl2Δ* strains and probed with the 3′ *APL2* probe confirmed that YKL136w expression is not altered by the disruption (Fig. 4B). Taking into consideration

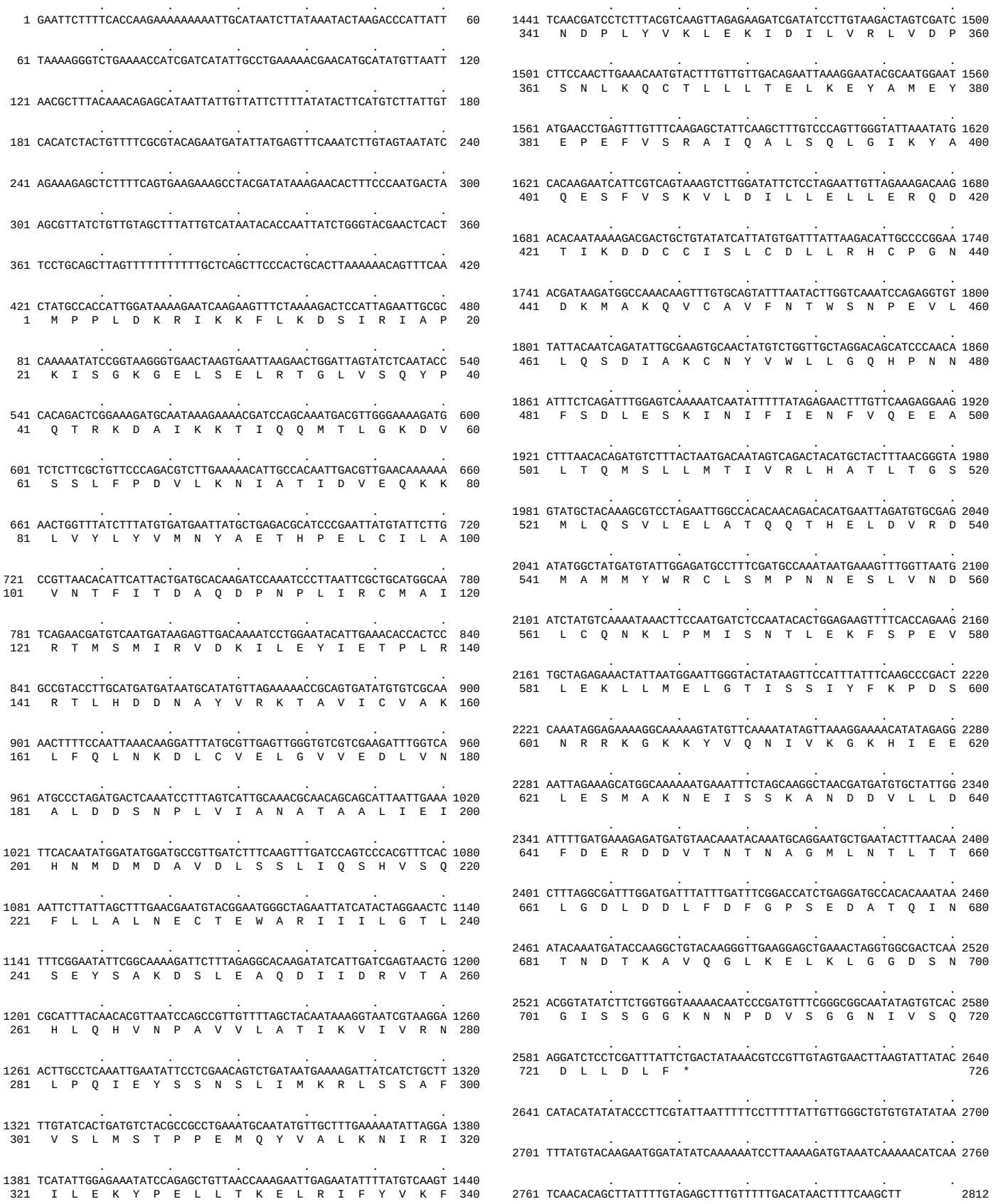

Fig. 1. Nucleotide sequence of the *Eco*RI-*Hin*dIII fragment containing *APL2* and the predicted amino acid sequence of Apl2p. These sequence data are in the EMBL Database under accession number Z30212.

```
        1                                                            50
β       ..........  .MTDSKYFTT N..KKGEIFE LKAELNNEK. ...KEKRKEA
APL2    mppldkrikk flk--iriap kisg---ls- -rtg-vsqy. ...pqt--d-
APL1    ..........  .-s-q-v-ar y..-an--vt dlqhfgvk-f ksnitr--n-

       51                                                           100
β       VKKVIAAMTV GK..DVSSLF PDVVNCMQ.T DNLELKKLVY LYLMNYAKSQ
APL2    i--t-qq--l --..------ ---lknia.- idv-q----- --v----eth
APL1    lr-i--nlvl -nygem-l-- sellkfw-ie -d--v-rich e-irvigalk

       101                                                          150
β       PDMAIMAVNS FVKDCEDPNP LIRALAVRTM GCIRVDKITE YLCEPLRKCL
APL2    -elc-l---t -it-aq---- ---cm-i--- sm------l- -iet---rt-
APL1    -qq-re-lpf imd-fksrde klqim-l--- vlvp-kelsd qafdciislv

       151                                                          200
β       KDEDP..YVR KTAAVCVAKL HDINAQMVED QGFLDSLRDL I..ADSNPMV
APL2    h-dna..--- ---vi----- fql-kdlcve l-vved-vna l..d----l-
APL1    nhks-peq-t r--iyalld- de-dher-.. l-lssi-h-i vkaqs-s-e-

       201                                                          250
β       VANAVAALSE ISESHPNS.N LLDLNPQNIN KLLTALNECT EWGQIFILDC
APL2    i---t---i- -hnmdmdavd -ss-iqshvs qf-l------ --ar-i--gt
APL1    iva-lht-ys -h-kna-m.e pfriplelaf dm-el-p-ln --nkatv-ev

       251                                                          300
β       LSNY.NPKDD REAQSICERV TPRLSHANSA VVLSAVKVLM KFLELLPKDS
APL2    --e-.sa--s l---d-id-- -ah-q-v-p- ---ati--iv rn-pqieys-
APL1    -ttsvv-qhy ldthemi-la l-y-qqv-ty ---nsl-fi- yl-nyvdvik

       301                                                          350
β       DYYNMLLKKL APPLVTLLSG EPEVQYVALR NINLIVQKR. PEILKQEIKV
APL2    n..slim-r- ssaf-s-m-t p--m-----k --ri-le-y. --l-tk-lri
APL1    e...t-ae-- snsvia--dk p--l-flv-- -vi-llls-e ssl-rld-sy

       351                                                          400
β       FFVKYNDPIY VKLEKLDIMI RLASQANIAQ ...VLAELKE YATEVDVDFV
APL2    -y--f---l- -----i--lv --vdps-lk- ctll-t---- --m-yepe--
APL1    --ie------ i-dt--ecly l--nketlpr ...i-e--eq ---di-iqms

       401                                                          450
β       RKAVRAIGRC AIKV.EQSA. .ERCVSTLLD LIQTKVNYVV QEAIVVIRDI
APL2    sr-iq-lsql g--y.a-esf vskvldi--e -le.rqdtik ddccislc-l
APL1    --s-----nl -v-ld-d-v. .hd--av--- -lefg-d--- --i-s-f-n-

       451                                                          500
β       FRKYPNKYE. .....SIIAT LCENLDSLDE PDARAAMIWI VGEYAERIDN
APL2    l-hc-gndkm akqvcavfn- wsnpevl-qs di-kcnyv-l l-qhpnnfsd
APL1    l-----nfk. .....anvte -vkhtevvq- -eskn----- itq-sdv-p-

       501                                                          550
β       ADELLESFLE GFHDESTQVQ LTLLTAIVKL FLKKPS.ETQ ELVQQVLSLA
APL2    leskini-i- n-vq-ealt- ms--mt--r- ...hat.l-g sml-s--e--
APL1    yl--frv-ss nmfn-tle-- fsi-nsai-f -irn-tk--e --cmdl-kgc

       551                                                          600
