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35. We thank Douglas Rees and Robert Almassy for computer programs and advice. This work was supported by NSF grant DMB85-01682. G.G.P. also thanks the Natural Sciences and Engineering Research Council of Canada for a postgraduate fellowship.

29 May 1987; accepted 10 September 1987

Clathrin Requirement for Normal Growth of Yeast

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Clathrin-coated membranes and coated vesicles take part in the selective transfer of proteins between different subcellular compartments of eukaryotic cells. To allow assessment of the role of clathrin in vesicular transport, genetic analysis of the clathrin heavy chain gene (*CHC1*) in *Saccharomyces cerevisiae* was initiated. The complete heavy chain gene was cloned, and the effects of deletion of this gene were studied. The null mutation (*chc1-Δ*) is lethal unless a *s*uppressor of *c*lathrin *d*eficiency (*scd1*) is present. Even in the presence of the suppressor gene, mutants lacking the clathrin heavy chain grow slowly, are genetically unstable, are morphologically abnormal, and show loss of or reduction in several yeast functions. These results indicate that clathrin is required for normal growth of yeast, and, therefore, most likely, for growth of all eukaryotic cells.

MANY PROTEINS ARE LOCALIZED IN SPECIFIC MEMBRANES or organelles within eukaryotic cells, and movement to and from these regions requires the budding, transport, and fusion of membrane vesicles (*1–3*). Clathrin-coated vesicles have been implicated in the initiation of membrane transfer during processes that include receptor mediated endocytosis (*1, 4, 5*) recycling of membranes (*6, 7*), transcellular transport (*5*), and vesicular transport within the secretory pathway (*2, 8*), such as during transfer of newly formed proteins from the Golgi apparatus to secretory vesicles and to the lysosome (*7, 9*). Coated vesicles have a striking polygonal surface lattice (*10*) composed of clathrin (*11*). Clathrin is a trimeric molecule, or triskelion, composed of three heavy chain arms of ~180,000 kilodaltons that radiate from a vertex and three light chains (usually of two types) of 30,000 to 40,000 kD, which bind noncovalently near the vertex of the triskelion, one per heavy chain arm (*12*). Isolated triskelions can assemble spontaneously into polygonal cages, and this property of clathrin is thought to be important in driving the formation of coated vesicles within cells (*3, 13*).

Clathrin-coated vesicles have been found in virtually every eukaryotic organism that has been examined, including the yeast *Saccharomyces cerevisiae* (*14*). Clathrin triskelions that have been isolated from this organism (*15, 16*) resemble structurally and functionally the molecule derived from mammals (*14–16*). That yeast also contains a well-developed glycosylation and secretory apparatus (*17*) and has been reported to undergo endocytosis (*18*) provided an opportunity to investigate coated vesicle function in an organism that is amenable to molecular and genetic manipulation. Since our data and the interpretation of the consequences of clathrin deficiency for yeast cells are somewhat at variance with earlier observations (*15*), we present our results on the cloning of the clathrin heavy chain gene and the effect of deletion of the gene in yeast.

Cloning and disruption of the clathrin heavy chain gene. To clone the clathrin heavy chain gene (*CHC1*), we used an immunological approach. A similar approach has been used by Payne and

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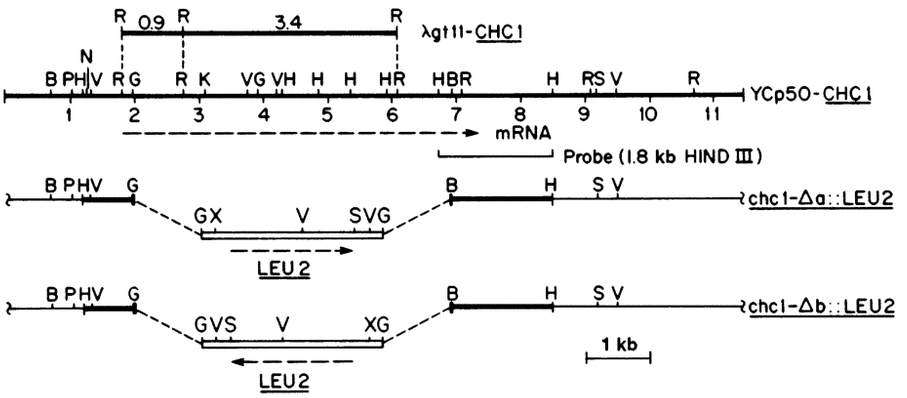

