A genetic screen identifies cellular factors involved in retroviral –1 frameshifting

(translation/*CUP1*/*IFS1*/paromomycin)

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ABSTRACT To identify cellular factors that function in –1 ribosomal frameshifting, we have developed assays in the yeast *Saccharomyces cerevisiae* to screen for host mutants in which frameshifting is specifically affected. Expression vectors have been constructed in which the mouse mammary tumor virus gag-pro frameshift region is placed upstream of the *lacZ* gene or the *CUP1* gene so that the reporters are in the –1 frame relative to the initiation codon. These vectors have been used to demonstrate that –1 frameshifting is recapitulated in yeast in response to retroviral mRNA signals. Using these reporters, we have isolated spontaneous host mutants in two complementation groups, *ifs1* and *ifs2*, in which frameshifting is increased 2-fold. These mutants are also hypersensitive to antibiotics that target the 40S ribosomal subunit. We have cloned the *IFS1* gene and shown that it encodes a previously undescribed protein of 1091 aa with clusters of acidic residues in the carboxyl-terminal region. Haploid cells lacking 82% of the *IFS1* open reading frame are viable and phenotypically identical to *ifs1-1* mutants. This approach could help identify potential targets for antiretroviral agents.

Ribosomal frameshifting, a mode of translational regulation, involves a directed change in reading frame at a specific site on the mRNA at a defined frequency. This allows the production of two or more proteins at fixed ratios from overlapping coding regions with a single translation initiation site (1, 2). The frequency of –1 frameshifting on retroviral RNA determines the ratio of gag to pol gene products, a ratio that dramatically affects viral assembly (3). Hence, the frameshifting is a step in the retroviral life cycle whose efficiency is thought to be tightly controlled. Experiments using *in vitro* translation in rabbit reticulocyte lysates have defined the cis-acting signals in retroviral mRNA directing –1 frameshifting. In all cases, the frameshifting signal is bipartite. Shifting occurs at a heptanucleotide sequence that conforms to the general motif X XXY YYZ (triplets represent the 0 frame), which allows the anticodons of tRNAs at both the P and A ribosomal sites to maintain two of three base pairs after shifting into the –1 frame (4). Frameshift sites also contain a higher-order RNA structure, either a stem-loop (5, 6) or a pseudoknot (7, 8), downstream of the shift sequence.

Here we report the development and use of a genetic approach to identify host factors involved in retroviral frameshifting. Using both a selectable (*CUP1*) and an enzymatic (*lacZ*) reporter, we demonstrate that yeast recapitulates retroviral –1 frameshifting. Using the mouse mammary tumor virus (MMTV) gag-pro frameshift site as a model substrate, we identified yeast mutants in which –1 frameshifting levels were specifically elevated. The gene defective in one of these mutants encodes a previously undescribed protein, Ifs1p.‡ Though the *IFS1* gene product is nonessential, deletion of the

gene causes increased frameshifting and a decrease in translational fidelity in response to antibiotics that target the 40S ribosomal subunit.

MATERIALS AND METHODS

Yeast Strains and Genetic Methods. *Saccharomyces cerevisiae* strains used in this study are listed in Table 1.

The numbering of the MMTV sequence is reported here with the first A residue of the heptanucleotide shifty site designated +1. The *CUP1* reporter plasmid contained nt –17 to +68 of MMTV inserted into the *Kpn* I site of pGM14 (J.G.U., unpublished data). β -Galactosidase was expressed from a *CYC1-lacZ* fusion gene cloned from pLGHY2 (12). The hemagglutinin (HA) epitope tag sequence (GATTACAAG-GACGATGACGATAAA) fused to nt –968 to +110 of the MMTV sequence was inserted into the *Bam*HI site of the *CYC1-lacZ* fusion gene.

Frameshift Assays. The copper-containing media and copper-resistance assay have been described (13). After replica plating, growth on copper was scored after 4 days at 30°C. β -Galactosidase assays of colonies *in situ* were performed as described (10).