β       TQDSDNPDLR DRGYIYWRLL S.......TD PVT...AKEV VLSEKPLISE
APL2    --qthel-v- -mamm---c- -mp.....nn esl...vndl cqnkl-m--n
APL1    -dhen----- -ktlm----- -ltktsrisn ai-fesl-s- ldg-l---em

       601                                                          650
β       ETDLIEPTLL DELICHIGSL ASVYHKP... .......PNA FVEGSHGIHR
APL2    tlekfs-ev- ek-lmel-ti s-i-f--dsn rrkgkkyvqn i-k-k-ieel
APL1    n-k-.d--v- e--eln--ti v-i-l--... .......vsh ifrlnktkll

       651                                                          700
β       KHLPIHHGST D....AGDSP V......GTT TATNLEQPQV IPSQGDLLGD
APL2    esmaknei-s k....-n-dv lldfderddv -n--agmlnt lttl--.-d-
APL1    pqs--lnpnk -llpvv-n-i p......p-g anrdrqnses qs-tksrkta

       701                                                          750
β       LLNLDLGPPV NVPQVSSMQM GAVDLLGGGL DSLVGQSFIP SSVPATFAPS
APL2    -..f-f--se dat-intndt k--qg-ke.. lk-g-d-ngi --ggknnpdv
APL1    m......... .........- ddy-kpaeki nq-k-krkss -nn-sklsrk

       751                                                          800
β       PTPAVVSSGL NDLFELSTGI GMAPGGYVAP KAVWLPAVKA KGLEISGTFT
APL2    sggni--qd- l---...... .......... .......... ..........
APL1    -stllrklsm krp-s..... .......... .......... ..........

       801                                                          850
β       HRQGHIYMEM NFTNKALQHM TDFAIQFNKN SFGVIPSTPL AIHTPLMPNQ
APL2    .......... .......... .......... .......... ..........
APL1    .......... .......... .......... .......... ..........

       851                                                          900
β       SIDVSLPLNT LGPVMKMEPL NNLQVAVKNN IDVFYFSCLI PLNVLFVEDG
APL2    .......... .......... .......... .......... ..........
APL1    .......... .......... .......... .......... ..........

       901                                                          950
β       KMERQVFLAT WKDIPNENEL QFQIKECHLN ADTVSSKLQN NNVYTIAKRN
APL2    .......... .......... .......... .......... ..........
APL1    .......... .......... .......... .......... ..........

       951                                                         1000
β       VEGQDMLYQS LKLTNGIWIL AELRIQPGNP NYTLSLKCRA PEVSQYIYQV
APL2    .......... .......... .......... .......... ..........
APL1    .......... .......... .......... .......... ..........

       1001
β       YDSILKN
APL2    .......
APL1    .......
```

Fig. 2. Amino acid sequence alignment of the rat β2 subunit and yeast Apl proteins. Apl residues identical to the rat β2 sequence are indicated by a dash (-). Dots (·) represent gaps introduced to maximize the alignments. The sequences were aligned using the University of Wisconsin programs GAP, LineUp and Pretty.

that elimination of a β AP subunit might result in a severe growth defect, *apl1Δ* was introduced into a diploid strain to generate diploid cells heterozygous for the disruption. Following meiosis and dissection into tetrads, no growth anomalies were observed (data not shown). Based on this observation, and the finding that disruptions of the *APS* genes do not affect growth of cells expressing wild-type Chc (Phan et al., 1994), *APL1* or *APL2* were disrupted directly in haploid strains. The synthetic effect of *aps1* and *chc1-ts* on cell growth and α-factor maturation (Phan et al., 1994) prompted us to disrupt the *APL* genes in congenic sets of *CHC1* and *chc1-ts* strains.

