Deoxyhypusine synthase activity is essential for cell viability in the yeast *Saccharomyces cerevisiae*.

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Deoxyhypusine synthase catalyzes the first step in the posttranslational synthesis of an unusual amino acid, hypusine (N^4-(4-amino-2-hydroxybutyl)lysine), in the eukaryotic translation initiation factor 5A (eIF-5A) precursor protein. The null mutation in the single copy gene, yDHS, encoding deoxyhypusine synthase results in the loss of viability in the yeast *Saccharomyces cerevisiae*. Upon depletion of deoxyhypusine synthase, and consequently of eIF-5A, cessation of growth was accompanied by a marked enlargement of cells, suggesting a defect in cell cycle progression or in cell division. Two residues of the yeast enzyme, Lys^308 and Lys^350, corresponding to Lys^51 and Lys^295, respectively, known to be critical for the activity of the human enzyme, were targeted for site-directed mutagenesis. The chromosomal ydhs null mutation was complemented by the plasmid-borne yDHS wild-type gene, but not by mutated genes encoding inactive proteins, including that with Lys^350 -> Arg substitution or with substitutions at both Lys^308 and Lys^350. The mutated gene ydhs(K308R) encoding a protein with diminished activities (<1% of wild type) could support growth but only to a very limited extent. These findings provide strong evidence that the hypusine modification is indeed essential for the survival of *S. cerevisiae* and imply a vital function for eIF-5A in all eukaryotes.

The biosynthesis of the unusual amino acid, hypusine (N^4-(4-amino-2-hydroxybutyl)lysine), represents a novel posttranslational modification that occurs exclusively in one cellular protein, the precursor of eukaryotic translation initiation factor 5A (eIF-5A)^1 (1, 2). In the first step of hypusine synthesis, deoxyhypusine synthase catalyzes the transfer of the butylamine moiety of the polyamine spermidine to the ε-amino group of a specific lysine residue of eIF-5A precursor (Lys^57 in the yeast proteins) to form a deoxyhypusine (N^4-(4-aminobutyl)lysine) residue (3, 4). Hydroxylation of the side chain of this intermediate by deoxyhypusine hydroxylase completes hypusine biosynthesis and eIF-5A maturation (5).

Deoxyhypusine synthase has been purified from rat testis (4), *Neurospora crassa* (6), HeLa cells (7), and yeast (8). Human (9, 10) and *N. crassa* (11) cDNAs for the enzyme have been cloned, and its gene has been identified (7, 11, 12) and cloned (8) in yeast. The amino acid sequence of deoxyhypusine synthase is highly conserved and enzymes from different species exist as homotetramers of 40 to 43-kDa subunits. In recent studies with human deoxyhypusine synthase, we have identified an active site lysine residue, Lys^329, that is involved in enzyme-substrate intermediate formation (13). Replacement of all the conserved lysine residues of the human enzyme (with Lys -> Arg or Lys -> Ala) by site-directed mutagenesis revealed that Lys^329 and Lys^295 are critical for enzyme activity (43).

Hypusine is ubiquitous in eukaryotes, occurring at one highly conserved residue of eIF-5A. The assignment of this protein as a putative translation initiation factor was based on its in *vitro* ability to stimulate methionyl puromycin synthesis (14). However, its true physiological function is as yet unknown. An increasing body of evidence supports the notion that eIF-5A and its hypusine modification play a pivotal role in eukaryotic cell proliferation (1, 2). In mammalian cells, inhibitors of either deoxyhypusine synthase (15) or deoxyhypusine hydroxylase (16) exert anti-proliferative effects. The arrest in cell proliferation by inhibitors of polyamine biosynthetic enzymes has been attributed to depletion of eIF-5A following deprivation of spermidine (17). The ability of spermidine analogues to suppress the growth of polyamine-deficient cells correlated with their competency to act as a substrate for deoxyhypusine synthesis (17, 18). Schnier et al. (19) and Wohlt et al. (20) independently demonstrated that expression of either one of the two eIF-5A genes in the yeast *Saccharomyces cerevisiae* is vital for yeast survival. Schnier et al. further showed that a yeast mutant protein, eIF-5A precursor(K51R), which cannot be modified to the hypusine form due to the substitution of Arg^51 for Lys, did not support the growth of yeast. Although suggesting the importance of the preservation of Lys^51 for hypusine synthesis, these results do not provide direct evidence of the essential requirement of the hypusine modification for yeast growth.

