Curr Genet (2015) 61:165–173
DOI 10.1007/s00294-014-0465-7

RESEARCH ARTICLE

Nonsense codon suppression in fission yeast due to mutations of tRNA$^{Ser.11}$ and translation release factor Sup35 (eRF3)

Reine U. Protacio · Aaron J. Storey · Mari K. Davidson · Wayne P. Wahls

Received: 10 October 2014 / Revised: 1 December 2014 / Accepted: 3 December 2014 / Published online: 18 December 2014

Abstract In the fission yeast *Schizosaccharomyces pombe*, *sup9* mutations can suppress the termination of translation at nonsense (stop) codons. We localized *sup9* physically to the *spctrnaser.11* locus and confirmed that one allele (*sup9-UGA*) alters the anticodon of a serine tRNA. We also found that another purported allele is not allelic. Instead, strains with that suppressor (renamed *sup35-F592S*) have a single base pair substitution (T1775C) that introduces an amino acid substitution in the Sup35 protein (Sup35-F592S). Reduced functionality of Sup35 (eRF3), the ubiquitous guanine nucleotide-responsive translation release factor of eukaryotes, increases read-through of stop codons. Tetrad dissection revealed that suppression is tightly linked to (inseparable from) the *sup35-F592S* mutation and that there are no additional extragenic modifiers. The Mendelian inheritance indicates that the Sup35-F592S protein does not adopt an infectious amyloid state ([*PSI*$^+$] prion) to affect suppression, consistent with recent evidence that fission yeast Sup35 does not form prions. We also report that *sup9-UGA* and *sup35-F592S* exhibit different strengths of suppression for opal stop codons of *ade6-M26* and *ade6-M375*. We discuss possible mechanisms for the variation in suppressibility exhibited by the two alleles.

Communicated by C. S. Hoffman.

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Keywords Genetics · Molecular biology · Translation · Phosphoribosylaminoimidazole carboxylase

Introduction

The *ade6* gene of fission yeast encodes a phosphoribosylaminoimidazole carboxylase required for the de novo synthesis of purines (Szankasi et al. 1988). Cells with mutations in *ade6* are auxotrophic for adenine, but grow as efficiently as wild type when adenine is added to the media (Gutz et al. 1974). On media with limiting amounts of adenine, wild-type cells form white colonies, whereas *ade6* mutants accumulate a red pigment (Gutz et al. 1974). Early work based on these properties identified nearly 400 different alleles of *ade6* and about 46,000 additional alleles were created in the following five decades [e.g. (Gutz 1971; Grimm et al. 1994; Steiner et al. 2009)]. These have provided powerful tools for analyses of many broadly conserved biological processes such as transcription; RNA processing and stability; chromatin remodeling; genetic recombination; DNA damage repair; and genome stability [e.g, (Hottinger and Leupold 1981; Bernardi et al. 1991; Allshire et al. 1994; Nimmo et al. 1994; Szankasi and Smith 1995; Kon et al. 1997; Mizuno et al. 1997; Kon et al. 1998; Mansour et al. 2001; Huang et al. 2005; Niwa et al. 2006; Hirota et al. 2008; Leem et al. 2008; Osman and Whitby 2009; Zhao et al. 2009; Chino et al. 2010; Gao et al. 2013)].

The *ade6-M375* and *ade6-M26* alleles are of particular interest. They each have a single G-to-T substitution that converts a glycine codon (GGA) to an opal stop codon (UGA), with *M375* and *M26* affecting adjacent codons (Fig. 1b) (Szankasi et al. 1988). Serendipitously, the *M26* mutation also creates a cyclic AMP responsive element