The growth rates of congenic strains carrying different combinations of *chc1-ts*, *apl1Δ*, *apl2Δ* and *aps1Δ* were compared by streaking cells onto agar plates and incubating the cells at 24°C, 30°C or 37°C. No observable effect of any of the *ap* mutations was apparent at 24°C or 30°C (Fig. 5 shows 24°C). However, at 37°C the presence of *apl2Δ* but not *apl1Δ* dramatically reduced the growth of *chc1-ts* cells (compare sectors B and D, Fig. 5, 37°C). This growth defect was more substantial than that observed for *aps1Δ* in *chc1-ts* cells (Fig. 5, 37°C, sector 5C). The effect of *apl2Δ* on growth of *chc1-ts* cells at 37°C was too severe to accurately measure a growth rate but we estimate the doubling time of *chc1-ts apl2Δ* cells in liquid medium was at least four times as long as *chc1-ts* cells (3 hours) and at least twice as long as *chc1-ts aps1* cells (data not shown; Phan et al., 1994). Analogous effects of *apl* alleles were observed in another set of congenic *chc1-ts* strains (FY1679-18B background, data not shown).

Disruption of *APL2* accentuates the α-factor maturation defect in *chc1-ts* cells

In wild-type *MATα* cells, α-factor is synthesized as part of a larger precursor that is proteolytically converted into the mature 13 amino acid peptide after the precursor reaches the Golgi apparatus and acquires extensive carbohydrate modifications (Fuller et al., 1988). A maturation anomally in *chc1Δ* cells results in secretion of the highly glycosylated precursor (Payne and Schekman, 1989). This defect appears to result from inefficient retention of Kex2p, the endoprotease that normally initiates maturation of α-factor in the Golgi apparatus. In *chc1Δ* cells, Kex2p reaches the cell surface instead of remaining in the Golgi apparatus (Payne and Schekman, 1989). Cells expressing *chc1-ts* display the same phenotype at 37°C as *chc1Δ* cells (Seeger and Payne, 1992a). At 30°C the α-factor maturation defect in *chc1-ts* cells is less severe and at 24°C maturation proceeds with wild type efficiencies of 97% or greater. When *aps1Δ* was introduced into *chc1-ts* cells, a slight processing defect was apparent at 24°C where mutant cells secreted approximately 10% of the pheromone in the precursor form (Phan et al., 1994). At 30°C the proportion of precursor secreted by *chc1-ts aps1Δ* cells was commensurate with that secreted by *chc1-ts APS1* cells at 37°C. Thus, the *aps1Δ* mutation accentuated the defect caused by the temperature-sensitive Chc.

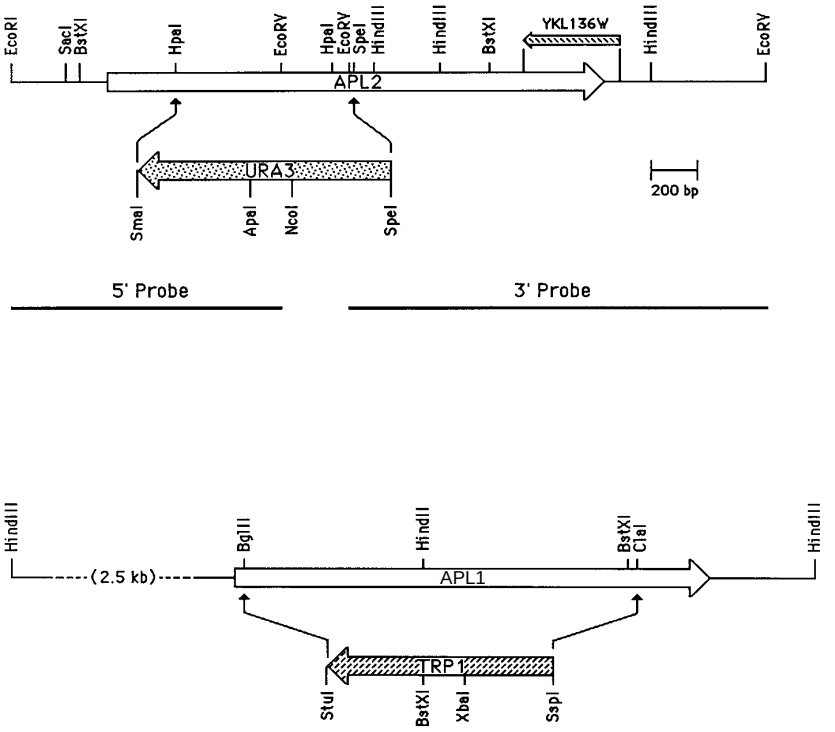

Fig. 3. The structures of *APL2* and *APL1* and the deletion constructs used to generate, *apl2-Δ1::URA3* and *apl1-Δ3::TRP1* mutants. Also shown is the location of the YKL136w ORF which overlaps *APL2*. Note that the disruption of *APL2* does not impinge on YKL136w. DNA fragments from the 5′ and 3′ regions of *APL2* used to probe poly(A)$^+$ RNA are delineated as 5′ Probe and 3′ Probe.

Following the precedent of *aps1Δ*, we introduced the *aplΔ* alleles into *chc1-ts* cells and assessed α-factor maturation at 24°C by monitoring the form of α-factor secreted into the culture medium. Congenic strains were incubated at 24°C for 45 minutes with ^{35}S-labelled amino acids and then α-factor was immunoprecipitated from the medium. Precipitated pheromone was subjected to SDS-PAGE and autoradiography (Fig. 6). In this gel system, mature α-factor migrates near the bottom of the gel and the highly glycosylated precursor migrates near the top of the gel. *CHC1 aps1Δ apl2Δ*, *chc1-ts*, and *chc1-ts apl1Δ* cells secreted only mature α-factor (Fig. 6, lanes 1-3). As reported previously *chc1-ts aps1Δ* cells secreted about 10% precursor pheromone (Fig. 6, lane 4). The presence of the *apl2Δ* allele in *chc1-ts* cells drastically reduced the efficiency of maturation, resulting in the secretion of 40% precursor (Fig. 6, lane 5). The *chc1-ts apl2Δ aps1Δ* cells secreted levels of precursor similar to that secreted by *chc1-ts apl2Δ* cells indicating that the effects of the two *ap* mutations are not synergistic (Fig. 6, lane 6).