We undertook a study to determine the role of deoxyhypusine synthase in the yeast *S. cerevisiae* through inactivation of its gene. Our data as well as those of Sasaki et al. (8), recently reported while this work was in progress, indicate that this gene is essential for cell viability in yeast. We extended the yDHS gene disruption studies by employing site-directed mutagenesis and plasmid shuffle techniques to compare the capabilities of wild type and mutant yDHS genes to support yeast growth. Single or double mutations were introduced into yDHS at the sites encoding Lys^308 and Lys^350 (corresponding to Lys^51 and Lys^295, respectively, of the human enzyme). Our results presented here show that the activity of deoxyhypusine synthase, as well as the gene product, is required for yeast viability.

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1 The abbreviations used are: eIF-5A, eukaryotic translation initiation factor 5A (eIF-5A defines the fully modified protein containing hypusine; the unmodified protein containing lysine in place of hypusine is termed eIF-5A precursor); ORF, open reading frame; yDHS, yeast deoxyhypusine synthase; PCR, polymerase chain reaction; 5-FOA, 5-fluoroorotic acid; bp, base pair(s); kb, kilobase pair(s).

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TABLE I
Yeast strains and plasmids

| Strains or plasmid | Description | Source or reference |
|--------------------|-------------|--------------------|
| JS10*              | MATa/MATa leu2–3,112/leu2–3,112 ura3–52/ura3–52 trpl–289/ trpl–289 his3–1/his3–1 | J. W. B. Hershey (19) |
| JSD1               | MATa/MATa leu2/leu2 ura3/ura3 trpl/trpl his3/his3 yDHS/yDHS::LEU2 | This work |
| JSD1[pRS316-yDHS]  | MATa/MATa leu2/leu2 ura3/ura3 trpl/trpl his3/his3 yDHS/yDHS::LEU2 [pRS316-yDHS] | This work |
| JSD1[YEp352T-yDHS] | MATa/MATa leu2/leu2 ura3/ura3 trpl/trpl his3/his3 yDHS/yDHS::LEU2 [YEp352T-yDHS] | This work |
| JSH1[pRS316-yDHS]  | MATa ura3 trpl his3 leu2 yDHS::LEU2 [pRS316-yDHS] | This work |
| JSH1[pRS316-yDHS]/ [YRp7-yDHS] | MATa ura3 trpl his3 leu2 yDHS::LEU2 [pRS316-yDHS] [YRp7-yDHS] | This work |
| JSH1[pRS316-yDHS]/ [YRp7-yDHS(K308R/K350R)] | MATa ura3 trpl his3 leu2 yDHS::LEU2 [pRS316-yDHS] [YRp7-yDHS(K308R/K350R)] | This work |
| JSH1[pRS316-yDHS]/ [YRp7-yDHS(K308R/K350R)] | MATa ura3 trpl his3 leu2 yDHS::LEU2 [pRS316-yDHS] [YRp7-yDHS(K308R/K350R)] | This work |
| YEp352             | 2µm ORI*/LEU2 Amp' Tc' | 21 |
| pRS316             | CEN4 ARSH4 URA3 Amp' | 23 |
| pRS316-yDHS        | CEN4 ARSH4 URA3 Amp' yDHS | This work |
| YEp352             | 2µm ORI URA3 Amp' | This work |
| YEp352T            | 2µm ORI TRPI Amp' | This work |
| YEp352T-yDHS       | 2µm ORI TRPI Amp' yDHS | This work |
| YRp7               | ARS1 TRPI Amp' Tc' | This work |
| YRp7-yDHS           | ARS1 TRPI Amp' Tc' yDHS | This work |
| YRp7-yDHS(K308R)    | ARS1 TRPI Amp' Tc' yDHS (K308R) | This work |
| YRp7-yDHS(K308R)    | ARS1 TRPI Amp' Tc' yDHS (K308R) | This work |
| YRp7-yDHS(K308R/K350R) | ARS1 TRPI Amp' Tc' yDHS (K308R/K350R) | This work |
| YRp7-yDHS(K308R/K350R) | ARS1 TRPI Amp' Tc' yDHS (K308R/K350R) | This work |
| YRp7-yDHS(K308R/K350R) | ARS1 TRPI Amp' Tc' yDHS (K308R/K350R) | This work |
| YRp7-yDHS(K308R/K350R) | ARS1 TRPI Amp' Tc' yDHS (K308R/K350R) | This work |
| YRp7-yDHS(K308R/K350R) | ARS1 TRPI Amp' Tc' yDHS (K308R/K350R) | This work |
| YRp7-yDHS(K308R/K350R) | ARS1 TRPI Amp' Tc' yDHS (K308R/K350R) | This work |
| YRp7-yDHS(K308R/K350R) | ARS1 TRPI Amp' Tc' yDHS (K308R/K350R) | This work |
| YRp7-yDHS(K308R/K350R) | ARS1 TRPI Amp' Tc' yDHS (K308R/K350R) | This work |