Fig. 1. Recovery of *CHC1* clones and restriction maps of the *CHC1* locus and disruption deletions. (i) λgt11-*CHC1*. Five recombinant phage were identified (*31*) in a λgt11 library (*19*) with the use of pooled mouse monoclonal antibodies to the yeast clathrin heavy chain (*16*). All contained the 3.4-kb Eco RI fragment; one contained the additional 0.9-kb fragment. (ii) YCp50-*CHC1*. The 0.9-kb Eco RI fragment purified (*32*) from the λgt11 recombinant phage was labeled (*33*) and used to probe a yeast genomic DNA library in YCp50 (*20*) by colony hybridization (*34*). The approximate region of transcription of *CHC1* as derived from RNA blot analysis of total yeast RNA (*32*) is indicated by the dashed arrow (*15, 21*). Partial DNA sequence analysis has confirmed the direction of transcription (*21*). (iii) *chc1-Δa::LEU2*, *chc1-Δb::LEU2*. The Bam HI site of pBR322 was deleted by filling in and religating cut DNA (*33*) to yield pBRΔBam, the 8.15-kb Pvu II–Sal I fragment containing *CHC1* from YCp50-*CHC1* was cloned into pBRΔBam to generate pBR-*CHC1*, and the 4.9-kb Bgl II–Bam HI *CHC1* fragment in pBR-*CHC1* was replaced by a 2.85-kb Bgl II fragment containing the *LEU2* gene (*20*). Recombinant plasmids with *LEU2* inserted in the same (p*chc1-Δa::LEU2*) or opposite (p*chc1-Δb::LEU2*) direction as transcription of *CHC1* were cut with Hind III to generate linear fragments containing the *LEU2* gene flanked by sequences homologous to the *CHC1* locus and used for gene transplacement (*22*). Leu$^+$ transformants of Leu$^-$ diploids were selected (*24*). Integration at heavy chain locus was confirmed by DNA blot analysis (*25*). Solid bars, plasmid-derived *CHC1* DNA sequences; open bars, *LEU2* sequences; solid lines, chromosomal sequences. Restriction sites: B, Bam HI, G, Bgl II; H, Hind III; K. Kpn I; N, Nru I; P, Pvu II; R, Eco RI; S, Sal I; V, Eco RV; X, Xho I.

Schekman (*15*). We immunized mice with yeast clathrin triskelions and obtained a set of eight monoclonal antibodies that recognize at least three distinct epitopes on the yeast clathrin heavy chain (*16*). The antibodies were pooled and used to identify recombinant phage in a yeast genomic DNA λgt11 expression library (*19*) (Fig. 1).

The complete clathrin heavy chain gene was recovered from a yeast genomic library inserted into the centromere plasmid, YCp50 (*20*). The restriction map for *CHC1* (Fig. 1) is identical to that described previously (*15*). A moderately abundant messenger RNA (mRNA) of 5.4 kb, which is large enough to encode the ~180,000-kD heavy chain, is transcribed from the gene. DNA blot analysis, even under reduced stringency hybridization, has uncovered only this clathrin heavy chain gene in *Saccharomyces cerevisiae* so far (*15, 21*).

In order to study the function of the clathrin heavy chain, a disruption deletion mutation was made by transplacement (*22*) of *CHC1* with a linear DNA fragment containing the *LEU2* gene flanked by sequences from the *CHC1* region (Fig. 1). To allow for the possibility that the null mutation would result in inviability of haploids, a *leu2/leu2* diploid (BJ3068) (*23*) was transformed to Leu$^+$ (*24*). Therefore, gene replacement in the *CHC1/CHC1* diploid yielded a heterozygote with one wild type and one mutant copy of *CHC1*. The phenotype of the null mutant was subsequently examined in haploids by sporulating such Leu$^+$ deletion heterozygotes and dissecting tetrads. If the null mutation were not lethal to cells, we would expect to obtain tetrads with two Leu$^-$ (Chc$^+$) and two Leu$^+$ (Chc$^-$) spores.

The results of the tetrad analysis (*23*) for three independent transformants (Table 1) indicate a large amount of spore inviability, with many tetrads providing only two or three viable spore clones. Among surviving spore clones, there were always two spore clones per tetrad that were Leu$^-$ and grew well, and the remaining viable spore clones were Leu$^+$ and grew very slowly. Similar results were obtained whether integration of *LEU2* was in the same or opposite orientation relative to transcription of *CHC1*.

DNA blot analysis (*25*) of tetrads confirmed that the Leu$^-$ spores contained the 5.3-kb Eco RV fragment corresponding to the wild-type version of *CHC1*, while the viable Leu$^+$ spores contained the 2.85-kb disruption-specific band (Fig. 2). The untransformed (*+/+*) and transformed (*+/Δ20* or *+/Δ32*) diploids yielded the expected patterns of single and double bands, respectively.