Western blotting was carried out on whole cell extracts. Equal amounts of total protein from each lysate were fractionated by SDS/PAGE. Monoclonal anti-HA antibodies (12CA5) were obtained from Babco (Richmond, CA). For quantitation, secondary antibodies conjugated to ¹²⁵I were used; the amount of gag-pro- β -galactosidase in each lysate was measured with a PhosphorImager (Molecular Dynamics) and normalized to a scale where the amount of the fusion protein expressed from the in-frame construct equaled 100% frameshifting.

The Screen for Increased Frameshifting Mutants. L5 cells, carrying a *CUP1* reporter with the UUUUUUA shifty site, which confers resistance to 0.2 mM copper, served as the parental strain. Four pools of 2 × 10⁶ cells each were plated at a density of 10⁶ cells per plate onto complete synthetic medium containing 0.4 mM copper sulfate. Each pool originated from a single colony grown to mid-logarithmic phase in complete synthetic minimal medium under appropriate selection. Twenty to 30 copper-resistant colonies from each pool were isolated and mated to L5 cells to determine whether the copper-resistance phenotype was dominant or recessive.

Cloning and Sequencing of *IFS1*. A centromere-based yeast genomic library (14) was used to transform H1 cells, and individual transformants were assayed for loss of nonsense codon suppression in paromomycin (Sigma) at 1 mg/ml. Two

Abbreviations: MMTV, mouse mammary tumor virus; HA, hemagglutinin.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. U28158).

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Table 1. Yeast strains

Strain	Genotype	Source
L5*	<i>MATa cup1Δ::ura3Δ[†] ura3-52 his3-Δ200 trp1 leu2 lys2 ade2</i>	K. Yamamoto
BJ2168	<i>MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 gal2</i>	Ref. 9
H1	Same as L5 except <i>ifs1-1</i>	This study
H2	Same as L5 except <i>ifs2-1</i>	This study
H3	Same as L5 except <i>MATa ifs1-1 ifs2-1</i>	This study
H4	Same as L5 except <i>MATa</i>	This study
P9	Same as L4 except <i>pep4Δ::LEU2</i>	This study
P10	Same as H1 except <i>pep4Δ::LEU2</i>	This study
D6	Diploid resulting from H1 × H4	This study
D7, D8	Same as D6 except <i>ifs1Δ::URA3/ifs1-1</i>	This study

*Derived from two backcrosses of K3aΔ*cup1* (10) to YPH252a (11).

[†]The *ura3* gene in the *cup1* locus has a 17-bp deletion. Hence this strain requires uracil for growth.

independent clones which complemented both the paromycin hypersensitivity and the increased frameshifting phenotypes were isolated. A common 5.5-kb *Xba* I–*Xho* I DNA fragment, found sufficient for full complementing activity, was sequenced entirely on both DNA strands from a double-stranded plasmid template. The *IFS1* open reading frame was identified by testing nested 5' and 3' deletion mutants of the 5.5-kb fragment for complementation. The 5' end of the mRNA was mapped by RNase protection analysis to within a 100-nt region containing a single in-frame AUG.

Disruption of *IFS1*. A fragment of the *IFS1* open reading frame corresponding to nt 103–542 (with respect to the ATG start codon) was amplified by PCR and inserted into the *Not* I and *Bam*HI sites of the *URA3*-marked integrating vector pRS306 (15) to generate pIFSΔ. This construct was linearized with *Spe* I and used to transform diploid strain D6 (*IFS1/ifs1-1*) to generate diploids D7 and D8.

RESULTS

Yeast Frameshifting Reporters. To assay –1 frameshifting in yeast, the MMTV *gag-pro* frameshift region was placed upstream of the *S. cerevisiae CUP1* or the *Escherichia coli lacZ* gene (Fig. 1). The reporter genes are in the –1 frame relative to the initiation codon positioned upstream of the frameshift site and expressed only when a translating ribosome shifts into the –1 frame in response to the MMTV frameshift signals.