a After the publication of its genotype, JS10 and its derivative strains, including all those listed in this table, turned out to be Gal−; the specific genotype for the Gal− phenotype is unknown.

b ORI, origin of replication.

and thus provide strong evidence of an essential role for eIF-5A and its hypusination modification in eukaryotic cell proliferation. Furthermore, the marked increase in the size of the cells at the cessation of growth upon depletion of deoxyhypusynase, and consequently of eIF-5A, invites speculation on the possible role of eIF-5A in the cell division phase of the cell cycle.

MATERIALS AND METHODS

Strains and Plasmids—The genotypes and sources of the S. cerevisiae strains used in this work are listed in Table I. S. cerevisiae strain JS10 (19), plasmid YEp13 (21), and plasmid YEp352 (22) were kindly supplied by Dr. John W. B. Hershey (University of California, Davis, CA). Plasmid pRS316 (23) was a generous gift from Drs. Nobuko Hamasaki, Celia Tabor, and Herbert Tabor (NIDDK, National Institutes of Health, Bethesda, MD), and plasmid pJ246 (24) from Dr. Reed Wickner (NIDDK, National Institutes of Health). Plasmid YRp7 (25) was purchased from American Type Culture Collection (Rockville, MD), and plasmid pOUCS-2 from Novagen (Madison, WI). Plasmid YEp352T was generated by inserting a 0.58-kb EcoRI-BglII fragment (TRPI gene) from pJ246 into the Stul-NdeI site of YEp352 by blunt end ligation.

Disruption and Replacement of yDHS Gene in Yeast—A 1.2-kb DNA fragment encompassing the whole open reading frame (ORF) (1164 bp) of yeast deoxyhypusynase synthase gene (yDHS) with two PstI sites near the 5’ and 3’ termini was prepared by PCR using yeast quick clone cDNA (CLONTECH) as a template and a primer set of D5–1 and D3–1 (Table II). After digestion with PstI, the 1.2-kb fragment was inserted into the PstI site of pUC19 to generate pYS1 (Scheme Ia). pYS1 was digested with SstI to remove a 700-bp fragment from the middle coding region of yDHS. Into this linearized plasmid, a LEU2 marker gene (2 kb), obtained from YEp13 by digestion with HpaI and Sall, was inserted by blunt end ligation, resulting in pYLs1. A 2.5-kb DNA fragment with yDHS disrupted by LEU2 was obtained after digestion of pYLs1 with PstI and was used for transformation of S. cerevisiae strain JS10 using the lithium acetate method (26). Leu+ transformants were isolated from minimal selection media lacking leucine.