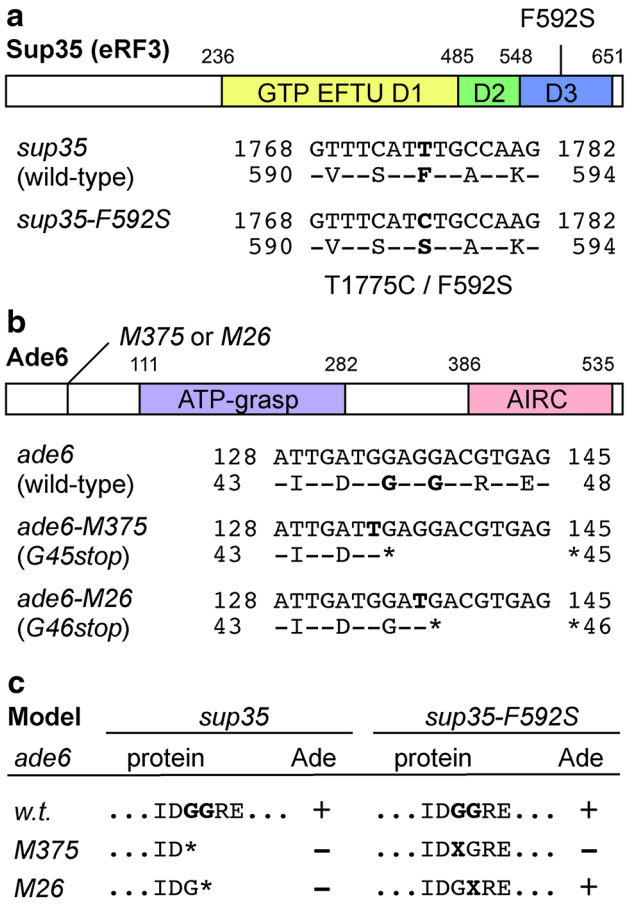

Fig. 1 Graphical summary and model. **a** Diagram of translation release factor Sup35 (eRF3) and its GTP elongation factor Tu domains (D1–D3). We identified an allele of *sup35* that has a single base pair substitution (numbered from start codon in cDNA) and encodes a protein with a single amino acid substitution. **b** Reporter alleles of *ade6* (*M375* and *M26*) each have a stop codon (*) in place of a glycine codon. **c** Efficient termination of translation by Sup35 at the internal stop codons abolishes production of Ade6 protein. Inefficient termination by Sup35-F592S causes read-through of each stop codon (insertion of a random amino acid, X). The phenotypes of identical stop codons at adjacent positions are differentially suppressed, revealing that the local context of the stop codon affects the efficiency of suppression

(*CRE*)-like DNA site (5′-ATGACGT-3′) (Schuchert et al. 1991) that is bound avidly by Atf1-Pcr1 (Mts1-Mts2) (Wahls and Smith 1994), which is a heterodimeric basic leucine zipper (bZIP) transcription factor of the ATF/CREB family (Wahls and Smith 1994; Kanoh et al. 1996; Kon et al. 1997). The *M375* allele does not have a binding site for Atf1-Pcr1 heterodimer and thus serves as a nucleotide substitution type-matched, codon type-matched negative control. Such controls supported discoveries that the Atf1-Pcr1-*M26* protein-DNA complex directly regulates multiple biological processes including chromatin remodeling (Davidson et al. 2004; Jia et al. 2004; Kim et al. 2004; Yamada et al. 2004), transcription of stress-responsive genes (Hirota et al. 2003; Davidson et al. 2004; Eshaghi et al. 2010), and the positioning of meiotic recombination at hotspots (Kon et al. 1997; Gao et al. 2008; Wahls and Davidson 2010).

Phenotypes caused by stop codons can be suppressed by mutations in tRNA genes that change the sequence of the anticodon loop (Rafalski et al. 1979; Willis et al. 1984, 1986). For example, a single nucleotide substitution in the anticodon of a serine tRNA (5′-UGA-3′ to 5′-UCA-3′) allows it to bind to a UGA (opal) stop codon and insert a serine into the nascent polypeptide, thus allowing the ribosome to read through the stop codon (Willis et al. 1984). Such suppressors have provided useful tools to analyze the biological functions of specific nonsense alleles, such as *ade6-M26* (Ponticelli et al. 1988).

