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Control of 5′,5′-Dinucleoside Triphosphate Catabolism by *APH1*, a *Saccharomyces cerevisiae* Analog of Human *FHIT*

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The putative human tumor suppressor gene *FHIT* (fragile histidine triad) (M. Ohta et al., Cell 84:587–597, 1996) encodes a protein behaving in vitro as a dinucleoside 5′,5‴-P^1,P^3-triphosphate (Ap_3A) hydrolase. In this report, we show that the *Saccharomyces cerevisiae APH1* gene product, which resembles human Fhit protein, also hydrolyzes dinucleoside 5′,5′-polyphosphates, with Ap_3A being the preferred substrate. Accordingly, disruption of the *APH1* gene produced viable *S. cerevisiae* cells containing reduced Ap_3A-hydrolyzing activity and a 30-fold-elevated Ap_3N concentration.

Aberrant transcripts of the human *FHIT* (fragile histidine triad) gene have been detected in nearly 50% of esophageal, stomach, and colon carcinomas (26). Accordingly, deletions in the *FHIT* gene, which is located at chromosome region 3p14.2, were found associated with many types of common human cancers, including lung (7, 37), skin (36), head and neck (22, 38), breast (20, 23), cervical (9), and colorectal (10) cancers. Actually, the *FHIT* gene encompasses *FRA3B*, the most highly inducible fragile site in the human genome (13, 26). The *FRA3B* breakpoints, including the renal carcinoma-associated one, t(3;8), fall within introns 3, 4, and 5 of the *FHIT* gene and are likely to be at the origin of the cancer-associated deletions (39). However, although the strong correlation between *FHIT* alterations and cancer development may indicate a suppressor function of the *FHIT* gene product, this function has not yet been proven (for reviews, see references 13, 16, and 27).

A marked similarity between the protein product of *FHIT* and the amino acid sequence of a *Schizosaccharomyces pombe* enzyme described as a diadenosine 5′,5‴-P^1,P^4-tetraphosphate (Ap_4A) asymmetrical hydrolase (12, 33) was noted early (26). Ap_4A belongs to an unusual family of ubiquitous dinucleoside polyphosphates, the possible functions of which are still debated. At one time, these nucleotides were suspected of being involved in the proliferation activity of animal cells (for a review, see reference 8). However, this hypothesis did not receive support from further studies (for a review, see reference 32). Because their intracellular concentration increases in response to oxidative stresses (for reviews, see references 15 and 28), dinucleoside polyphosphates were also proposed to participate to cellular adaptation. Finally, extracellular functions have been envisaged. For instance, dinucleoside polyphosphates were reported to interfere with cardiovascular and neurotransmission activities (for reviews, see references 2 and 24).

The Fhit protein could be produced from cDNA in *Escherichia coli*. In agreement with its resemblance to an Ap_4A hydrolase, this protein accelerated the in vitro hydrolysis of various dinucleoside polyphosphates, including Ap_4A (1). The most efficient substrate was diadenosine 5′,5‴-P^1,P^3-triphosphate (Ap_3A). In light of the putative cancer suppression function of the *FHIT* product, the observations described above supported the idea of some role of dinucleoside polyphosphates in tumor development. Whether dinucleoside triphosphates rather than dinucleoside tetraphosphates would be involved is still an open question.

In *Saccharomyces cerevisiae*, Ap_4A is transformed into ATP plus ADP by two Ap_4A phosphorylases encoded by two distinct genes, *APA1* and *APA2* (30, 31). To recycle Ap_3A, which is fully resistant to the two Ap_4A phosphorylases, the yeast cell produces an Ap_3A hydrolase capable of converting Ap_3A into ADP plus AMP (3). The enzyme also slowly hydrolyzes Ap_4A into ATP plus AMP.

The *S. cerevisiae* genome sequence data indicates a gene (*APH1*) product highly similar to the Ap_4A hydrolase of *S. pombe* (12, 26). To determine whether this protein product behaves like the *S. pombe* Ap_4A hydrolase or whether it corresponds to the previously described *S. cerevisiae* Ap_3A hydrolase, we have undertaken its expression in *E. coli*. In vitro characterization of the obtained protein strongly indicates that it corresponds to the already isolated Ap_3A hydrolase, therefore resembling the Fhit protein. In addition, gene disruption experiments unambiguously show that the *APH1* product is the main actor in *S. cerevisiae* Ap_3N (N = A, C, G, or U) catabolism. This conclusion emphasizes the putative predominance of dinucleoside triphosphates in any *FHIT*-mediated function.