Immunoblots of protein extracts from cells confirmed that the Leu$^+$ spores contained no detectable clathrin heavy chain, while the

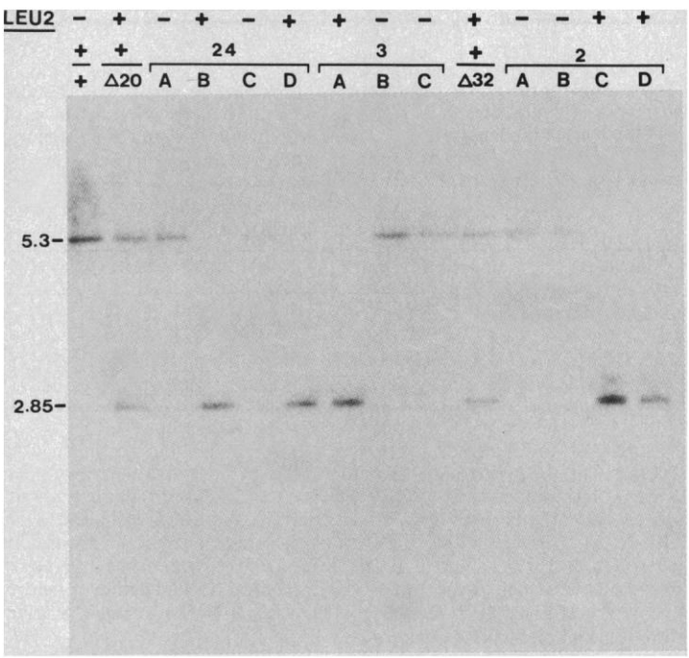

Fig. 2. DNA blot analysis (*25*) of tetrads from deletion heterozygotes. Yeast genomic DNA was prepared (*32*) and cut with Eco RV, fractionated on 0.8 percent agarose gels, transferred to nitrocellulose, and probed with the ^{32}P-labeled 1.8-kb Hind III fragment that encodes the COOH-terminal region of the clathrin heavy chain (see Fig. 1B). The wild-type gene yields a fragment of 5.3 kb, and the disruption deletion (*chc1-Δa::LEU2*) yields a 2.85-kb Eco RV fragment that hybridizes to the probe. The Leu phenotype for each strain is indicated above each lane. Lanes: +/+ is transformation recipient, BJ3068; +/Δ20 is disruption heterozygote BJ3119; 24A–D is tetrad 24 derived from BJ3119; 3A–C is tetrad 3 derived from BJ3119; +/Δ32 is disruption heterozygote BJ3120; 2A–D is tetrad 2 derived from BJ3120.

untransformed diploid (*+/+*), the heterozygous diploid (*+/Δ20*), and all Leu$^-$ spores produced the 180,000-kD protein (Fig. 3).

Examination of germination of spores in 16 tetrads indicated that all spores germinated, but those that had been scored dead were arrested as microcolonies with 20 to 40 cells having a swollen and misshapen appearance. Presumably enough clathrin or its mRNA was partitioned into each spore from the parent heterozygote to

sustain growth of the dying Chc$^-$ spore clones for four to six doublings. This suggests that clathrin or its mRNA is relatively stable in cells.

Hypothetical segregation of a suppressor of clathrin deficiency (*scd1*). The most important results from the tetrad analysis (Table 1) were that approximately one-fourth of the spores died, that all of the dead spores were Leu$^+$Chc$^-$, and that these dead spores constituted about half of all possible Chc$^-$ spores (112 out of 214). The ratio of tetrad types was approximately 1:4:1 for tetrads in which four, three, and two spores survived to produce colonies. To account for the pattern of spore viability in the tetrads and for the selective loss of half of the Chc$^-$ spores, we postulated that a second gene (*scd1*) that suppressed the lethality associated with clathrin deficiency was segregating independently in these tetrads. According to this hypothesis, Chc$^-$ cells that bear the suppressor (of *chc1-Δ::LEU2 scd1* genotype) are viable, but Chc$^-$ cells that lack the suppressor (of *chc1-Δ::LEU2 SCD1* genotype) die. The expected classes and frequencies of tetrad types given in Table 2 are based on the assumption that *CHC1* and *SCD1* are unlinked and, of course, agree with the results reported in Table 1.

Table 1. Segregational analysis of diploids heterozygous for the clathrin disruption. Strain BJ3068 (*23*) was transformed to Leu$^+$ as described in the legend to Fig. 1C to generate deletion heterozygotes of genotype: *MATα leu2/MAT***a** *leu2 chc1-Δ::LEU2/CHC1 ura3-52/URA3 trp1/TRP1 his1/HIS1 ade6/ADE6*. Three independent transformants were analyzed with *LEU2* inserted in the same (BJ3119, *+/chc1-Δ20a*; BJ3120, *+/chc1-Δ32a*) *or opposite (BJ3359, +/chc1-Δ33b*) orientation relative to transcription of *CHC1*. Data entries are the number of tetrads that gave the stipulated ratio of viable to dead spores.

Diploid genotype	Ratio of viable to dead spores in tetrads		
	4:0	3:1	2:2
+/chc1-Δ20a	9	19	6
+/chc1-Δ32a	1	29	9
+/chc1-Δ33b	7	20	7

Table 2. Hypothetical segregation of a suppressor to give observed spore viabilities. The numbers in parentheses represent the expected frequencies of tetrad types on the basis of independent segregation of two unlinked genes, *CHC1* and *SCD1*. Underlined spores are dead; others are viable.