Two findings argue that *APL2* and *APL1* are not functionally redundant for α-factor maturation. First, *chc1-ts apl1Δ apl2Δ* cells displayed a maturation defect equivalent to that of *chc1-ts apl2Δ* cells. Second, overexpression of Apl1p from a multicopy plasmid carrying *APL1* does not improve the maturation defect in *chc1-ts apl2Δ* cells (data not shown).

These results demonstrate that neither Apl protein is required for α-factor maturation in cells expressing wild-type clathrin heavy chain. However, in cells expressing the temperature-sensitive Chc, the substantial maturation defect at 24°C caused by *apl2Δ* suggests that the absence of Apl2p reduces clathrin function at the Golgi apparatus.

Disruption of *APL2* partially suppresses the vacuolar precursor sorting defect in *chc1-ts* cells

Another clathrin-dependent function of the Golgi apparatus is

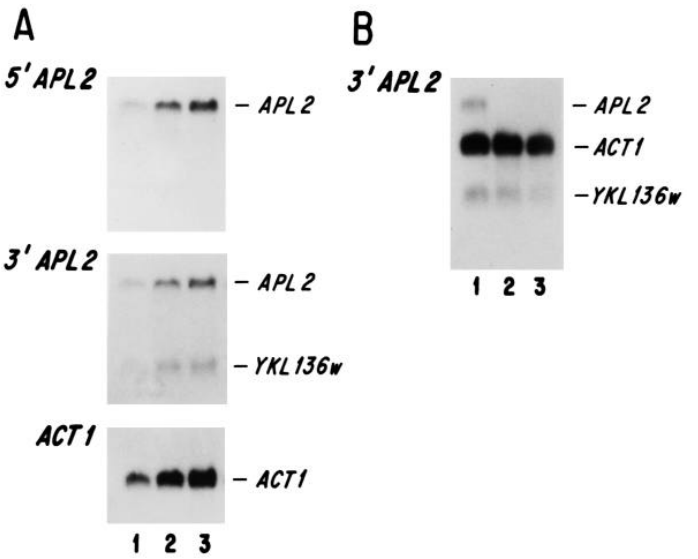

Fig. 4. *APL2* and YKL136w are expressed. (A) Poly(A)$^+$ RNA was prepared from wild-type strain FY1679-18B grown under different conditions. The RNA was fractionated on a 1% formaldehyde-agarose gel, transfered to nylon membrane and sequentially probed with labelled DNA fragments from *APL2* 5′, *APL2* 3′, and *ACT1* (see Materials and Methods). RNA from cells grown in: lane 1, 2% dextrose; lane 2, 0.1% dextrose; lane 3, 2% ethanol/glycerol. RNA was probed for the actin transcript to control for variations in loading. (B) Poly(A)$^+$ RNA was prepared and analysed from wild-type (FY1679-18B, lane 1) and *apl2Δ* strains (FY1679-18B *apl2Δ::URA3*, lane 2; GPY74-15 *apl2Δ::URA3*, lane 3) as described in (A). The RNA was probed simultaneously with the *APL2* 3′ and *ACT1* probes.

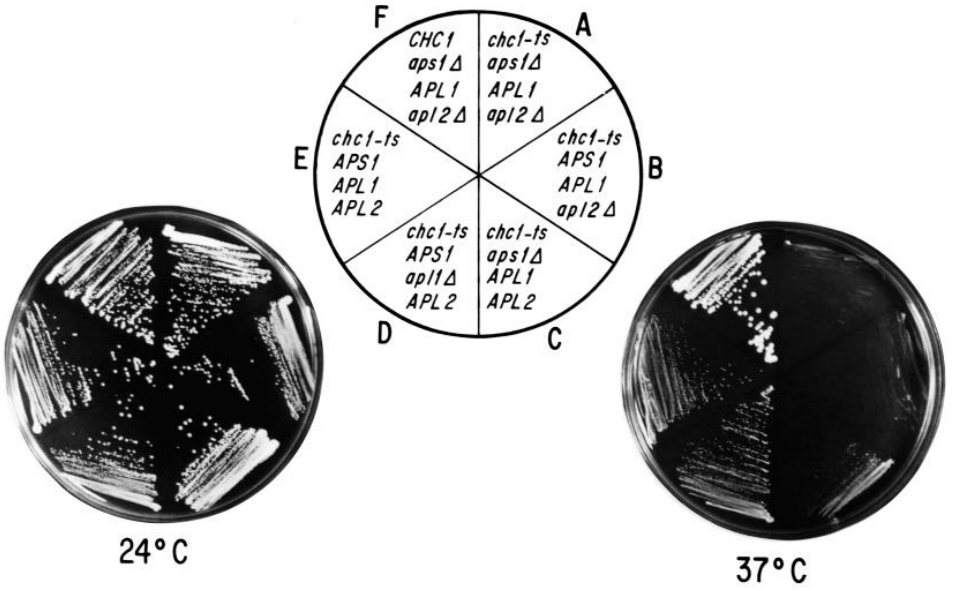

Fig. 5. The growth defect of *chc1-ts* cells is exacerbated by *apl2Δ* but not *apl1Δ*. Congenic strains GPY907 (A), GPY 906 (B), GPY719 (C), GPY721 (D), GPY418 (E) and GPY905 (F) were streaked onto supplemented SD agar plates and incubated at 24°C or 37°C.