MATERIALS AND METHODS

Yeasts, bacteria, and plasmids. The strains used are listed in Table 1. Bacterial and yeast transformations were performed by electroporation and by the lithium acetate method of Ito et al. (14), respectively. YPD and minimal sporulation media were as described before (34). Plasmid pBluescript SK(−) was from Stratagene (San Diego, Calif.).

Nucleotides. Ap_4C and Ap_4G were extracted from *E. coli* PAL2103D (18) as described previously (30). Ap_3C was enzymatically synthesized with purified *E. coli* lysyl-tRNA synthetase (29). Other nucleotides were from Boehringer (Ap_4A and Ap_5A), Sigma (Ap_3A), or Pharmacia (Ap_3G, Gp_3G, and Gp_4G). [^{3}H]Ap_4A (reference batch TRQ.4405, 159 GBq/mmol, radiochemical purity of >98%) was from Amersham.

Preparation of *S. cerevisiae* crude extracts. For the preparation of *S. cerevisiae* crude extracts, cells were grown until the optical density at 650 nm of the culture reached 2 ± 0.5. After centrifugation at 12,000 × *g* for 15 min, the cell pellet was suspended in 50 mM Tris-HCl buffer (pH 7.8) containing 0.1 mM EDTA, 10 mM 2-mercaptoethanol, and 1 mM phenylmethylsulfonide fluoride (PMSF). Cells were sonicated twice for 5 min at 0°C, and cell debris was removed by centrifugation at 17,000 × *g* for 15 min. The total amount of protein in the supernatant was determined by using the Bio-Rad protein assay reagent.

Enzymatic assays. Hydrolysis of [^{3}H]Ap_4A was used to monitor enzyme activity; the reaction mixture (100 μl) contained 50 mM Tris-HCl (pH 7.8), 5 mM $MgCl_2$, 0.1 mM EDTA, 50 μM [^{3}H]Ap_4A (2 GBq/mmol), and a 140-U/ml concentration of alkaline phosphatase from calf intestine (2,000 U/mg; Boehringer). After an incubation ranging from 10 to 90 min at 37°C, the [^{3}H]adenosine produced was counted as described previously (31).

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TABLE 1. Bacterial and yeast strains used in this study

Strain	Genotype	Reference
E. coli JM101TR	Δ(*lac-pro*) *supE thi recA56 srl-300*::Tn*10* (F′ *traD36 proAB lacI*^{q} *lacZ*ΔM15)	11
S. cerevisiae		
CMY214	*trp1-Δ1/trp1-Δ1 his3Δ200/his3Δ200 ura3-52/ura3-52 ade2-101/ade2-101 lys2-801/lys2-801 can1/CAN1*	21
YPALHU	CMY214 *APA1/apa1Δ::HIS3 APA2/apa2Δ::URA3*	30
YPALHUT	YPALHU *APH1/aph1Δ::TRP1*	This work
YPALS	*trp1-Δ1 his3Δ200 ura3-52 ade2-101 lys2-801 can1*	30
YPALSH	YPALS *apa1Δ::HIS3*	30
YPALSU	YPALS *apa2Δ::URA3*	30
YPALSHU	YPALS *apa1Δ::HIS3 apa2Δ::URA3*	30
YPALST	YPALS *aph1Δ::TRP1*	This work
YPALSHT	YPALS *apa1Δ::HIS3 aph1Δ::TRP1*	This work
YPALSUT	YPALS *apa2Δ::URA3 aph1Δ::TRP1*	This work
YPALSHUT	YPALS *apa1Δ::HIS3 apa2Δ::URA3 aph1Δ::TRP1*	This work

When Ap_3A hydrolysis was used to monitor enzyme activity, the same reaction mixture was made, except that unlabeled 50 μM Ap_3A was the substrate. After a 10- to 120-min incubation at 37°C, the reaction was stopped by the addition of perchloric acid (10%, wt/vol) and the mixture was centrifuged at 17,000 × *g* for 15 min. The supernatant was neutralized by K_2CO_3 and further centrifuged for 5 min at 17,000 × *g*. The supernatant was diluted 20-fold in Tris-HCl buffer (20 mM, pH 7.8) containing 0.1 mM $ZnCl_2$ and 1 mM $MgCl_2$. The concentration of the Ap_3A remaining at the end of the reaction was determined with the help of the luminescence assay described below.