Expected genotypes of spores in tetrads at the ratio of viable to dead spores:					
4:0 (1/6)		3:1 (2/3)		2:2 (1/6)	
CHC1	*SCD1*	*CHC1*	*SCD1*	*CHC1*	*scd1*
CHC1	*SCD1*	*CHC1*	*scd1*	*CHC1*	*scd1*
chc1-Δ::LEU2	*scd1*	*chc1-Δ::LEU2*	*scd1*	*chc1-Δ::LEU2*	*SCD1*
chc1-Δ::LEU2	*scd1*	*chc1-Δ::LEU2*	*SCD1*	*chc1-Δ::LEU2*	*SCD1*

Table 3. Test for the existence of a suppressor of clathrin deficiency by tetrad analysis of a strain homozygous for *scd1*. To test the suppression hypothesis spores 24B (*MAT***a** *leu2 trp1 his1 ade6 chc1-Δ20a::LEU2 scd1*) and 35D (*MATα leu2 ura3-52 CHC1 scd1*) from the tetrad analysis of disruption heterozygote BJ3119 (*+/chc1-Δ20a::LEU2*) were crossed. The genotype of the Chc$^-$ spore clone (24B) represents its presumed haploid genotype before knowledge of polyploidy. Data entries are the number of tetrads that gave the stipulated phenotypic ratio.

Phenotype	Expected ratio	Observed phenotypic ratio in tetrads		
		4:0	3:1	2:2
Viable:dead	4:0	18	1	1
Chc$^+$:Chc$^-$	2:2	0	0	18*
Trp$^+$:Trp$^-$	2:2	0	0	18
His$^+$:His$^-$	2:2	0	0	18
Ade$^+$:Ade$^-$	2:2	0	0	18
Ura$^+$:Ura$^-$	2:2	18	0	0
		4:0	3:0	1:1
Leu$^+$:Leu$^-$	2:2	18	1	1†
α:**a**	2:2	α:**a**:nonmaters, 1:41:35 scored)		(Summation of spores
		[2**a**::2 nonmaters, [1α:3**a**		17 tetrads] 1 tetrad]†

*For several of the observed phenotypes the results of only the 18 four-spore tetrads are given. Results of the three- and two-spore tetrads were consistent with the polyploidy outcome. †These uncommon tetrads are accounted for by crossovers during meiosis I.

Test for the existence of a suppressor of clathrin deficiency by analysis of a strain homozygous for *scd1*. In the first experiment, a strain homozygous for *scd1*, but heterozygous for *chc1-Δ::LEU2*, was generated by crossing a Chc$^-$ spore from a tetrad in which four spores survived (postulated genotype *chc1-Δ::LEU2 scd1*) to a wild-type spore from a tetrad with two surviving spores (postulated genotype *CHC1 scd1*). We expected that all markers would show 2:2 segregation and four viable spores would be recovered in all tetrads because of the presence of the suppressor in homozygous form. This result was not found (Table 3). Although there was virtually 100 percent spore viability, Leu, Ura, and mating type showed unusual segregation patterns. We can account for the segregation in this cross by postulating that the original mating was between a triploid Chc$^-$ cell and a haploid Chc$^+$ cell that yielded a tetraploid zygote.

The Chc$^+$ nonmating spores provided confirmation of tetraploidy, since these spores all sporulated and showed marker segregations appropriate for diploids. These also provided the necessary diploids to allow testing of the suppression hypothesis. The expected genotype of these diploid spores was *MAT***a** *leu2/MATα leu2 chc1-Δ::LEU2/CHC1 scd1/scd1*, which was exactly the genotype that we were trying to generate when the original cross of the Chc$^-$ and Chc$^+$ cells was made with the intent of obtaining a strain homozygous for *scd1*. The predicted segregation for these diploids was four viable haploid spores per tetrad and the genotypic ratio of two *chc1-Δ::LEU2 scd1* (Chc$^-$Leu$^+$) to two *CHC1 scd1* (Chc$^+$Leu$^-$). In the tetrad analysis of five Chc$^+$ nonmating spores (Table 4) 36 out of 39 tetrads had four viable spores, and every tetrad with four surviving spores showed the predicted segregation for all markers. These results support the suppression hypothesis and indicate that cells of genotype *chc1-Δ::LEU2* can survive if they carry *scd1*.

None of the Chc$^-$ spores that were nonmaters sporulated, despite the fact that, from the predicted segregation pattern of mating type, most of the Chc$^-$ cells were probably *MAT***a**/*MATα* rather than *MAT***a**/*MAT***a** or *MATα*/*MATα*. It was not possible in all cases to distinguish spores homozygous at *MAT* from those that were true *MAT***a**/*MATα* nonmating diploid spores, because many Chc$^-$ cells show reduced mating ability (*21, 26*). Nevertheless, these results suggest that *MAT***a**/*MATα chc1/chc1 scd1/scd1* diploids are sporulation-defective.