The *CUP1* gene encodes a metallothionein that allows cells to grow in the presence of otherwise lethal concentration of copper (11, 13), and differences in copper resistance as little as 2-fold can be detected. *lacZ* fusions in yeast provide very sensitive and relatively linear assays of activity over a 10,000-fold range (12).

Recapitulation of Frameshifting in Yeast. We first asked whether yeast ribosomes shift reading frames in response to retroviral frameshift signals. Cells carrying the MMTV *gag-pro* frameshift site (Fig. 2A; AAAAAAC) fused to either the *lacZ* or the *CUP1* reporter display levels of reporter activity above those of cells carrying a vector lacking a *CUP1* gene (Fig. 2A; no reporter), suggesting that –1 frameshifting is indeed occurring in yeast. To calculate frameshifting levels, the β-galactosidase activity from cells carrying either the in-frame *lacZ* or a *lacZ*[–] vector was set equal to 100% or 0% frameshifting, respectively. The enzymatic activity of cells carrying the *lacZ* gene fused to the MMTV *gag-pro* overlap sequence corresponded to a yeast frameshifting frequency of 1.7% at this retroviral frameshift site.

To ascertain that the observed expression of the copper resistance and β-galactosidase was due to ribosomal frameshifting, we determined whether expression required the exact bipartite frameshifting signal defined in mammalian transla-

FIG. 1. Yeast frameshifting reporters. The copper-resistance reporter contains the *S. cerevisiae CUP1* sequence on a high-copy 2μ vector. The mRNA is transcribed from the glyceraldehyde-3-phosphate dehydrogenase (*GPD*) promoter and spliced (ss = splice site) at the actin intron (13). The β-galactosidase reporter contains the *E. coli lacZ* sequence transcribed from the iso-1-cytochrome *c* (*CYC1*) promoter (12) on a CEN/ARS plasmid (for colony screens on filters) or on a 2μ vector (for quantitation of frameshift levels in whole cell extracts). The HA epitope tag was introduced adjacent to the start codon.

tion extracts (16). We first introduced mutations into the AAAAAAC shifty site of the MMTV *gag-pro* overlap (Fig. 2A). Substitution with the UUUUUUA shifty sequence, which had previously been reported to serve as an efficient shifty site in yeast (13), resulted in even higher frameshifting frequencies. In contrast, introduction of a scrambled heptanucleotide sequence (AAAUCGA) that does not fit the XXXYYYZ configuration (Fig. 2A; scrambled) abrogated β-galactosidase activity or copper resistance.

Mutational studies were also used to demonstrate the requirement for the downstream pseudoknot structure in yeast frameshifting (Fig. 2B). Mutations that disrupt base pairing in either stem of the MMTV *gag-pro* pseudoknot downstream of the UUUUUUA shifty site decrease frameshifting about 4-fold. Compensatory mutations that restored base pairing increased frameshifting to near wild type (S2 compensated) or above wild type (S1 compensated) levels. The observation that both stems must be present is consistent with a pseudoknot structure promoting efficient frameshifting. These studies were extended to the human immunodeficiency virus (HIV) *gag-pol* frameshift site, which displays a hairpin structure downstream of the shifty site (5). As in mammalian systems (6), efficient frameshifting at the HIV *gag-pol* overlap in yeast requires an intact stem-loop (data not shown). Thus, –1 frameshifting in yeast displays requirements similar to those in mammals, suggesting a conserved mechanism.

We assayed the levels of the translation products from the *lacZ* reporter by probing total yeast lysates with anti-HA antibodies (Fig. 2C). Proteins corresponding in size to the shifted (150-kDa) and the nonshifted (40-kDa) products are detected in cells carrying the *lacZ* frameshift construct (Fig. 2C, lanes 1–7) but not in cells lacking the reporter (Fig. 2C, lane 8). Frameshifting levels, calculated by measuring amounts of shifted protein in each lysate, are consistent with those calculated by using β-galactosidase assays.