To compare the activity of the *APH1* gene product in response to various substrates, the incubation mixture (105 μl) contained 40 mM Tris-HCl (pH 7.8), 0.5 mM $MgCl_2$, 20 μM EDTA, 200 μg of bovine serum albumin per ml, and a 20 μM concentration of the substrate under study. After a 10-min incubation at 25°C, the reaction was stopped by freezing the samples in liquid nitrogen. The intact substrate remaining was quantitated after separation from the reaction products on a high-pressure liquid chromatography column (0.46 by 20 cm) packed with Lichrosorb RP18. The column was isocratically eluted at a flow rate of 1.5 ml/min with 50 mM potassium phosphate (pH 5.3). The concentrations of the nucleotides under study were deduced from their absorbancy in the column effluent at 254 nm.

Determination of Ap_4N and Ap_3N concentrations in *S. cerevisiae* crude extracts. Nucleotides were extracted with perchloric acid from 30-ml aliquots of the cultures, as described previously (31). The sample was then neutralized by K_2CO_3 and diluted fivefold in a Tris-HCl buffer (20 mM, pH 7.8) containing 0.1 mM $ZnCl_2$ and 1 mM $MgCl_2$. After digestion of ATP with alkaline phosphatase from calf intestine (3,000 U/mg, enzyme immunoassay grade; Boehringer), two successive luminescence assays were performed (4). In the first one, a 10-μl extract sample was mixed with 100 μl of the ATP-bioluminescence HS mixture from Boehringer. After extinction of the luminescence background due to ATP contamination, snake venom phosphodiesterase (Boehringer) was added at a final concentration of 5 μg/ml and the luminescence was recorded. In this assay, only Ap_4N nucleotides contribute to the signal. In the second assay, 10 μl of sample was mixed with 100 μl of the luminescence mixture containing, in addition, 0.25 mM phosphoenolpyruvate (from Sigma) and 35 U of rabbit muscle pyruvate kinase (Boehringer) per ml. After extinction of the signal caused by contaminating ATP and ADP, phosphodiesterase was added at a final concentration of 5 μg/ml and the luminescence was recorded. Under these conditions, both the Ap_3N and Ap_4N compounds contributed to the light signal. The Ap_3N concentration was deduced from the difference between the two assays (4, 25).

Cellular concentrations of Ap_4N and Ap_3N were calculated by assuming that, in the yeast culture, one optical density unit at 650 nm corresponds to 0.4 μl of intracellular volume (6).

Cloning of the *S. cerevisiae APH1* gene. The *APH1* gene of *S. cerevisiae* was amplified by PCR using 25 pmol of the oligonucleotides ATABam (5′-CTTAG AATGCAGCGGATCCTTGGGATTAGC-3′) and ATAXho (5′-TTTACTGTT GAGTCTCCTCGAGGAAAGTAG-3′) and 3.4 μg of genomic DNA from strain CMY214 (30 cycles; 95°C for 1 min, 40°C for 1 min, 72°C for 2 min). The sequences of the two primers encompass the *Bam*HI and *Xho*I sites flanking the *APH1* gene (Fig. 1). The amplified DNA fragment of 1.3 kbp was purified with the Qiagen Plasmid Mini Kit 100. After digestion by *Bam*HI and *Xho*I, the purified DNA fragment was inserted into the corresponding sites of plasmid pBluescript SK(−) to give plasmid p35.

Disruption of the *APH1* gene in *S. cerevisiae*. To inactivate the *APH1* gene, a 75-bp *Nsi*I-*Bgl*II fragment internal to the gene was deleted and substituted by the selectable marker *TRP1*. For this purpose, the *TRP1* marker carried by plasmid pRS314 (35) was amplified by PCR with primers TRPNsi (5′-GAGTGATGCA TAAACGACATTACTATATATA-3′) and TRPBgl (5′-GTCACAGATCTGG CAAGTGCACAAACAATA-3′) (30 cycles; 95°C for 1 min, 45°C for 1 min, 72°C for 2 min). The resulting DNA was digested by *Nsi*I and *Bgl*II before insertion into p35.