Construction of Yeast-Escherichia coli Shuttle Vectors Expressing Deoxyhypusynase Synthase or Mutant Enzymes—The yDHS gene with NdeI and BamHI sites introduced at the 5’ and 3’ termini, respectively, of the ORF was constructed according to Scheme IB—yDHS-U (400-bp 5’-untranslated region with NdeI and NdeI sites) and yDHS-D (450-bp 3’-untranslated region with BamHI and HindIII sites) were generated by PCR of the genomic DNA of S. cerevisiae strain JS10 using the primer set D5–3/3D–4 and consequently of yDHS-U and the 5’ terminus of yDHS-D are complementary, the two products purified from a gel were used as templates for the second round of PCR with the primer set D5–3/3D–4 to generate yDHS-UD, a 0.85-kb fusion product. After digestion with Sall and HindIII, this PCR product was inserted into the SalI-HindIII site of pOUCS2 to generate pOUD1. pOUD1 was digested with NdeI and BamHI and ligated with the yDHS ORF, 1.2-kb NdeI-BamHI fragment produced from pET11a-yDHS (12), to generate pOyDHS. The 2.1-kb SalI-HindIII fragment of pOyDHS was inserted into the SalI-HindIII site of the centromeric plasmid pRS316 to form pRS316-yDHS, or into the Smal-Sall site of the yeast episomal plasmid YEp352T (by blunt end ligation) to generate YEp352T-yDHS. A 1.5-kb EcoRI fragment of YRP7 containing ARS1 and TRPI was inserted into the EcoRI site of pOyDHS to generate YRP7-yDHS. For the construction of plasmids expressing mutant enzymes in yeast, the 1.2-kb NdeI-BamHI fragment of the above recombinant plasmids containing wild type yDHS gene was replaced with a 1.2-kb NdeI-BamHI fragment of pET11a recombinant plasmids

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The numbers in parentheses indicate the nucleotides (nt) of the coding or complementary sequences of yeast deoxyhypusine synthase gene based on the sequence of locus YSCH 8025 (GenBank accession no. U00061); these sequences are denoted in uppercase letters. Italicized sequences are those that matched the partial amino acid sequences determined for the enzymes from rat (4), human (7), or N. crassa cDNA sequences (11). The identity of the deoxyhypusine synthase gene was verified by demonstrating enzymatic activity after expression of its ORF in E. coli (12). Independently, Sasaki et al. purified deoxyhypusine synthase from yeast and cloned the identical gene (8). There appears to be a single gene for this enzyme in yeast. Hybridization of yeast genomic DNA digested with EcoRI or HindIII with the radiolabeled ORF of yDHS displayed one radiolabeled band in each case, of 5.83 or 3.75 kb, respectively (data not shown). Furthermore, no other sequence related to the ORF of yDHS is found in the whole genome of S. cerevisiae (28), when searched by the BLAST network service of NCBI (National Center for Biotechnology Information).

To determine whether the deoxyhypusine synthase gene is essential for cell viability in yeast, we constructed a plasmid, pYLS1, containing yDHS with the middle 60% of the ORF deleted and replaced with LEU2, as shown in Scheme IA. A 2.5-kb PetI fragment with disrupted yDHS was used to transform the Leu<sup>−</sup> diploid strain JS10 (Table I). Leu<sup>−</sup> transformants were selected; disruption of chromosomal yDHS by homologous recombination was confirmed by PCR. As shown in Fig. 1, most Leu<sup>−</sup> transformants carried normal yDHS on one allele and a disrupted ydhs::LEU2 on the other allele. No difference in the pattern of growth and sporulation of JS10 and of the heterozygous ydhs/yDHS diploids was observed, suggesting that disruption of one chromosomal allele is not detrimental in yeast cells. One such transformant, JSD1, was subjected to sporulation followed by tetrad dissection (Fig. 2A). Only two of the four spores from each ascus grew to viable colonies (8 out of 18 dissected ase1 strain) Fig. 2A). All growing colonies were Leu<sup>+</sup>; none was Leu<sup>−</sup>, an indication that the haploid with the ydhs::LEU2 genotype is not viable. Microscopic examination of those spores unable to form visible colonies revealed that they did in fact germinate but ceased to grow after eight or nine
Site-directed Mutagenesis of Yeast Deoxyhypusine Synthase