A Screen for Increased Frameshifting (*ifs*) Mutants. A strain bearing the *CUP1* reporter with the UUUUUUA shifty site in the context of the MMTV *gag-pro* overlap (copper resistance = 0.2 mM) was grown on medium containing 0.4 mM copper to select for spontaneous mutants that increase

FIG. 2. (A) Frameshifting in yeast requires a shifty sequence. The MMTV heptanucleotide shifty sequence (AAAAAAC) was mutated and the resulting levels of frameshifting were measured by using the *lacZ* and the *CUP1* reporters. (B) Frameshifting in yeast requires a downstream pseudoknot. Mutations introduced into the pseudoknot sequence are indicated by the black box. Schematic representations of the resulting structures are drawn with the frameshifting levels, measured by the β -galactosidase assay, reported below each structure. (C) Translation products from the *lacZ* reporter. Western blot of whole cell extracts containing a *lacZ* reporter with the MMTV *gag-pro* frameshift site mutation indicated above each lane was probed with an anti-HA antibody. Arrowheads on the right indicate the shifted (*gag-pro-beta-gal*) and the nonshifted (*gag*) translation products. Migration of molecular mass standards (in kDa) is indicated on the left. Frameshifting levels of the same constructs determined by measuring β -galactosidase activity are also indicated.

frameshifting. Of the 21 copper-resistant isolates identified, 13 were recessive, suggesting that they are host mutants.

Isolates that fit the criteria for frameshift mutants fell into two complementation groups designated *ifs1* (5 isolates) and *ifs2* (1 isolate). Frameshifting was increased at both the UUUUUUA and the AAAAAAC shifty sequence with the wild-type MMTV pseudoknot downstream (Table 2). Increased expression of the reporter gene was due to increased frameshifting frequency in these mutants, since the introduction of an in-frame construct yields the same reporter levels in both the wild type and the *ifs* strains. The β -galactosidase assay demonstrates about 2-fold elevated levels of frameshifting in both *ifs1-1* and *ifs2-1*. The ratio of *gag-pro-beta-galactosidase* to the *gag* translation products was noted to be increased approximately 2- to 3-fold in *ifs1-1* cells by Western blotting (data not shown). Increased frameshifting also requires the MMTV pseudoknot. Introduction of a synthetic pseudoknot sequence, PK5 (17), known not to promote frameshifting in mammalian translational extracts (18), fails to promote frameshifting in either the wild type or the *ifs* mutant strains.

Neither *ifs1-1* nor *ifs2-1* strains display temperature or cold sensitivity or altered growth in high salt or nonfermentable carbon sources. Since we expected to find components involved in translation in a screen for frameshifting factors, we tested the sensitivity of *ifs* mutants to translational inhibitors. Drugs that target either the small subunit (paromomycin, G418, and hygromycin) or the large subunit (cycloheximide, anisomycin, and verrucarin A) of the ribosome were tested for their effects on the *ifs* strains. None of the compounds tested specifically affected growth of the mutants.

However, both *ifs1-1* and *ifs2-1* display nonsense suppression at concentrations of paromomycin that do not affect the translation fidelity of the wild-type strain (Fig. 3). Because these strains carry *ade2-101^{UAG}* and *lys2-801^{UAA}* nonsense mutations, they normally require adenine and lysine for growth. However, in the presence of paromomycin at 1 mg/ml, the *ifs* mutants grew in the absence of lysine (Fig. 3) or adenine (data not shown), indicating that they are expressing the *LYS2* or *ADE2* gene products by suppressing the nonsense mutations in those genes. Such general loss of translational fidelity was

Table 2. Frameshifting assays in *ifs* mutants

Strain	Copper resistance, mM			β -Galactosidase, %	
	UUUUUUA MMTV	AAAAAAC MMTV	In-frame* MMTV	UUUUUUA MMTV	UUUUUUA PK5
Wild type	0.2	0.05	0.4	12	0.8
<i>ifs1-1</i>	0.6	0.10	0.4	22	0.9
<i>ifs2-1</i>	0.6	0.10	0.4	24	1.0

Column headings give the frameshift site and the pseudoknot.