The resulting plasmid was digested by *Bam*HI and *Xho*I and used to transform the diploid yeast strain YPALHU. PCR amplifications using either ATABam and ATAXho, or ATABam and TRPBgl, as primers were performed to verify that the resulting transformed strain, YPALHUT, actually harbored both a wild-type and a disrupted copy of the *APH1* gene.

Recombination of the *APH1* gene for overexpression in *E. coli* cells. Deletion of the intron harbored by the *APH1* gene and insertion of a canonical Shine-Dalgarno sequence upstream of this gene was performed as described in the legend to Fig. 1. Briefly, two PCR amplifications were performed with plasmid p35 as the template and either ATA3 (5′-GCGGGATCCCTAGAAAGGAGG TACGATCATGAATAAGCCAATATATTTCAGCA-3′) plus ATA1 (5′-ATA TTTTGACTTATAGAAAACTTGTTCAGTTACAAG-3′) or ATA2 (5′-CAA GTTTTCTATAAGTCAAAATATACGTATGCATTG-3′) plus ATAXho as the primer pair (30 cycles; 95°C for 1 min, 50°C for 1 min, 72°C for 2 min). After Qiagen purification, the two resulting DNA fragments were mixed and further subjected to 30 cycles of amplification using ATA3 and ATAXho as primers. The final purified DNA was cut by *Bam*HI and *Xho*I and inserted into the corresponding sites of plasmid pBluescript SK(−), to give plasmid p19. The nucleotide sequence of the insert was verified.

Purification of the *APH1* gene product. JM101TR cells harboring plasmid p19 were grown in 125 ml of LB medium containing 100 μg of ampicillin per ml and 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). When the optical density at 650 nm reached 2.0, the cells were harvested by a 30-min centrifugation at 12,000 × *g*. The cell pellet, resuspended in 8 ml of a 50 mM Tris-HCl buffer (pH 7.8) containing 10% glycerol and 0.5 mM PMSF, was sonicated for 4 min at 0°C. After removal of cellular debris by centrifugation (30 min, 12,000 × *g*), nucleic acids were precipitated by the addition of streptomycin sulfate (3% [wt/vol] final concentration) and centrifuged (30 min, 12,000 × *g*). The supernatant (7.5 ml) was dialyzed against 1 liter of 20 mM potassium phosphate (pH 7.0)–10% glycerol–0.1 mM PMSF and applied to a DEAE Sephadex column (1 by 5 cm) equilibrated in 20 mM potassium phosphate (pH 7.0). The column was eluted by use of 20 to 200 mM linear gradient of potassium phosphate (80 ml, pH 7.0) at a flow rate of 6 ml/h. During the purification, the activity of the *APH1* gene product was monitored through hydrolysis of either Ap_4A or Ap_3A. The fractions containing activity were pooled and concentrated by ammonium sulfate precipitation. At the end of this step, the specific Ap_3A degradation activity of the sample had increased ~16-fold in comparison to that of the crude extract.

RESULTS AND DISCUSSION

Expression of the *S. cerevisiae APH1* gene in *E. coli*. In 1996, the sequencing of the *FHIT* cDNA revealed homologies between the Fhit protein and two yeast proteins, an Ap_4A hydrolase produced by the *S. pombe aph1* gene and the product of an *S. cerevisiae* gene (26). The latter gene was named *APH1* because of its homology to the *S. pombe aph1* gene. It was also designated *HNT2* by other authors (see accession no. U28374 in the EMBL and GenBank databases) and received the name YDR305c in the systematic genome sequence designation. Two possible in-frame initiator codons are found in the 5′ region of the *S. cerevisiae APH1* gene; they correspond to either MILSK or MNKPI N-terminal sequences. The second