In this study we used the opal stop codons of *ade6-M26* and its control allele, *ade6-M375*, as tools to elucidate the molecular basis of two nonsense codon suppressors. There are two key findings. First, we localized the *sup9* suppressor gene physically to the *spctrnaser.11* locus and confirmed that the mechanism of suppression involves mutation of the anticodon of a serine tRNA (Willis et al. 1984). Second, we localized another suppressor gene to the *sup35* locus and we determined that the mechanism of suppression involves an amino acid substitution in a GTP elongation factor Tu domain of the ubiquitous eukaryotic translation release factor 3 (eRF3). And as has been reported for translational read-through suppression in other organisms (Dalphin et al. 1997; Poole et al. 1998; Namy et al. 2001), we show that the local context of the stop codon also affects the efficiency of suppression in fission yeast.

Materials and methods

Yeast strains and culture

The genotypes of strains used in this study are provided in Table 1. Relevant sequences of the *ade6* (Szankasi et al. 1988) and *sup35* alleles are provided in the main text. The *ade6-D1* allele lacks the *ade6* ORF, allowing us to use *ade6* as a selectable marker in plasmids and eliminating potential effects of chromosomal alleles on selection. Culture media, culture conditions, genetic crosses, and scoring of genetic markers were as described (Gutz et al. 1974; Forsburg and Rhind 2006; Gao et al. 2008; Kan et al. 2011). For rich media we used yeast extract liquid (YEL) or agar (YEA); for minimal media we used nitrogen base liquid (NBL) or agar (NBA) supplemented as necessary with amino acids and nucleobases at 100 μg/ml; and for mating we used sporulation agar (SPA). For initial

Table 1 Genotypes of *S. pombe* strains used in this study

Strain	Genotype
WSP 0003	*h*$^-$ *ade6-M26 sup35-F592S*
WSP 0006	*h*$^+$ *ade6-M375*
WSP 0578	*h*$^+$ *ade6-M375 his3-D1 ura4-D18 leu1-32*
WSP 4096	*h*$^-$ *ade6-M26 sup9-UGA*
WSP 5131	*h*$^+$ *ade6-D1 sup35-F592S*
WSP 5755	*h*$^+$ *ade6-D1 sup35-F592S pat1-114 ura4-D18 leu1D::prALacI*
WSP 5795	*h*$^-$ *ade6-M375 ura4-D18*
WSP 5800	*h*$^-$ *ade6-M375 ura4-D18 sup9-UGA*
WSP 5806	*h*$^-$ *ade6-M26 ura4-D18 sup9-UGA*
WSP 5811	*h*$^-$ *ade6-M26 ura4-D18*
WSP 5814	*h*$^-$ *ade6-M26*
WSP 5816	*h*$^-$ *ade6-M26 sup35-F592S*
WSP 5818	*h*$^-$ *ade6-M375*
WSP 5820	*h*$^-$ *ade6-M375 sup35-F592S*

strain constructions and experiments prior to identifying the gene responsible for the sup† phenotype, we used test crosses and tetrad spore colony phenotyping (ability to suppress adenine auxotrophy caused by nonsense codons in *ade6*) to follow the markers. Once we identified the *sup†* mutation (*sup35-T1775C* of cDNA sequence from start codon), we used PCR and DNA sequencing to genotype alleles at the *sup35* locus. Alleles of *spctrnaser.11* were also analyzed by PCR and DNA sequencing.

Tetrad dissection

Procedures for mating, isolation of conjugants and tetrad dissection using a Singer MSM300 microdissection apparatus were according to instructions of the manufacturer (Singer Instrument Co. Ltd., Somerset, UK). Spores from each tetrad were plated in grids on YEA and incubated for four to five days at 32 °C.

Molecular biology

Standard methods were used for PCR, for constructing plasmids, and for DNA sequencing. Oligonucleotide primers were designed using tools of, and were synthesized by, Integrated DNA Technologies (Coralville, Iowa). Primer sequences are available upon request. Relevant features of all plasmids are a fission yeast origin of replication and an allele of *ade6* (wild-type p*ade6*, p*M375* and p*M26*). A subset of the plasmids contain in addition the *ura4* gene (p*ade6-ura4*, p*M375-ura4* and p*M26-ura4*). Cells were transformed using the LiOAc procedure (Ito et al. 1983), transformants were plated on NBA lacking adenine or uracil, and plates were incubated at 32 °C for four to six days.