*The in-frame *CUP1* reporter used in these studies contained a splice site mutation and hence confers lower copper resistance than the in-frame *CUP1* reporter containing a wild-type intron (Fig. 24).

FIG. 3. *ifs* strains suppress nonsense mutations (e.g., *lys2-801^{UAA}*) in the presence of paromomycin. Yeast were grown on complete medium containing paromomycin and then replica-plated onto medium lacking lysine and either containing (+) or lacking (-) paromomycin.

not observed in the wild-type strain at the same paromomycin concentration. Similarly, in the presence of low concentrations of other translation-inhibiting drugs directed at the small subunit—i.e., G418 at 50 μ g/ml or hygromycin at 40 μ g/ml, nonsense suppression was observed only with the *ifs* mutants and not with the wild-type strain (data not shown). Thus the translational fidelity of *ifs* cells was hypersensitive only to drugs that target the small ribosomal subunit, responding like wild-type cells to large ribosomal subunit inhibitors.

Genetic analyses confirmed that the increased frameshifting and the paromomycin hypersensitivity phenotypes are linked. *IFS1/ifs1-1* diploids were sporulated and the resulting tetrads were tested for frameshifting efficiency and nonsense suppression in paromomycin. Analogous studies were carried out with *IFS2/ifs2-1* diploids. For each diploid, in 10 of 10 four-spore tetrads, the increased frameshifting and the drug hypersensitivity phenotypes cosegregated. Finally, in all growth conditions and frameshift assays, *ifs1-1*, *ifs2-1*, and the *ifs1-1 ifs2-1* double mutants behaved identically.

The *IFS1* Gene Encodes a Previously Undescribed Protein and a Targeted Mutation Recapitulates the *ifs1-1* Phenotype. The *IFS1* gene was isolated from a yeast genomic library (14). Deletion analyses and nucleotide sequencing defined an open reading frame of 1091 codons predicted to encode a protein of 128 kDa (Fig. 4). The predicted Ifs1p is not related to any previously reported in the sequence data bases. The carboxyl-terminal region of the protein is strongly acidic, with several uninterrupted stretches of glutamic and aspartic acid residues (Fig. 4, underlined letters).

To ask whether a defect in the cloned *IFS1* gene produced the *ifs1-1* phenotype, we disrupted one of the copies of the

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1  MYQQDGRKKE LEDLNTRAWN GEEVFLKSK KLDSSIKRWT GFIRKLRKCF
51  VKGESSLLK DLSEASLEKY LSEIIVTYTE CLNVLKNDK DVIAAVEIIS
101 GLGRFNGRF TSPLLGAFIQ AFENPSVDIE SERDELRIT RVKGNLRVFT
151 ELYLVGVFRT LDDIESKDAI PNFLOKQKGR KDPILLFSLR EILNYKFKLG
201 FTTIATAFI KKFAPLFRDD DNSWDDLIYD SKLKGALQSL FNFIDATFA
251 RATELHKVN KLQREHQRCK IRTGKLRDEY VEEDKLLPI FIRFKTSAT
301 LGEFFKLEIP ELEGASNDL KETASPMITN QILFPNQLW ENEDTRKFEY
351 ILPDISKTVE ESQSSKTEKD SNVSKNINL FTFDLEMACD KDIDDDLSNR
401 YWSSYLNDKA TNRILKFTM ETQDWSKLPV YSRFIATNSK YMPFVSEFI
451 NYLDNGRSQ LHSNKINVN IIFFSKMKP QLIPSFMIH KIRTLIMQ
501 VPPNVEILTV LLEHSGKFLN NKPEYKELME KQVQLIKDK MDRQLNMMK
551 SALENIITLL YPPSVKSLNV TVKTIPTPEQQ FYRILIRSEL SSLOFKHIV
601 LVKKAHDDV AIQKVLFSLF SKPKKISYQN IPLLTKVLGG LYSYRDFVI
651 RCIDQVLENI ERGLINDYG QMMHRISNVR YLTFIFNFM IKSDVLDTI
701 YHIRFGRHN NQPNPFYLYN SOPPDNYFRI QLVTTILLNI NRTPAAPTKK
751 CKLLLRFFY YTFIKQPLP KETEFKVSST FKRYENIFGN TKFERSNLV
801 ESASRLSEL KSLNAIKSD DRVKGSSASI HNGKESAVPI ESITEDDEDE
851 DDENDGDVL LKQEDDAIS TPNTSAPCK HQAKQDESD EDDDDDDDD
901 DDDDDDDDD GEGEDDDDE DDDDDDDDE EKKDSQDLK YGGDLADRD
951 TEMRMYYEY ERKLKDEER KAKKELERQF QRMQESIDA RKSEKVVASK
1001 IFVSKFVSF QKPLLRKSE EPSSSKTYE ELSKPKKIAF TFLTKSGKKT
1051 QSRILQLPTD VKFVSDVLEE EKKLTERNK IKRIVLRKSF D