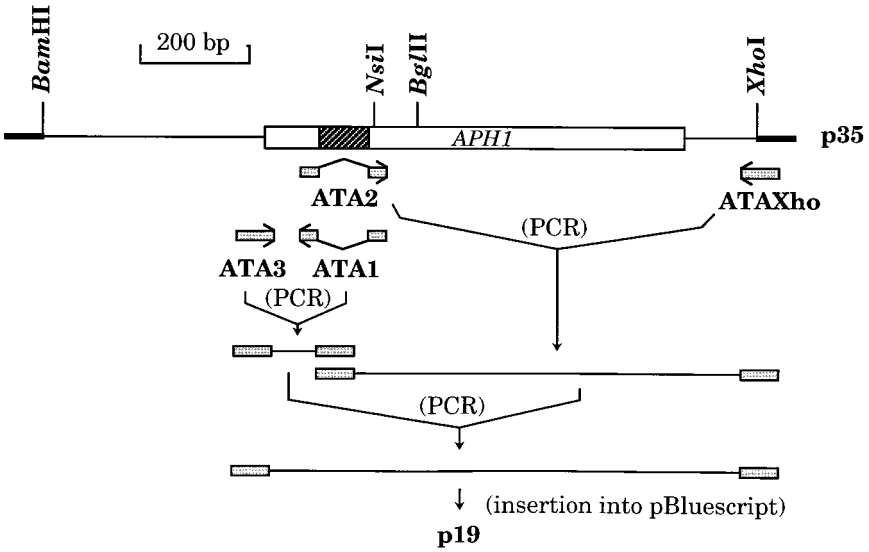

FIG. 1. Construction of plasmid p19. The p35-inserted *APH1* gene is represented by a rectangle with the intron shaded. Two PCR amplifications were performed with plasmid p35 as the template and either ATA3 plus ATA1, or ATA2 plus ATAXho, as the primer pair. Primers ATA1 and ATA2 are complementary to the exonic sequences on both sides of the intron. The two amplified DNA fragments were mixed and further subjected to PCR with ATA3 and ATAXho as the primers. The resulting DNA was cut with *Bam*HI and *Xho*I, the sites of which are present in ATA3 and ATAXho, respectively, and inserted into plasmid pBluescript SK(−), to give plasmid p19.

one is likely to be used as the translational start because it corresponds to the initiator methionine in *S. pombe*. The coding sequence of the *APH1* gene is interrupted by a single intron of 89 bp. The 5′ boundary of this intron is located 56 bp downstream of the putative translational start. The protein encoded by the *APH1* gene is expected to contain 206 amino acids (M_r = 23,541).

To compare the *S. cerevisiae APH1* gene product and the previously isolated Ap_3A hydrolase (3), chromosomal DNA of strain CMY214 was used to amplify the *APH1* DNA by PCR. The resulting DNA was inserted into plasmid pBluescript under control of the Plac promoter. The intron of the gene was then deleted, and the sequence upstream of the initiation codon was replaced by a powerful Shine-Dalgarno sequence (5, 17), yielding plasmid p19 (Fig. 1).

Upon transformation by plasmid p19, *E. coli* JM101TR clearly showed a new protein band on sodium dodecyl sulfate-polyacrylamide gels. This band migrated with an apparent M_r of 26,000 ± 2,000 (data not shown). This molecular ratio fits with that expected for the *APH1* product. Moreover, a crude extract of the p19-transformed *E. coli* strain contained 500- and 20,000-fold-larger amounts of Ap_4A- and Ap_3A-hydrolyzing activities, respectively, than an extract of the control strain containing pBluescript. Upon chromatography through a DEAE-Sephadex column, both the Ap_3A- and Ap_4A-hydrolyzing activities comigrated with the 26-kDa polypeptide described above. However, lability of activity precluded further purification of the enzyme. Such a behavior resembles that of the previously isolated Ap_3A hydrolase, the activity of which also vanished during purification (3). The partially purified *APH1* product could be stored after ammonium sulfate precipitation, and various dinucleotides could be assayed as substrates (Table 2). Strikingly, the relative rates of hydrolysis by APH1 of the assayed substrates were nearly identical to those already reported in the case of Ap_3A hydrolase from *S. cerevisiae* (3). In particular, the dinucleoside triphosphates Ap_3A, Ap_3G, and Ap_3C were the most efficient substrates.