DISCUSSION

The present study provides definitive evidence that deoxyhypusine synthesis activity is vital for growth of the yeast S. cerevisiae. The yDHS gene disruption experiments presented here and in an earlier report (8) complement the earlier eIF-5A gene inactivation studies in yeast (19, 20). Considering the functional interchangeability of human and yeast eIF-5A’s (31), and the high sequence conservation of eIF-5A (1), these results taken together lead to the conclusion that both the expression

Further evidence for the dependence of the ydhs null haploid on plasmid-borne yDHS for growth was obtained from comparison of plasmid retention of the LeuG haploid JSH1[pRS316-yDHS] and the LeuG haploid JSH2[pRS316-yDHS], both derived after sporulation and tetrad dissection of JSD1[pRS316-yDHS], in rich medium. After culture of the above two haploid strains in YPAD media over 3 days, significant loss (~90%) of the Ura phenotype (pRS316-yDHS plasmid) occurred for LeuG haploid containing undisrupted yDHS, but no sign of loss of the plasmid was observed for the LeuG haploid, the ydhs::LEU2 strain, suggesting the mandatory nature of yDHS for yeast survival.

We next addressed the question of whether the role of the yDHS gene in cell growth is, indeed, to provide deoxyhypusine synthesis activity and thereby hypusine modification in eIF-5A. Using mutated yDHS genes, we sought to see if a correlation exists between the deoxyhypusine synthesis activity of the mutant proteins and their ability to support growth in yeast. Since two lysine residues (Lys326 and Lys329 of the human enzyme) have been identified as critical for deoxyhypusine synthase activity (13, 43), we targeted the two corresponding residues in the yeast enzyme (Lys350 and Lys352, respectively) for mutations. The expression of the five mutant proteins with mutations at Lys350 and/or Lys352 were equally high in BL21(DE3) cells (Fig. 4A). Deoxyhypusine synthase activity of each mutant protein was determined after its partial purification using three substrate proteins, the human eIF-5A precursor, and the two yeast eIF-5A precursors, A and B (Fig. 4B). The mutant protein yDHS(K308R) exhibited low but definite activity (less than 1% of the wild type enzyme activity) toward the two yeast eIF-5A precursor proteins, but not with the human precursor protein. No activity was detectable with yDHS(K308R) or with the three double mutant proteins, yDHS(K308R/K350R), yDHS(K308R/K350E), and yDHS(K308R/K350P), with any of the substrate proteins, indicating that Lys350 plays a critical role in catalysis. The mutated genes or the wild type gene were inserted into the YRp7 plasmid carrying TRP1. After transformation of the haploid ydhs::LEU2 strain, JSH1[pRS316-yDHS], with the recombinant YRp7 plasmids, TrpG UraG colonies containing both plasmids were selected and tested for their growth on 5-FOA plates (Fig. 5). Since 5-FOA selects for cells that have lost the UraG plasmid, only YRp7-derived plasmids remain in the cells. After 4 days’ incubation, thick growth was observed for the ydhs::LEU2 haploid containing YRp7-yDHS encoding the wild-type enzyme. There was no sign of growth for those plasmids containing plasmids encoding inactive proteins, i.e. yDHS(K308R) or any of the double mutant proteins. Only marginal growth was observed under the microscope with the haplids carrying YRp7-yDHS(K308R).