Test for the existence of a suppressor of clathrin deficiency by

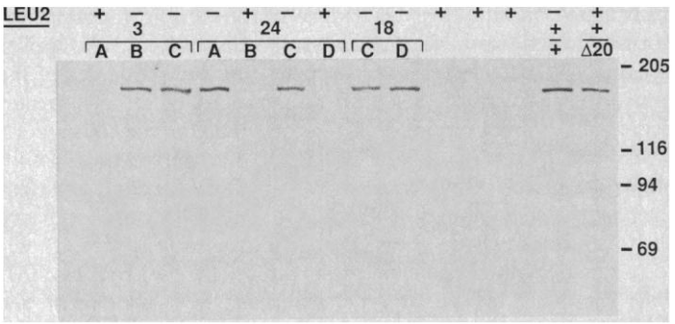

Fig. 3. Immunoblot analysis of tetrads from deletion heterozygotes. Yeast extracts were prepared from cells in the mid-to-late log phase of growth (*35*). The protein (40 μg) was fractionated on 7.5 percent SDS polyacrylamide gels, transferred to nitrocellulose, and probed with the pooled monoclonal antibodies to the yeast heavy chain (*16*). The Leu phenotype is indicated above each lane. All tetrads were derived from deletion heterozygote +/Δ20 (BJ3119). Lanes 3, A to C; 24, A to D; and 18, C and D are spore progeny from tetrads that gave three, four, and two survivors, respectively. (+) Lanes with no other label are three different viable Chc$^-$ spores derived from deletion heterozygote +/Δ32 (BJ3120). Molecular size markers are: myosin (205 kD), β-galactosidase (116 kD), phosphorylase b (94 kD), bovine serum albumin (69 kD).

analysis of a diploid lacking *scd1*. In order to obtain a diploid strain that lacked the suppressor but was heterozygous at *CHC1*, we first generated a *CHC1/CHC1* diploid lacking the suppressor by crossing two wild-type spores, presumed to be of genotype *CHC1 SCD1*, that were derived from tetrads with four surviving spores from the original dissections (Table 1). Two such strains (*leu2/leu2 CHC1/CHC1 ura3-52/ura3-52 SCD1/SCD1*) were then transformed to Ura$^+$ with YCp50-*CHC1*, which carries a wild-type copy of *CHC1* as well as *URA3* as a selectable marker. Introduction of an additional one to two copies of *CHC1* on the relatively stable centromere plasmid served to prevent selection for suppressor mutations in the *chc1* heterozygote by ensuring that at least two copies of *CHC1* were always present in the diploid. A chromosomal copy of *CHC1* was then disrupted by transplacement (Fig. 1), and three independent Leu$^+$ disruption diploids carrying YCp50-*CHC1* were sporulated directly for tetrad analysis (Table 5). Derivative Ura$^-$ diploids lacking the plasmid were isolated, and then these were immediately sporulated.

When transformant *+/chc1-Δ36b* was analyzed in the absence of YCp50-*CHC1* (Table 5), all tetrads gave only two surviving spores and all viable spores grew well and were Leu$^-$. No Leu$^+$ spores were obtained, indicating that the Chc$^-$ spores were all dead. When all three deletion heterozygotes bearing YCp50-*CHC1* were analyzed, tetrads that gave four, three, and two surviving spores were recovered. All tetrads contained two Leu$^-$ spores that were phenotypically Chc$^+$ (some were also Ura$^+$ if they carried the plasmid). No Leu$^+$Ura$^-$ spores were recovered. Since Leu$^+$Ura$^+$ spores were recovered, indicating that Leu$^+$ spores could be rescued by the presence of a wild-type copy of *CHC1* on YCp50, we conclude that all Leu$^+$Ura$^-$ spores were dead. Examination of germination of spores supported these results. Spores that died had germinated and then arrested growth at four to six doublings as described above for inviable heavy chain minus spores. These results support the suppression hypothesis and indicate that cells of genotype *chc1-Δ::LEU2* are inviable in the absence of the suppressor allele, *scd1*.

Two major lines of evidence suggest that the initial diploid (BJ3068) used as the recipient for the disruption was already heterozygous for *scd1* and that *scd1* was not a mutation selected to compensate for the presence of one-half of the normal amount of heavy chain in the *chc1*-Δ heterozygote, a concern because gene dosage effects have been observed for genes that encode other structural proteins in yeast (*27*). First, in *chc1*-Δ heterozygotes that lack the suppressor, there has been no evidence for selection for suppressors even after long-term propagation in the absence of an extra copy of *CHC1* carried on YCp50. Second, segregational analysis of crosses between each of the haploid parents that were used to generate the original diploid transformation recipient (BJ3068) and strains of *chc1*-Δ *SCD1* (YCp50-*CHC1*) genotype indicates that one of the haploid parents contained the suppressor, *scd1*, and the other parent contained the *SCD1* allele (*28*). Therefore, BJ3068 was of genotype *scd1/SCD1* prior to disruption of *CHC1*.