Disruption of the *APH1* gene. In *S. cerevisiae*, Ap_4A catabolism is mainly sustained by two Ap_4A phosphorylases produced by the *APA1* and *APA2* genes (30, 31). Strains fully devoid of Ap_4A phosphorylase activity have been described. In crude extracts of such a strain, a weak Ap_4A-hydrolyzing activity was measurable (3). This hydrolysis could be assigned to an Ap_3A hydrolase having a rather broad specificity. To examine whether the weak Ap_4A hydrolysis described above was associated with the *APH1* gene, we have undertaken the knockout of this gene in an *apa1 apa2* context and measured dinucleoside tri- and tetraphosphate-hydrolyzing activities in cell extracts.

In the diploid *apa1/APA1 apa2/APA2* strain YPALHU, one copy of each of the *APA1* and *APA2* genes are inactivated by insertion of a *HIS3* and a *URA3* cassette, respectively. To

TABLE 2. Comparison of the substrate specificities of the *APH1* gene product and of the *S. cerevisiae* Ap_3A hydrolase

Substrate	Relative rate of hydrolysis (%)	
	APH1 gene product[a]	Ap_3A hydrolase[b]
Ap_3A	100	100
Ap_4A	11 ± 1.6	13
Ap_5A	6 ± 1.1	4
Gp_3G	80 ± 3	74
Gp_4G	51 ± 2	61
Ap_3C	94 ± 6	94
Ap_3G	92 ± 7	95
Ap_4G	73 ± 9	76
Ap_4C	7.8 ± 1.3	4.3

[a] The reaction mixture (105 μl) contained 40 mM Tris-HCl (pH 7.8), 0.5 mM $MgCl_2$, 20 μM EDTA, 200 μg of bovine serum albumin per ml, a 20 μM concentration of the substrate under study, and catalytic amounts of enzyme. Initial rates of hydrolysis are expressed as percentages (means ± standard deviations) of the rate measured with Ap_3A. Each measurement was done in duplicate.

[b] Data from reference 3.

TABLE 3. Degradation activities and dinucleotide concentrations in various *S. cerevisiae* strains[a]

Strain	Relative degradation activity[b]		Relative dinucleotide concentration[c]	
	Ap_4A	Ap_3A	Ap_4N	Ap_3N
YPALS (control)	1	1	1	1
YPALST (*aph1*)	0.8 ± 0.07	<0.05	2.5 ± 0.2	31 ± 1
YPALSHU (*apa1 apa2*)	0.011 ± 0.007	1 ± 0.1	50 ± 3	NM[d]
YPALSHUT (*apa1 apa2 aph1*)	0.003 ± 0.001	<0.05	750 ± 50	NM

[a] Yeast cells were grown in rich YPD medium. When the optical density of the culture reached 2 ± 0.5 at 650 nm, two samples of the culture were removed for either enzymatic or dinucleotide measurements (100 and 30 ml, respectively). Each measurement was done in duplicate. Ap_4A and Ap_3A degradation activities were measured in crude extracts obtained by sonication. The reaction mixtures contained 50 mM Tris-HCl (pH 7.8), 5 mM $MgCl_2$, 0.1 mM EDTA, 140 U of alkaline phosphatase per ml, and a 50 μM concentration of the substrate under study. Ap_4N and Ap_4N plus Ap_3N concentrations were assayed by bioluminescence as described in Materials and Methods. Values are given as means ± standard deviations.

[b] With the control strain YPALS, Ap_3A degradation activity was 0.8 μmol/min/g of protein. Ap_4A degradation activities were 6.9 μmol/min/g in the absence of added phosphate and 14.0 μmol/min/g in the presence of 1 mM phosphate. Comparison of the latter values indicates that Ap_4A phosphorylases in the extracts from YPALS and YPALST mainly contribute to the measured Ap_4A degradation activities. The values in the table correspond to the activities in the absence of phosphate.

[c] In the control strain YPALS, Ap_4N and Ap_3N concentrations amounted to 0.4 μM each.