the sorting of newly-synthesized soluble vacuolar precursors from the secretory pathway to a route directed to the vacuole (Seeger and Payne, 1992b). We examined the effects of the *aplΔ* mutations on the sorting of the soluble vacuolar protein carboxypeptidase Y (CPY) in *chc1-ts* cells at both permissive and non-permissive temperatures. In wild-type cells, and in *chc1-ts* cells at 24°C, CPY is synthesized in a precursor form that is translocated into the ER and subjected to signal sequence cleavage and core glycosylation (Stevens et al., 1982). The resulting 67 kDa p1 precursor is delivered to the Golgi apparatus where further limited addition of mannose residues yields the 69 kDa p2 form. p2 CPY is sorted in a late Golgi compartment and delivered to the vacuole by way of an endosomal compartment (Vida et al., 1993). Upon reaching the vacuole the precursor is proteolytically processed to produce the active, mature (m) form. In *chc1-ts* cells shifted to 37°C for short periods of time, sorting of CPY is blocked and the p2 form is secreted into the culture medium (Seeger and Payne, 1992b). A curious, and unexplained, feature of this block is that it is not evident in *chc1-ts* cells shifted to 37°C for much longer times, or in *chc1Δ* cells. Thus, some type of adaptation allows mutant cells to regain an ability to sort soluble vacuolar precursors. The loss of *APS1* does not noticeably affect the CPY sorting defect or the sorting recovery in *chc1-ts* cells (Phan et al., 1994).

Sorting of CPY was monitored in *chc1-ts apl* mutants under various temperature treatments by analyzing the forms of cell-associated and extracellular CPY in pulse-chase experiments. The results obtained from *chc1-ts* and *chc1-ts apl2Δ aps1Δ* cells are shown in Fig. 7. At 24°C, after a 10 minute labelling period, all of the CPY was contained within the cells in the p1 and p2 forms (Fig. 7A, lanes 1-2, 5-6). Forty minutes after the chase was initiated, all of the CPY was sorted and delivered to the vacuole where it was processed to the mature form (Fig. 7A, lanes 3-4, 7-8). CPY sorting was also unaffected at 30°C in each of the strains (data not shown).

In contrast, when cells were shifted to 37°C for 5 minutes and then subjected to the pulse-chase regimen, a sorting defect was apparent in each strain (Fig. 7B). In the *chc1-ts* cells after

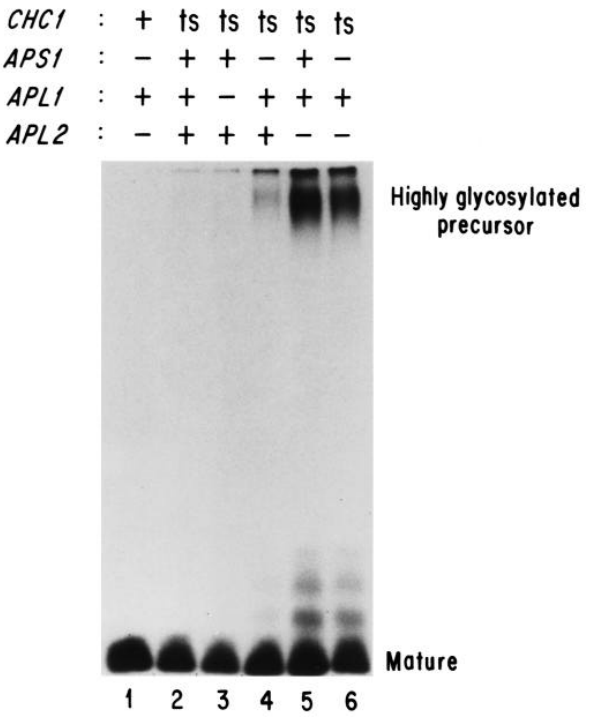

Fig. 6. Increased α-factor maturation defect in *chc1-ts apl2Δ* cells. Cells were metabolically labelled with [^{35}S]amino acids for 45 minutes at 24°C. α-Factor was immunoprecipitated from the culture supernatant and analyzed by SDS-PAGE and autoradiography (see Materials and Methods). + and – indicate the presence or absence of a gene (*CHC1*, *APS1*, *APL1*, *APL2*). ts indicates the presence of the temperature sensitive allele of *CHC1*.

the 10 minute labelling, the bulk of the CPY was intracellular as the p1 and p2 forms with only a slight amount of p2 in the extracellular fraction (Fig. 7B, lanes 1-2; the extracellular CPY is visible in the original autoradiogram). By 40 minutes of chase, a significant amount of p2 CPY was secreted and the intracellular fraction contained mostly p2 CPY (Fig. 7B, lanes 3-4). Somewhat different results were obtained with the *chc1-*