Results

The sup9-e (sup9-UGA) phenotype is due to a mutation of *spctrnaser.11* that alters the anticodon of tRNA$^{Ser.11}$

We obtained, from different sources, strains that harbored alleles of *sup9*, a well-characterized opal stop codon suppressor (Willis et al. 1984, 1986). Genetic and haploidization mapping experiments placed *sup9* close to (5.8 cM away from) *arg1* on Chromosome III (Kohli et al. 1977).

The first allele, designated *sup9-UGA* or *sup9-e*, was cloned by plasmid transformation and found to harbor a mutation in a gene encoding a serine tRNA located adjacent to a gene encoding a methionine tRNA (Willis et al. 1984). Our inspection of the genome sequence (Wood et al. 2002) revealed that, of the seven genes encoding a tRNA^{Ser} that are located on Chromosome III, only *spctrnaser.11* is located next to a tRNA^{Met} gene (*spctrnamet.07*). This physical location is consistent with the reported genetic map location (Kohli et al. 1977). We therefore amplified DNA from the *spctrnaser.11* locus and identified a single base pair substitution that alters the anticodon of tRNA$^{Ser.11}$ from 5′-UGA-3′ to 5′-UCA-3′. Our sequence of the mutated tRNA$^{Ser.11}$ gene matches that reported for the serine tRNA encoded by the plasmid clone (Willis et al. 1984), thus localizing the original mutation to the *spctrnaser.11* locus. This mutation, which provides a molecular basis for recognition of UGA (opal) stop codons and for suppression, was not observed in wild-type strains.

The second strain, originally designated as *sup9* (Ponticelli et al. 1988), was reported to harbor an opal nonsense suppressor. However, when we sequenced the *spctrnaser.11* (*sup9*) locus of this strain we did not detect any mutations within the *spctrnaser.11* gene. Thus, nonsense codon suppression in this second strain is not due to changes in the tRNA$^{Ser.11}$. We therefore designated this strain temporarily as genotype *sup†* and sought to determine the molecular basis for its sup† phenotype.

Strains with the sup† phenotype express a mutated translation release factor, Sup35 (eRF3)

We found that sup† strains do not harbor any mutations in the *spctrnaser.11* locus. However, our genetic mapping data (some of which are presented below) indicated that the *sup†* marker also resides in that region of chromosome III. Inspection of the genome sequence (Wood et al. 2002) revealed six candidate genes in that region, including five encoding tRNAs (tRNA$^{Lys.12}$, tRNA$^{Ser.11}$, tRNA$^{Met.07}$, tRNA$^{Asn.06}$ and tRNA$^{Ser.12}$). Whole-genome sequencing of the sup† strain that we conducted for another project (Gao et al. 2013) revealed no mutations in any of the tRNA candidate genes.