Chc$^-$ spores that germinate and survive are not normal. Suppressed Chc$^-$ cells (*chc1-Δ::LEU2 scd1*) show a slow and temperature sensitive growth on YEPD plates (yeast extract, peptone, dextrose), they are virtually dead on YEP-glycerol at 30° and 37°C, and growth on synthetic medium is very slow and restricted. However, the sensitivity to these conditions varies from isolate to isolate, and growth appears to improve with propagation. Small colonies from suppressed Chc$^-$ cells generate small and large colonies upon restreaking (*29*), generate faster growing papillae on a patch, and give growth rates in culture that are heterogeneous. Doubling times of Chc$^-$ cultures vary from 4 hours to more than 8 to 12 hours at 30°C (2 hours or less being normal for Chc$^+$ haploids), but, after time in culture, slow growing isolates often develop faster growth rates. The simplest explanation of these observations is that there is selection for healthier derivatives of clathrin deficient cells that result from additional suppressor mutations, although some of the instability and heterogeneity in these populations is probably due to changes in ploidy, since we have observed that suppressed Chc$^-$ cells become polyploid at a high frequency.

Chc$^-$ cells also have aberrant morphology and nuclear division (Figs. 4 and 5). Mutant cells (Fig. 4D) are larger and rounder in shape than wild-type cells (Fig. 4C), and they have a more granulated appearance. In liquid culture, many dead Chc$^-$ cells are found and growth in aggregates of as many as 40 to 60 cells that require sonication for dispersion for microscopy is common. In normal

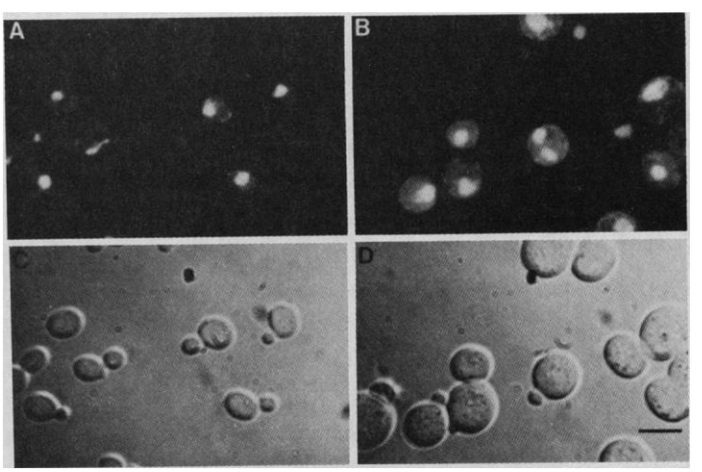

Fig. 4. Chc$^-$ cells show aberrant morphology and nuclear division. Yeast cells were grown to log phase in YEPD at 30°C and 10 to 20 A_{600} were washed in 0.1*M* KH_2PO_4, *p*H 6.5, and fixed in 3.7 percent formaldehyde in the same buffer for 60 minutes at 25°C. The cells were washed twice with the phosphate buffer and sonicated briefly to disperse aggregates; they were centrifuged, resuspended, and then stained in 0.05*M* KH_2PO_4, *p*H 7.0, containing DAPI (1 μg/ml) for 10 minutes. Cells were washed once in KH_2PO_4 buffer (*p*H 7.0) and once with water, applied in H_2O to slides that had been coated with poly-L-lysine, and viewed with a Zeiss Epi-fluorescence microscope at ×1000. (**A** and **C**) Chc$^+$ strain BJ3240 (*MATa leu2 CHC1 ura3-52 ade6*); (**B** and **D**) Chc$^-$ strain BJ3254 (*MATa leu2 chc1-Δ20a::LEU2 trp1 ade6 scd1*) (*36*); (A and B) DAPI fluorescence (C and D) Nomarski optics. Scale bar, 5 μm.

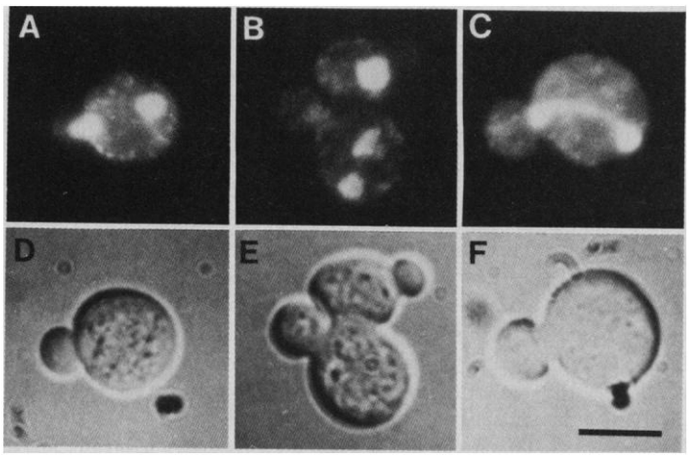

Fig. 5. Examples of aberrant nuclear division in *chc1-Δ scd1* cells. Chc$^-$ cells were prepared for microscopy as described in Fig. 4. (**A** and **D**) BJ3250 (*MAT***a** *leu2 chc1-Δ20a::LEU2 ura3-52 scd1*) (*36*) (**B, C, E, F**) BJ3254. (A to C) DAPI fluorescence; (D to F) Nomarski optics. Scale bar, 5 μm.