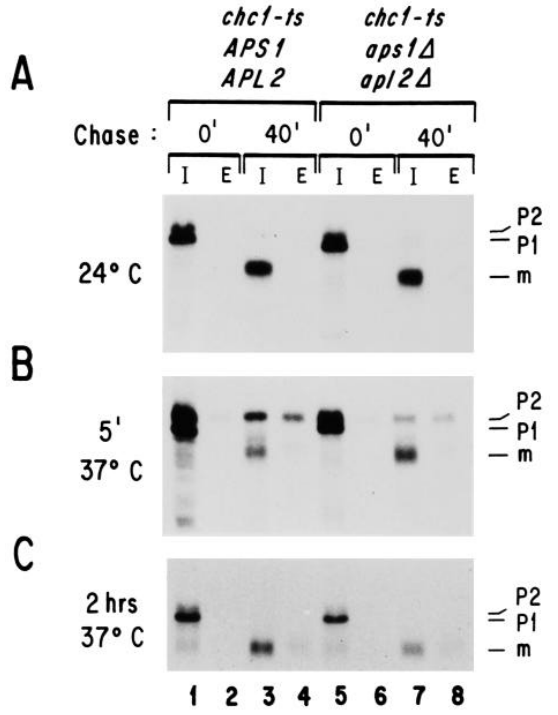

Fig. 7. Partial suppression of the *chc1-ts* CPY sorting defect by *apl2Δ*. Strains GPY418 (*chc1-ts*) and GPY907 (*chc1-ts aps1Δ apl2Δ*) were grown at 24°C and then either retained at 24°C (A), shifted for 5 minutes to 37°C (B) or shifted for 2 hours to 37°C (C). Following the temperature shifts the cells were pulse-labelled for 10 minutes with [^{35}S]amino acids after which excess unlabelled amino acids were added and an aliquot was harvested (0′ chase). The remaining cells were incubated for an additional 40 minutes (40′ chase). Intracellular (I) and extracellular (E) fractions were prepared and CPY was immunoprecipitated from each fraction and analyzed by SDS-PAGE and autoradiography (see Materials and Methods). The ER and early Golgi form is labelled p1, the late Golgi form is labelled p2 and the mature form is labelled m.

ts apl2Δ aps1Δ strain (Fig. 7B, lanes 5-8). In these cells the sorting defect was reproducibly not as strong as that observed in the *chc1-ts* cells (compare Fig. 7B, lanes 3-4 to lanes 7-8), although compared to the 24°C samples (Fig. 7A, lanes 7-8) there was clearly some missorting of p2 CPY. Quantitation of the data shown in Fig. 7 indicates that in the *chc1-ts apl2Δ aps1Δ* strain 71% of the CPY was correctly processed to the mature form after 40 minutes (Fig. 7B, lanes 7-8) whereas in the *chc1-ts* strain only 34% of CPY was found in the mature form (Fig. 7B, lanes 3-4). The reduced sorting defect in the *chc1-ts apl2Δ aps1Δ* cells was due to the presence of the *apl2Δ* allele since similar results were obtained with *chc1-ts apl2Δ* cells (data not shown).

We also examined whether *apl2Δ* interfered with the recovery of sorting in *chc1-ts* cells by first shifting cells to 37°C for 2 hours prior to labelling (Fig. 7C). By this time, both strains displayed efficient sorting with the bulk of the CPY present intracellularly as the m form after the 40 minute chase (Fig. 7C, lanes 3, 4, 7 and 8). The small amount of mCPY present in the extracellular fraction most likely was due to a slight degree of cell lysis during the removal of the cell wall. When the same CPY sorting experiments were carried out with *chc1-ts apl1Δ* cells, no difference was observed between these cells and *chc1-ts* cells (data not shown). Taken together, these results show that neither *apl2Δ* or *apl1Δ* accentuate the CPY sorting defect in *chc1-ts* cells. However, the presence of *apl2Δ* appears to enhance the ability of *chc1-ts* cells to properly sort CPY immediately after a shift to the non-permissive temperature.

Endocytosis of mating pheromone is not altered by *apl* mutations

Clathrin also plays a role in the uptake of mating pheromone receptors in yeast (Tan et al., 1993). In *chc1-ts* cells, α-factor internalization occurs at wild-type rates at 24°C and 30°C but immediately after a shift to 37°C internalization is reduced 2- to 3-fold to the level of endocytosis observed in *chc1Δ* cells. These findings indicate that clathrin-independent endocytosis of pheromone can occur, but clathrin is required for optimal uptake rates. The effect of *apl* mutations on clathrin-mediated and clathrin-independent endocytosis was determined by comparing uptake of radiolabelled α-factor in *chc1-ts* cells to uptake by *chc1-ts aplΔ* cells at 24°C, 30°C or 37°C. For these experiments, cells were grown overnight at 24°C and then allowed to bind to radiolabelled ligand at 0°C in the absence of glucose. Unbound pheromone was removed and cells were shifted to 24°C, 30°C or 37°C for five minutes still without glucose (preshift). In the absence of glucose, cells lack sufficient energy stores to carry out endocytosis. Thus, when carried out at the non-permissive temperature, the preshift allows imposition of the temperature-sensitive defect in Chc cells without membrane traffic. Following the 5 minute preshift, glucose was added and endocytosis was measured after 5 and 20 minutes by treating cells with a low pH buffer to remove surface-bound α-factor. Fig. 8 compares the levels of α-factor internalization at 30°C by congenic *chc1-ts*, *chc1-ts apl1Δ*, and *chc1-ts apl2Δ* strains in two separate experiments. Although the total uptake of α-factor in different experiments varied somewhat, within a given experiment there was no effect of the *apl* mutations on uptake at this temperature, or at 24°C (data not shown). The *apl* mutations did not reduce the residual, clathrin-independent endocytosis that occured at 37°C (data not shown). Similar results were obtained using *chc1-ts* cells carrying both *apl1Δ* and *apl2Δ* (data not shown). Therefore, the Apl1 and Apl2 proteins are not necessary for either clathrin-dependent or clathrin-independent endocytosis.

DISCUSSION

We have discovered a gene, *APL2*, on yeast chromosome XI that encodes a homologue of the β subunits of mammalian clathrin AP complexes. We have carried out the first in vivo analysis of β subunit function with this gene and the previously identified *APL1* (Kirchhausen, 1990). Deletions of these genes in cells expressing fully functional clathrin cause no detectable phenotypes. In contrast, *apl2Δ* accentuates the growth and α-factor maturation defects, and partially suppresses the CPY sorting defect, in *chc1-ts* cells. Such genetic interactions can indicate that two gene products act in common processes and, combined with sequence comparisons, argue that Apl2p is a yeast cognate of mammalian clathrin AP β subunits.