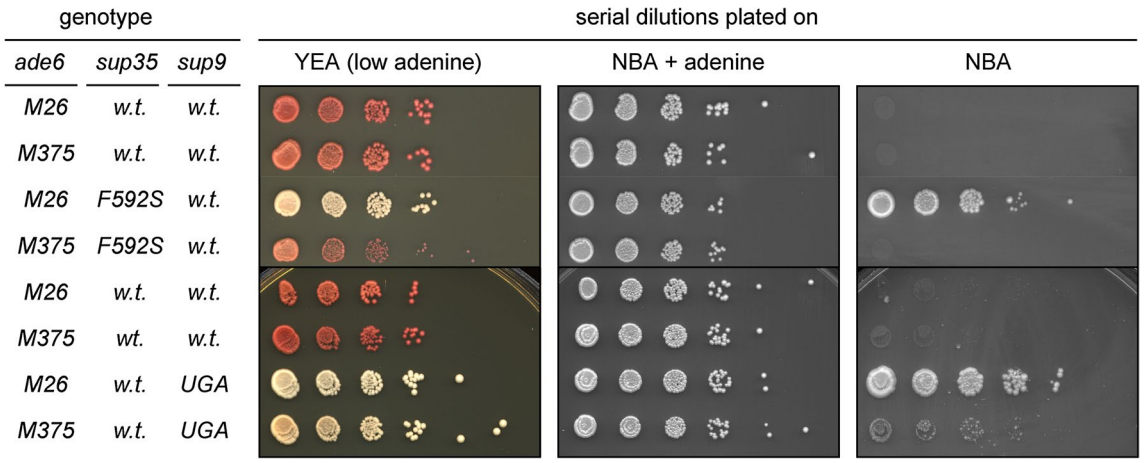

Fig. 2 Adenine auxotrophy caused by stop codons of *ade6-M375* and *ade6-M26* is differentially suppressed by both *sup35-F592S* and *sup9-UGA*. Serial dilutions of cells taken from rich liquid medium were plated on the indicated solid media. The *ade6* mutants produce a *red* pigment when adenine is limiting (YEA) and fail to grow without adenine (NBA). Efficient suppression confers both adenine prototrophy (NBA) and *white colony color* (YEA); whereas weak suppression confers weak prototrophy and nearly *white colony color*

However, whole-genome sequencing detected a mutation in the *sup35* gene of strains with the sup† phenotype. The encoded protein, Sup35, is also known as eRF3 (eukaryotic translation release factor 3). PCR amplification and conventional sequencing of the locus from strains with the sup† phenotype confirmed the presence of the mutation first identified by whole genome sequencing. The mutation is a single base pair substitution (T1775C, numbered in the cDNA from the start codon) that results in a phenylalanine to serine substitution at position 592 of the protein (Fig. 1a). We therefore refer to this allele as *sup35-F592S*.

To determine whether suppression is due to this mutation, we set up heterozygous *sup35-F592S* crosses, prepared genomic DNA from 16 individual spore colonies, used PCR to amplify the *sup35* locus, and sequenced the PCR products. In each case, sequences from spore colonies without suppression were wild-type at *sup35* and sequences from spore colonies exhibiting suppression contained the T1775C substitution within the *sup35* gene.

Together, these findings provided strong evidence that the Sup35-F592S protein is responsible for nonsense codon suppression of sup† strains. The Sup35 (eRF3) protein is the universal eukaryotic translation release factor that ensures the fidelity of termination at stop codons recognized by eRF1 (Kisselev et al. 2003; Salas-Marco and Bedwell 2004; Alkalaeva et al. 2006). The suppression phenotype exhibited by the mutant strain is therefore a logical consequence of the amino acid substitution, which affects a functional domain involved in GTP-binding and translation termination (Fig. 1, for additional details see "Discussion").

The degree of phenotype suppression by Sup35-F592S varies for opal codons at different locations in the same gene

The mutation type-matched, codon type-matched *M375* and *M26* alleles of *ade6* harbor UGA stop codons at amino acid positions 45 and 46, respectively (Fig. 1b). To determine the suppressibility of these alleles, we constructed strains that harbored *sup35-F592S* together with *ade6-M375* or *ade6-M26*. We then plated serial dilutions of strains on rich media with limiting amounts of adenine, on minimal media supplemented with adenine, and on minimal media lacking adenine (Fig. 2). As long as adenine was present, all strains plated efficiently. As expected, strains of genotype *ade6-M375* and *ade6-M26* failed to plate in the absence of adenine. Interestingly, while *sup35-F592S ade6-M26* strains plated efficiently in the absence of adenine (efficient suppression), the *sup35-F592S ade6-M375* strains did not.