The data of Figs. 2B and 3 show that plasmid-borne yDHS can complement the ydhs null mutation on the chromosome. JSD1 transformed with the centromeric plasmid pRS316-yDHS carrying URA3 and yDHS, or a high copy number episomal plasmid YEpl52T-yDHS carrying TRP1 and yDHS, was sporulated and tetrads were analyzed. Unlike the 2:2 viable/ non-viable segregation of JSD1, tetrads from JSD1 carrying either plasmid could yield more than two viable haploid colonies after dissection. Dissection of 16 ascii derived from JSD1[pRS316-yDHS] yielded visible colonies as follows: 1 (1 ascus), 2 (10 ascii), 3 (4 ascii), 4 (1 ascus). Tetrads from JSD1[YEpl52T-yDHS] gave rise to a higher number of viable colonies, i.e. 4 (6 ascii), 3 (1 ascus), 2 (1 ascus) (Fig. 2B). All viable LeuG haploid colonies derived from JSD1[pRS316-yDHS] were TrpG; all LeuG haploids derived from JSD1[YEpl52T-yDHS] were yDHS; all LeuG haploid colonies carrying pRS316-yDHS (Fig. 3A), derived from sporulation and tetrad dissection of JSD1[pRS316-yDHS], were patched onto minimal media containing uracil and 5-FOA, which selects against UraG cells, only LeuG cells grew (Fig. 3C), indicating that they can lose the URA3 plasmid. In contrast, no LeuG colonies could grow on 5-FOA plates, suggesting that they cannot grow without yDHS from the plasmid.

Further evidence for the dependence of the ydhs null haploid on plasmid-borne yDHS for growth was obtained from comparison of plasmid retention of the LeuG haploid JSH1[pRS316-yDHS] and the LeuG haploid JSH2[pRS316-yDHS], both derived after sporulation and tetrad dissection of JSD1[pRS316-yDHS], in rich medium. After culture of the above two haploid strains in YPAD media over 3 days, significant loss (~90%) of the Ura phenotype (pRS316-yDHS plasmid) occurred for LeuG haploid containing undisrupted yDHS, but no sign of loss of the plasmid was observed for the LeuG haploid, the ydhs::LEU2 strain, suggesting the mandatory nature of yDHS for yeast survival.

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of eIF-5A precursor protein and its hypusine modification are required for proliferation of all eukaryotes. Although Sasaki et al. (8) recently reported evidence that the yDHS gene is essential for viability of \textit{S. cerevisiae}, the possibility could not be ruled out that the yDHS gene itself, or its encoded protein, in addition to contributing deoxyhypusine synthesis activity, may have an independent function vital for yeast cells. To investigate whether yeast growth depends on deoxyhypusine synthase activity, and, if so, how, we generated mutations at Lys308 and Lys350 of the enzyme. Unlike those expressing the wild-type enzyme, no growth was observed for the cells carrying the mutated genes \textit{ydhs(K350R)}, \textit{ydhs(K308R/K350R)}, \textit{ydhs(K308R/K350E)}, or \textit{ydhs(K308R/K350P)} that encode mutant proteins that are totally inactive in deoxyhypusine synthase. Only slow growth was observed with the \textit{ydhs::LEU2} haploid carrying YEp352T-yDHS (1 mg/ml) (C). The plates were incubated at 30 °C for 2 days.

For human deoxyhypusine synthase, which shares 58% sequence identity and 73% similarity with the yeast enzyme, Lys329 has been identified as the active site residue involved in the enzyme-substrate intermediate formation (13), and Lys329 of the human enzyme appears to be essential for deoxyhypusine synthesis activity. Therefore, it seems reasonable to assume that in the yeast mutant proteins yDHS(K308R) and yDHS(K350R) there is no gross disruption of global structure or of auxiliary function, if any, outside the active site. Judging from the total lack of deoxyhypusine synthetic activity of yDHS(K350R), Lys350 of the yeast enzyme, like the corresponding lysine residue (Lys329) of the human enzyme, appears to be essential for deoxyhypusine synthesis activity.