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FIG. 4. Sequence of the *IFS1* protein. The protein sequence was derived by translating the DNA sequence of a 5.5-kb fragment sufficient for full complementing activity. Acidic residues between amino acid residues 845 and 980 are underlined.

gene in an *IFS1/ifs1-1* diploid stably integrating a *URA3* marker at the *IFS1* locus and creating a partial duplication which produces two truncated copies of the *IFS1* open reading frame—the first missing the carboxyl-terminal 910 codons and the second missing the upstream region and the first 34 codons. Approximately half the diploid transformants obtained with this construct displayed an *ifs1-1* phenotype, as would be expected for integration and disruption of the wild-type allele at the *IFS1* locus. The correct site of integration for the *ifsΔ::URA3/ifs1-1* diploids was confirmed by a PCR assay.

Two disrupted strains were sporulated and dissected (Fig. 5) and found to produce three- or four-spore tetrads at the same frequency as the parental *IFS1/ifs1-1* strain (Fig. 5B). The *URA3* marker segregated 2:2 in each of at least 20 tetrads with four viable spores (Fig. 5C). Thus, the *IFS1* gene does not encode an essential protein. Since all the uracil-requiring spores displayed the *ifs1-1* phenotypes of paromomycin sensitivity and increased frameshifting, the integrated construct is, indeed, linked to the *IFS1* locus. Eighty uracil-independent segregants also displayed increased frameshifting (data not shown) and paromomycin sensitivity (Fig. 5D and E). In summary, deletion of 82% of the *IFS1* open reading frame results in a viable strain displaying the *ifs1-1* phenotype.

DISCUSSION

We have demonstrated that yeast ribosomes shift into the -1 frame in response to signals defined in mammalian translation extracts (Fig. 2). A limited screen for spontaneous frameshifting mutants yielded two complementation groups, *ifs1-1* and *ifs2-1*, both displaying a 2-fold increase in frameshifting levels

FIG. 5. Analysis of tetrads from *ifsΔ::URA3/ifs1-1* diploids. (A) Schematic of strains on plates in B-E. The strain name is designated in boldface type and the relevant genotype is listed above. Strains D7-1a-d are the meiotic products of a representative tetrad from strain D7. (B-E) The strains described above were patched onto a YEPD (yeast extract/peptone/dextrose) plate and then replica-plated to YEPD (B), SD (synthetic/dextrose) - uracil (C), SD - lysine (D), and SD - lysine + paromomycin at 1 mg/ml (E). Plates B and C were incubated for 3 days at 30°C, and plates D and E were incubated for 5 days at 30°C.