[d] NM, not measurable.

achieve disruption of *APH1*, a 75-bp fragment of this gene was deleted on plasmid p35 and replaced by the selectable marker *TRP1* (35). The corresponding plasmid was linearized and used to transform strain YPALHU. Resulting diploid cells were left to sporulate, and haploids were selected as canavanine-resistant clones. Among the can^r cells obtained, 14% proved to be *his*$^-$ *ura*$^-$ *trp*$^-$ (*APA1 APA2 APH1*), 12% were *his*$^+$ *ura*$^-$ *trp*$^-$ (*apa1*), 8% were *his*$^-$ *ura*$^+$ *trp*$^-$ (*apa2*), 17% were *his*$^-$ *ura*$^-$ *trp*$^+$ (*aph1*), 9% were *his*$^+$ *ura*$^+$ *trp*$^-$ (*apa1 apa2*), 16% were *his*$^+$ *ura*$^-$ *trp*$^+$ (*apa1 aph1*), 11% were *his*$^-$ *ura*$^+$ *trp*$^+$ (*apa2 aph1*), and 12% were *his*$^+$ *ura*$^+$ *trp*$^+$ (*apa1 apa2 aph1*). These numbers show that the *APH1* product is not essential to cell viability, even in the absence of Ap_4A phosphorylase activity. Whereas the growth of the cells in YPG solid or liquid medium was not sensitive to inactivation of one or two of the three genes *APA1*, *APA2*, and *APH1*, it was slightly but significantly impaired by simultaneous inactivation of all three genes. Generation times of strains YPALS, YPALST, and YPALSHU in liquid medium were 101 ± 4 (n = 4), 103 ± 4 (n = 4), and 102 ± 3 (n = 4) min, respectively. In contrast, the generation time of strain YPALSHUT was 150 ± 5 min (n = 4).

In crude extracts of strain YPALST (*aph1*) and YPALSHUT (*apa1 apa2 aph1*), the specific Ap_3A hydrolase activity was reduced more than 20-fold, as compared to that of YPALS, the control strain. In addition, the Ap_4A hydrolase activity of a crude extract of strain YPALSHUT (*apa1 apa2 aph1*) was reduced nearly fourfold, as compared to that of strain YPALSHU (*apa1 apa2*). These measurements establish that *APH1* actually directs hydrolytic activity towards Ap_3A as well as Ap_4A. They also show that *APH1* is responsible for the major part of the weak Ap_4A-hydrolyzing activity detected in a YPALSHU extract.

Ap_3N and Ap_4N concentrations in various *aph1* mutants. In the control strain YPALS (*APA1 APA2 APH1*), Ap_3N and Ap_4N concentrations are equal to 0.4 μM. These concentrations were increased 30- and 2.5-fold, respectively, upon disruption of the *APH1* gene only (Table 3). The variation of Ap_4N may appear relatively small in view of the broad specificity of the *APH1* product. However, in the YPALS and YPALST strains, two Ap_4A phosphorylases which are likely to control Ap_4N concentration occur, thereby counterbalancing the effect of the absence of Ap_3A hydrolase activity on the cellular pool of these nucleotides.

The *APH1* gene was also disrupted in a strain lacking the two Ap_4A phosphorylase genes. This time, the lack of Ap_3A hydrolase activity raised the Ap_4N pool 15-fold. Ap_3N concentrations could not be reliably measured in extracts of these strains (YPALSHU and YPALSHUT) because of an excessive contribution of Ap_4N to the pyruvate kinase-containing luminescence assay.

The combination of these results with the above finding that the growth rate of YPALST (*aph1*) is indistinguishable from that of YPALS (*APH1*) suggests that the *S. cerevisiae* cell cycle is not under the control of the Ap_3N pool. In the case of YPALSHUT (*apa1 apa2 aph1*), the observed reduction of the growth rate can be simply related to the nucleotide unbalance caused by the ~300 μM cellular Ap_4N concentration.

Conclusions. Altogether, the present results establish that *APH1*, an analog of *FHIT* in *S. cerevisiae*, encodes a protein product involved mainly in Ap_3N catabolism. This product corresponds to the Ap_3A hydrolase already characterized (3). The similarity between the in vitro functions of the *APH1*- and *FHIT*-encoded polypeptide products sustains the view that Ap_3N concentration in human cells also might be deregulated by *FHIT* inactivation.

Regarding the putative role of *FHIT* in tumor suppression, an abnormally high Ap_3N cellular concentration resulting from inactivation of one *FHIT* allele in the diploid human cell can be hypothesized to directly or indirectly impair growth control or induce tumorigenesis. In this context, involvement of the Fhit protein as well as of the *APH1* product in an as-yet-unrecognized nucleotidyl transferase reaction at the expense of the Ap_3N substrate (19) must not be neglected. The present study, however, has not made it possible to relate *APH1* inactivation to a growth defect in *S. cerevisiae.*

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