With the prior identification of *APL1*, there are now two

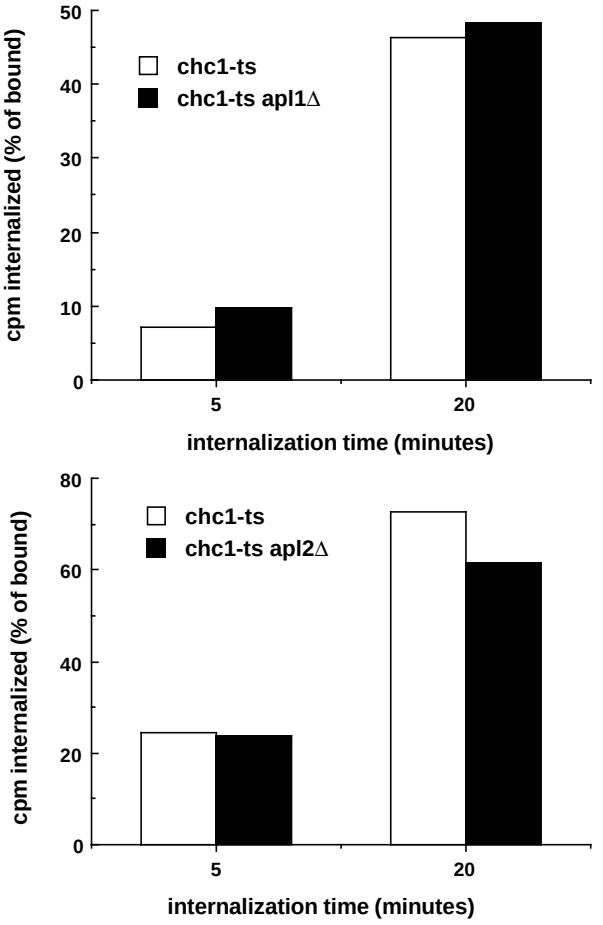

Fig. 8. Deletions of *APL1* and *APL2* do not affect endocytosis of α-factor. Cells were grown at 24°C, allowed to bind to ^{35}S-labelled α-factor, and then shifted to 30°C in the absence of glucose. After 5 minutes, glucose was added to 2% and samples were harvested after 5 and 20 minutes and internalized pheromone was measured (see Materials and Methods). For each time point duplicate samples were analyzed and the results averaged. The top and bottom panels come from separate experiments.

known genes in yeast capable of expressing β subunits, as well as two possible σ subunit genes (*APS1* and *APS2*; Kirchhausen et al., 1991; Nakai et al., 1993; Phan et al., 1994) and one μ subunit gene (*APM1*; Nakayama et al., 1991). To date there are no published candidates for genes expressing α or γ subunits. In the case of the *APS* genes, comparison of the predicted amino acid sequences with mammalian σ sequences allowed a tentative assignment of the Aps1 protein as a σ1 subunit and Aps2p as a σ2 subunit. Biochemical characterization of Aps1p combined with phenotypic analysis of *chc1-ts aps1Δ* strains provided support for the idea that Aps1p functions as part of an AP-1 complex at the Golgi apparatus (Phan et al., 1994). Establishing preferential relationships between the Apl proteins and the mammalian β1 or β2 subunits is precluded by the high degree of sequence identity (84%) between the mammalian β1 and β2 subunits. However, the similar phenotypes displayed by *chc1-ts* cells harboring either *apl2Δ* or *aps1Δ* offer genetic evidence that Apl2p and Aps1p function in the same processes. Preliminary chemical cross-linking experiments also suggest that Aps1p and Apl2p interact (H.P. and G.P., unpublished). Based on this evidence we propose that Apl2p is the β subunit of a Golgi-localized AP complex which also contains Aps1p.

Disruption of either *APL* gene in cells expressing fully-functional Chc produced no observable effects on cell growth, α-factor maturation, sorting of CPY to the vacuole, or pheromone endocytosis. It is possible that the innocuous nature of individual *APL* disruptions is due to functional redundancy between Apl1p and Apl2p. The mammalian β subunits are capable of interchange between AP complexes (W. Boll, K. Clairmont and T. Kirchhausen, unpublished), but they share a much higher degree of identity (84%) than the Apl proteins (24%). Furthermore, functional redundancy between the Apl proteins seems unlikely because mutation of both genes in *CHC1* cells does not effect cell growth, α-factor maturation or α-factor endocytosis (E. Bensen, P.T. and G.P., unpublished). Also, the α-factor maturation defect resulting from the combination of *chc1-ts* and *apl2Δ* is not worsened by including *apl1Δ*, nor improved by overexpression of Apl1p. However, we cannot discount the existence of other β subunits that could provide overlapping function. Another plausible interpretation is that the yeast AP-1 complex can assemble and function in the absence of a β subunit. In this view, the remaining subunits in the complex could provide sufficient clathrin assembly and cargo-clustering activity to avoid detectable defects in clathrin-dependent processes unless Chc function is also compromised. This hypothesis is potentially at odds with the proposal that the β subunits are chiefly responsible for the clathrin coat assembly activity of AP complexes, a model which derives from the finding that recombinant mammalian β1 or β2 alone will drive clathrin cage assembly in vitro (Gallusser and Kirchhausen, 1993). However, the assembly activity of AP complexes lacking the β subunit has not been tested in vivo, and a study using partially purified β2 subunits raises the possibility that α subunits may also play a role in clathrin assembly (Prasad and Keen, 1991). Furthermore, α subunits bind to plasma membranes previously stripped of clathrin and AP-2 while β2 subunits do not (Chang et al., 1993), suggesting that in yeast, β-deficient AP complexes could still associate with membranes It will be necessary to isolate the yeast gene encoding the γ subunit in order to evaluate the role of each of the large subunits in the function of the AP-1 complex in vivo.