nuclear division of wild-type haploids as visualized by DAPI (4′, 6-diamidino-2-phenylindole) staining, the nucleus migrates to the neck of the bud and elongates between mother and daughter cells (Fig. 4A). Such normal division has been observed for suppressed Chc$^-$ cells; however, occasionally these cells show unusual nuclear patterns. Chc$^-$ mother cells with two nuclei (Figs. 4B and 5B), or with two nuclei, one of which is prematurely in the neck of the bud (Fig. 5A), or with nuclear division taking place completely within the mother cell (Fig. 5C), are examples. These unusual nuclear patterns are seen in, at most, 1 percent of asynchronously growing populations; however, many suppressed Chc$^-$ cells may have already undergone abnormal division and fusion of nuclei prior to the time of observation. It is possible that these cells that undergo abnormal nuclear division die; alternatively, this apparent failure to coordinate cell division and nuclear division may provide part of the mechanism for the increases in ploidy that have been observed in suppressed Chc$^-$ cells.

The importance of clathrin heavy chains in yeast. Our evidence leads us to conclude that the clathrin heavy chain is important for normal growth and viability of yeast cells. Recently, a similar deletion of *CHC1* in yeast was reported and the properties of the mutant were described (*15*). Our results indicated a much greater degree of inviability amongst Chc$^-$ spores than the previous report. The 5′ end of our deletion was identical to one of those in (*15*), and, although our deletion extended farther in the 3′ direction than did theirs, it was still within the open reading frame of the clathrin heavy chain gene as determined by DNA sequence analysis (*21*). Therefore, it is unlikely that our deletion disrupted an adjacent essential gene left intact by the largest deletion in (*15*). In none of our deletion mutants were we able to detect the 180,000-kD heavy chain or a related peptide, in agreement with previous work (*15*). This argues against the presence of a partially functional truncated heavy chain. Therefore, it is clear that yeast cells can survive without clathrin, but in our strains a suppressor gene must be present for them to do so.

Thus far we have little information concerning the suppressor of clathrin deficiency, *scd1*. On its own, in a cell with normal clathrin, *scd1* has no obvious phenotypic consequences. Preliminary analyses of crosses of *chc1-Δ scd1* cells to *CHC1 SCD1* cells that resulted in tetraploid zygotes indicate that *scd1* is recessive (*21*). Even if *scd1* is recessive, we cannot determine whether *SCD1* or *scd1* is the wild-type allele.

One of the most striking characteristics of cells of *chc1-Δ scd1* genotype is their heterogeneity and genetic instability, with polyploidy being an important signature of this instability. We cannot distinguish whether the polyploidy is a result of the *chc1* mutation alone or the combination of the *chc1* and *scd1* alleles since only the *chc1 scd1* genotype is compatible with viability. However, the *scd1* allele has no effect on ploidy in Chc$^+$ cells. The polyploidy itself is not a requirement for survival of *chc1 scd1* cells, since we observed a normal diploid segregation pattern in one cross of a *chc1-Δ scd1* strain to a *CHC1 SCD1* strain; but it is possible that cells of higher ploidy have a growth advantage relative to haploids.

We have limited our phenotypic characterization of *chc1-Δ scd1* cells to that required for genetically characterizing them (ploidy, mating, sporulation, and obvious growth properties). Once we obtain appropriate gene fusions and conditional mutants for *CHC1*

Table 4. Analysis of tetrads from Chc$^+$ diploid spores. Diploid spores were derived from the analysis described in Table 3 and were derivatives of the tetraploid strain. Diploid spore genotypes were inferred from tetrad analysis. All spores were *MAT***a** *leu2/MATα leu2 chc1-Δ20a::LEU2/CHC1 scd1/scd1*. In addition, spore 2B was *ura3-52/URA3 trp1/TRP1 his1/his1 ade6/ADE6*; spore 7B was *ura3-52/URA3 trp1/trp1 his1/HIS1 ade6/ADE6*; spore 5D was *URA3/URA3 trp1/trp1 his1/His1 ade6/ADE6*; spore 10D was *ura3-52/URA3 trp1/trp1 his1/HIS1 ade6/ADE6*; and spore 15B was *URA3/URA3 trp1/TRP1 HIS1/HIS1 ade6/ADE6*. Data entries are the number of tetrads that gave the stipulated phenotypic ratio.