(Table 2). It is unlikely that the increased readout with the frameshift reporters was due to enhanced gene copy number, expression, or product stability in *ifs* cells, since expression was elevated with both the *lacZ*, CEN/ARS vector (data not shown) and the *CUP1*, 2 μ vector (Table 2). RNase protection analysis of total RNA in wild-type and *ifs1-1* mutant cells revealed that the level of *IFS1* mRNA was equivalent in both strains. Moreover, the elevated frameshifting levels cannot reflect a general loss of translational fidelity in *ifs* mutants, since reporter activity levels were unaffected when expression did not involve -1 frameshifting in response to retroviral signals—e.g., with in-frame and the PK5 constructs. Strains known to be defective in translational fidelity—e.g., *suf12*, *suf13*, and *suf14* mutants (19, 20)—or with mutations in known translational RNA helicases—e.g., *SSL2* (21) or initiation factor eIF4A (22)—and in elongation factors (23) also failed to affect frameshifting levels in response to retroviral mRNA signals (data not shown). Thus, the genetic screen reported here identified cellular genes whose products, while most likely involved in translational elongation, also specifically affect -1 frameshifting.

Yeast mutants with altered -1 frameshifting efficiencies in L-A double-stranded RNA have been reported (24). These *mof* (maintenance of frame) mutants comprise eight genes, but their relationship to the *ifs* genes reported here has not yet been determined. Factors possibly involved in frameshifting include ribosomal components (either rRNAs or proteins) crucial for keeping tRNAs in frame, polysome-associated RNA helicases, new translational elongation factors (GT-Pases), or regulatory kinases.

Antibiotics that bind to the small ribosomal subunit increase miscoding in *ifs1-1* and *ifs2-1* strains (Fig. 3), suggesting that the *IFS* gene products may be components of the small ribosomal subunit or translation factors interacting with it. This is consistent with the anticipated characteristics of a frameshifting factor, since the codon–anticodon interaction occurs in the small subunit and is somehow disrupted during -1 frameshifting.

Cloning and sequencing of the *IFS1* gene reveals that the gene product is a previously undescribed protein with no identifiable functional domains. Southern blots using a probe containing the entire *IFS1* open reading frame suggest that this gene exists in a single copy in the yeast haploid genome (data not shown). *IFS1* displays none of the properties of ribosomal protein genes, lacking any RPG or HOMOL1 sequences (25). The protein displays no homology to known yeast ribosomal proteins (26, 27), translation factors (28), or omnipotent suppressor gene products (20, 29–32). The clusters of acidic residues in the carboxyl-terminal region of the *IFS1* protein (Fig. 4) are reminiscent of the acidic domains in nucleolin, a highly conserved protein involved in ribosome biogenesis (33, 34). However, the *IFS1*-encoded protein does not display RNA-binding motifs, a glycine/arginine-rich region (the GR domain), or a nuclear localization signal, all of which are found in nucleolin. The function of the nucleolin acidic domain is unknown. Haploid cells lacking 82% of the *IFS1* open reading frame are viable and phenotypically identical to *ifs1-1* mutants. Therefore, *ifs1-1* strains likely contain an *IFS1* null allele, but we have not determined the sequence of the spontaneously generated mutant alleles.

The small-scale genetic screen reported here demonstrates that frameshift reporters that require -1 ribosomal frameshifting can be used to look for mutations in cellular genes that affect frameshifting efficiency even subtly. Components involved in -1 frameshifting represent new targets for antiretroviral drugs that would be aimed at disrupting a very tightly regulated step in the viral life cycle.

Note. During publication of this manuscript a gene involved in nonsense-mediated mRNA decay, called *UPF2* (35) or *NMD2* (ref. 36; GenBank accession no. U14974), was found to be identical to *IFS1*. Sequence comparison revealed that we had incorrectly identified the *IFS1* initiation codon. The corrected sequence of *Ifs1p* has MD instead of MYQQ on the amino terminus (Fig. 4).

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