There are features of the yeast Apl proteins that contrast with the mammalian β subunits. First, the Apl proteins are significantly shorter than their mammalian counterparts. When viewed by electron microscopy mammalian AP-2 appears as a brick-like complex with two appendages (Heuser and Keen, 1988). Proteolytic dissection of AP complexes revealed that the core is composed of the N-terminal domains of the large subunits and the μ and σ subunits (Zaremba and Keen, 1985; Kirchhausen et al., 1989; Matsui and Kirchhausen, 1990; Schroder and Ungewickell; 1991). The C-terminal domains of the large subunits constitute the appendages. The amino acid sequences of the mammalian large subunits also reflect a two domain structure (Kirchhausen et al., 1989; Robinson, 1990). For example, the highly conserved amino-terminal domains of β1 and β2 are connected to more divergent C-terminal domains by proline and glycine rich linker sequences (Kirchhausen et al., 1989). Sequences at the C-terminal domain may be necessary for clathrin assembly since protease-generated AP cores, as well as a truncated form of recombinant β2 missing the C-terminal domain, are unable to assemble clathrin into

coats in vitro (Zaremba and Keen, 1985; Keen and Beck, 1989; Matsui and Kirchhausen, 1990; Gallusser and Kirchhausen, 1993). Also, proteolytic treatment of AP-2-containing clathrin cages releases the AP complexes concomittant with cleavage of the β appendage suggesting that high affinity binding of AP complexes to clathrin may require both β domains (Schroder and Ungewickell, 1991). However, an alternative proposal attributes clathrin assembly activity to mammalian AP-2 cores based on the unaltered activity of proteolytically-treated AP-2 subunits in an assay for clathrin coat assembly and invagination of plasma membrane fragments (Peeler et al., 1993). When the yeast Apl proteins are aligned with the mammalian β subunits to maximize sequence conservation, it is apparent that the amino-terminal domains are conserved but the yeast proteins have much shorter C-terminal domains. Thus, if the Apl proteins are involved in clathrin assembly then either the C-terminal domains are functionally analogous to the longer mammalian C-termini, or the N-terminal core domains are able to function in the assembly process.

It is also curious that the Apl proteins have diverged (24% identity) substantially more than mammalian β1 and β2 (84% identity). The degree of sequence conservation between the mammalian β subunits, which is higher than that between any of the other AP subunits, suggests that they perform conserved functions in AP-1 and AP-2. Because the common feature of the two AP complexes is an interaction with clathrin, it has been argued that the β subunits are primarily involved in clathrin assembly, not cargo collection (see Gallusser and Kirchhausen, 1993, for discussion). In comparison to the β subunits the Apl proteins are not distinctively conserved; in fact the two yeast Aps proteins display a higher sequence conservation (39%; Phan et al., 1994). However, although there is evidence that Apl2p is homologous to β1, it is premature to assume that Apl1p is a homologue of β2 since there is no phenotypic effect of *apl1Δ* in *chc1-ts* cells, and we have not established whether Apl1p is associated with clathrin-coated vesicles. Another Apl with more similarity to Apl2p may function at the plasma membrane. Further characterization of Apl1p, and attempts to isolate additional *APL*-related genes will be needed to assess this possibility.

We have found that *apl2Δ* unexpectedly causes a partial suppression of the CPY sorting defect that occurs in *chc1-ts* cells immediately after imposing the non-permissive temperature. Previously, we noted that *chc* mutations by themselves have unusual effects on sorting of soluble vacuolar precursors. Cells carrying *chc1Δ* alleles sort soluble vacuolar precursors to the vacuole nearly as well as wild-type cells (Payne et al., 1988). In contrast, *chc1-ts* cells display a severe sorting defect immediately after a shift to the non-permissive temperature, but regain sorting ability after longer periods at 37°C (Seeger and Payne, 1992b). Two general models were presented to explain these observations. In one, clathrin lattices on Golgi membranes cannot progress to coated vesicles at the non-permissive temperature and thereby block the formation of transport vesicles targeted to the vacuolar pathway. During extended incubation at 37°C, the coats disassemble or are destroyed to allow resumption of vesicular traffic. In a second model, the clathrin lattices disassemble quickly at the non-permissive temperature and an alternative pathway to the vacuole becomes operative over time. Support for the first model has been obtained in recent experiments by analyzing the disposition of clathrin in the *chc1-ts* cells using indirect immunofluorescent detection of clathrin light chain. In *chc1-ts* cells the punctate staining of membrane-associated clathrin light chain is not altered immediately after shift to 37°C but gradually disappears after extended incubations (Mary Seeger and G.P., unpublished). The first model offers a straightforward explanation of the effect of *apl2Δ* on CPY sorting in *chc1-ts* cells. If coats assembled from temperature-sensitive Chc impede vesicle formation then a reduction in clathrin assembly caused by the absence of Apl2p would partially alleviate the block and thereby suppress the sorting defect. Given the difference between the effects of *apl2Δ* and *aps1Δ* on α-factor maturation by *chc1-ts* cells, it is not surprising that a suppressive effect of *aps1Δ* on sorting was not detected even though Aps1p and Apl2p are likely to be components of the same complex.

Our results show that *apl2Δ* affects vacuolar protein sorting and α-factor maturation in cells expressing the *chc1-ts* allele. The likely cause of the decrease in α-factor maturation is a defect in the retention of the Golgi-localized processing protease Kex2p. Although we have not directly ascertained whether Kex2p is mislocalized, ample precedent indicates that the level of α-factor maturation is a sensitive and reliable indicator of proper Kex2p localization (Payne and Schekman, 1989; Seeger and Payne, 1992a; Wilsbach and Payne, 1993b). Therefore, the AP complex containing Aps1p and Apl2p appears to participate in both clathrin-dependent Golgi functions in yeast, consistent with models that posit a functional connection between vacuolar protein sorting and Golgi membrane protein retention (Wilsbach and Payne, 1992a; Nothwehr and Stevens, 1994). However, while the vacuolar protein sorting defect is transient in *chc1-ts* cells, the mislocalizaiton of Golgi membrane proteins is not. Our results do not offer new insights into this difference. Clathrin also acts at the plasma membrane in yeast to facilitate pheromone receptor endocytosis. Deletions of *APL1* and *APS2* do not alter endocytosis in *chc1-ts* cells at permissive or non-permissive temperatures. Thus, the components of the AP complex participating with clathrin in endocytosis await identification.

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