Spores	Ratio of viable to dead spores in tetrads:		
	4:0	3:1	2:2
2B	7	0	0
7B	8	1	0
5D	10	0	0
15B	6	2	0
10D	5	0	0
Total	36	3	0

Table 5. Test for the existence of a suppressor of clathrin deficiency by tetrad analysis of a diploid lacking the suppressor. Diploid transformation recipients BJ3475 (*MAT***a**/*MATα leu2/leu2 CHC1/CHC1 ura3-52/ura3-52 trp1/TRP1 his1/HIS1 ade6/ADE6 SCD1/SCD1*) and BJ3473 (*MAT***a**/MATα *leu2/leu2 CHC1/CHC1 ura3-52/ura3-52 his1/HIS1 ade6/ADE6 SCD1/SCD1*) were obtained by crossing two Chc$^+$ spores from tetrads that contained four viable spores from the analysis (Table 1) of BJ3359 and BJ3119, respectively. BJ3475 and BJ3473 were transformed to Ura$^+$ with YCp50-*CHC1* and then to Leu$^+$ by gene transplacement (see Fig. 1) to generate their respective deletion heterozygotes, BJ3535 (*+/chc1-Δ36b*(YCp50-*CHC1*)) and BJ3530 and BJ3532 (*+/chc1-Δ37a*(YCp50-*CHC1*) and *+/chc1-Δ38a*(YCp50-*CHC1*)). Genotypes were confirmed by DNA blot analysis and plasmid loss experiments. The results were identical whether *LEU2* was inserted in the opposite (BJ3535) or the same (BJ3530 and BJ3532) direction of transcription as *CHC1*. The occurrence of four, three and two viable spores in tetrads was reflective of segregation of *chc1-Δ* combined with segregation and plasmid loss of *CHC1* on a centromere plasmid like YCp50 (*30*).

Diploid genotype	Number of tetrads at ratio of viable to dead spores:			Number of spores at phenotype			
	4:0	3:1	2:2	Leu$^-$Ura$^\pm$	Leu$^+$Ura$^+$	Leu$^+$Ura$^-$	Dead
*+/chc1-Δ36b**	0	0	23	46	0	0	46
+/chc1-Δ36b(YCp50-*CHC1*)	3	11	10	48	17	0	31
+/chc1-Δ37a(YCp50-*CHC1*)	6	18	4	56	30	0	26
+/chc1-Δ38a(YCp50-*CHC1*)	10	10	10	60	30	0	30

*A mitotic recombination in BJ3475(YCp50-*CHC1*) occurred, resulting in homozygosity for *ade6*. Therefore *+/chc1-Δ36b*(±YCp50-*CHC1*) was also *ade6/ade6*.

function we should be able to directly analyze the role of clathrin in processes like secretion, intracellular targeting, and endocytosis and assess the effect of the suppressor gene.

The mechanism by which this suppressor gene acts is unknown, but possible mechanisms include those directly related to clathrin function as well as those involving processes only very indirectly related to clathrin function. For example, the suppressor allele could encode a protein that has the ability to bypass or substitute for the clathrin function or it could act by a mechanism that permits expression of a gene or genes at levels that allow bypass or substitution for the function. Alternatively, the mechanism of suppression could involve removal of a function that is deterimental or lethal only when clathrin is absent. This would include suppression resulting from a defect in a function that opposes that of clathrin, so that the two mutations would mutually compensate.

In spite of our lack of understanding of the mechanism of suppression by *scd1*, the identification of this gene provides exciting prospects for studies of clathrin in *S. cerevisiae*. In addition to identifying another gene that may play a role in membrane vesiculation in yeast, we have been provided with the ability to construct strains that are inviable in the absence of clathrin. This should permit us to obtain conditional lethal mutations in the heavy chain gene, which further extends the possibilities of standard suppression analysis.

In conclusion, we have evidence that null mutations for the clathrin heavy chain gene are lethal unless a suppressor gene is present. This finding has presented the possibility that clathrin, while not essential for viability, may serve an essential function in yeast cells, and that the lack of this function can be compensated for by other genes. However, even in the presence of the suppressor gene we have studied, cells without clathrin heavy chains are clearly not normal. They grow poorly, are genetically unstable, have aberrant morphology and show loss of or decreases in several yeast functions. Our results emphasize the importance of clathrin for normal growth and development of yeast, and therefore, most likely, all eukaryotic cells.

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28. The original transformation recipient (Table 1) was made by crossing BJ2738 (*MATα leu2 ura3-52 trp1*) and BJ2700 (*MATa leu2 ade6 his1*). The cross of BJ2738 to BJ3573 [*MATa leu2 chc1-Δ36::LEU2 ura3-52 his1 ade6 SCD1* (YCp50-*CHC1*)] yielded germinating spores of phenotypes: 28 $Leu^-Ura^{\pm}$; 17 Leu^+Ura^+; 6 Leu^+Ura^-; 4 dead; that of BJ2700 to BJ3574 [*MATα leu2 chc1-Δ36::LEU2 ura3-52 trp1 ade6 SCD1* (YCp50-*CHC1*)] yielded germinating spores of phenotypes: 27 $Leu^-Ura^{\pm}$; 11 Leu^+Ura^+; no Leu^+Ura^-; 13 dead.
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37. We thank Aaron Mitchell and Richard Russell for helpful discussions; Robert Preston for his critical reading of this manuscript; members of our laboratory for comments and encouragement; and Kathleen Conley and Carol Freund for technical assistance. Supported by Public Health Service grants AM18090 and GM29713 (E.W.J.) and AI06884 and GM36995 (S.K.L.).

16 April 1987; accepted 21 September 1987