For human deoxyhypusine synthase, which shares ~58% sequence identity and 73% similarity with the yeast enzyme,
catalysis as the site of enzyme-substrate intermediate formation. 2

eIF-5A is the only protein known to contain hypusine. Since eIF-5A is an abundant protein with a long half-life (32), the lack of deoxyhypusine synthase in the yDHS null mutant would be expected to result in the slow depletion of only this protein, i.e. mature eIF-5A, with the concomitant accumulation of the eIF-5A precursor protein. 2 The growth of yDHS null spores derived from the yDHS/yDHS diploid, JSD1, ceased gradually (Fig. 2C), presumably as residual deoxyhypusine synthase and eIF-5A inherited from the parent diploid became limiting due to degradation and dilution through 8–9 cell divisions. In the case of disruption of the eIF-5A gene TIF51A, tif51A null spores derived from tetrad dissection appeared to stop multiplying after approximately five generations of growth (20). The fact that a yDHS null haploid can grow for perhaps several generations longer than a tif51A null haploid may reflect the extra time required for the depletion of deoxyhypusine synthase before the subsequent reduction in eIF-5A takes place. Judging from this extra time required for enzyme depletion, and the lack of accumulation of eIF-5A precursor protein in normal cells (32), it is probable that deoxyhypusine synthase is also a stable protein present in amounts far exceeding the minimum level necessary to modify all the newly translated eIF-5A gene products (34).

The arrest of growth of yDHS null mutants with abnormally enlarged cells (Fig. 2C) with no detectable budding suggests that cessation of growth upon deprivation of deoxyhypusine synthase is caused by specific defects in cell cycle progression or division. Interestingly, growth arrest was accompanied by a similar enlargement of cells in the S. cerevisiae strain UBYH-R upon depletion of a functional but unstable eIF-5A fusion protein, R-eIF-5A (34). Thus, it is reasonable to assume that in both cases the cessation of growth is mediated by common cellular defects resulting from exhaustion of eIF-5A. A striking increase in cell size was also observed in cells whose growth was arrested following depletion of the polyamines, spermidine and spermine, in a S. cerevisiae strain with a null mutation of the SPE2 gene encoding S-adenosylmethionine decarboxylase (35). In addition to enlargement, decrease in budding, accumulation of vesicles, and abnormal distribution of actin-like and chitin-like materials were observed (35). These characteristics are similar to the abnormalities reported for a cell division cycle (cdc) mutant (36, 37). Polyamine-deficient cells also exhibit several physiological changes, including loss of functional mitochondria (38), increase in +1 ribosomal frameshifting (39), and sensitivity to damage by oxygen (38) and heat (40). Although hypusine has been implicated as a key element of the polyamine requirement in eukaryotic cells (20), it is not known which aspects of these cellular defects are caused by eIF-5A deprivation.

Despite the essential nature of hypusine and eIF-5A in cell proliferation, the true physiological function of eIF-5A remains an enigma. In experiments where a rapid depletion of eIF-5A was achieved in yeast, only a small reduction in overall protein synthesis and a slight change in polysome profile were observed (34). This finding led to the conclusion that eIF-5A is not a general protein synthesis initiation factor but rather that it may act as a factor selective for a subset of specific mRNAs. In line with this idea is the observation that certain metal chelator inhibitors of deoxyhypusine hydroxylase, the enzyme that catalyzes the final step of hypusine synthesis, cause arrest of mammalian cells at the G1/S boundary of the cell cycle (16). Recently, eIF-5A was reported to be a cellular cofactor essential for Rev function in human immunodeficiency virus type 1 replication (41) or Rex function in human T-cell lymphotrophic virus replication (42), thus promoting speculation on its role in the recognition and nuclear export of specific mRNAs. The fact that yeast cells deprived of polyamines and those depleted of eIF-5A or of deoxyhypusine synthase display a similar enlargement suggests the intriguing possibility that eIF-5A may play a role in cell division cycle control. If so, eIF-5A may be either directly involved in this process or it may function indirectly by controlling the expression of a subset of proteins critical for cell division.

Dissection and analysis of the molecular events that occur in eIF-5A deficient cells should shed further light on the physiological function of this unique protein.

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