The differential suppression of phenotypes for *ade6* alleles was also apparent using a colorimetric readout. On media with limiting adenine, the *ade6-M375* and *ade6-M26* mutants each accumulated a red pigment (Fig. 2) due to defects in the adenine biosynthesis pathway (Gutz et al. 1974). Cells of genotype *sup35-F592S ade6-M26* formed white colonies (as do wild-type cells), indicating suppression of the biosynthetic defect caused by the *ade6-M26* mutation. However, cells of genotype *sup35-F592S ade6-M375* remained red (Fig. 2). We conclude that while adenine auxotrophy caused by the opal stop codon of *ade6-M26* is efficiently suppressed by *sup35-F592S*, that of *ade6-M375* is at best weakly suppressed.

Differential suppression also occurs for the sup9-UGA (tRNA$^{Ser.11}$) suppressor

To determine whether the differential suppression is specific to Sup35-F592, we also constructed strains combining *sup9-UGA* with the reporter alleles *ade6-M26* and *ade6-M375*. Similar results were obtained with this tRNA suppressor as with the Sup35 suppressor (Fig. 2). Cells of genotype *sup9-UGA ade6-M26* were able to grow efficiently on media without adenine, whereas *sup9-UGA ade6-M375* cells were not. However, the *sup9-UGA ade6-M375* cells plated slightly more efficiently than the *sup35-F592S ade6-M375* cells, indicating partial (albeit very weak) suppression of *ade6-M375* by *sup9-UGA*. Plating of strains on media with limiting adenine, which provides a colorimetric readout of suppression, provided further support of this conclusion. While strains of genotype *sup9-UGA ade6-M375* were severely hypomorphic for the biosynthesis of adenine (inefficient plating without adenine), the low level of suppression was sufficient to render an almost white colony color when plated on media with limiting adenine (Fig. 2).

Differential suppression is not due to synthetic toxicity

To further characterize the differential suppression detailed above, a different assay was used. A strain harboring a deletion of the *ade6* gene and the *sup35-F592S* mutation was transformed with plasmids bearing wild-type *ade6* (p*ade6*), *ade6-M26* (p*M26*) or *ade6-M375* (p*M375*). The resulting transformants were then plated on media lacking adenine (Fig. 3a). Transformations with p*ade6* provided a positive control and, as expected, yielded Ade$^+$ colonies. Transformations with plasmid-born *ade6-M26* always produced Ade$^+$ colonies, whereas transformations with plasmid-born *ade6-M375* never produced Ade$^+$ colonies. We resequenced the plasmids and confirmed that their *ade6* DNA sequences contain only the *M26* or *M375* mutations, excluding the possibility that the differential plating efficiencies were due to other mutations in or flanking *ade6*. Again, *sup35-F592S* could efficiently suppress the phenotype of *ade6-M26* but not that of *ade6-M375*.

For the plating experiments with endogenous alleles (previous section) and for the plasmid transformation experiments, it remained possible that some feature of the genetic background in our *sup35-F592S* strains was synthetically toxic when coupled with *ade6-M375*. To test this possibility, we transformed *sup35-F592S ade6-D1 ura4-D18* cells with plasmids bearing the different *ade6* alleles together with a *ura4* marker (p*ade6-ura4*, p*M26-ura4* and p*M375-ura4*). We then selected for transformants using media lacking uracil but containing adenine (Fig. 3a). All three plasmids were capable of producing Ura$^+$ transformants, eliminating the possibilities that plasmids bearing *ade6-M375* fail to enter cells or that the *ade6-M375* allele is toxic in the presence of *sup35-F592S*. When the Ura$^+$ transformants were subsequently transferred to media lacking adenine, the cells harboring plasmids with *ade6-M26* plated efficiently while cells harboring plasmids with *ade6-M375* plated inefficiently (Fig. 3b). This recapitulated the results obtained with *ade6* alleles at the endogenous chromosomal locus (Fig. 2).

Our finding that phenotypes caused by identical stop codons at different (adjacent) positions in the same reporter gene are differentially suppressed by both *sup9-UGA* and *sup35-F592S* was intriguing, but not unprecedented (see "Discussion"). More importantly, understanding the nature of the reporter allele-specific differences allowed us to make sense of suppression phenotypes that seemed, initially, to be complex.

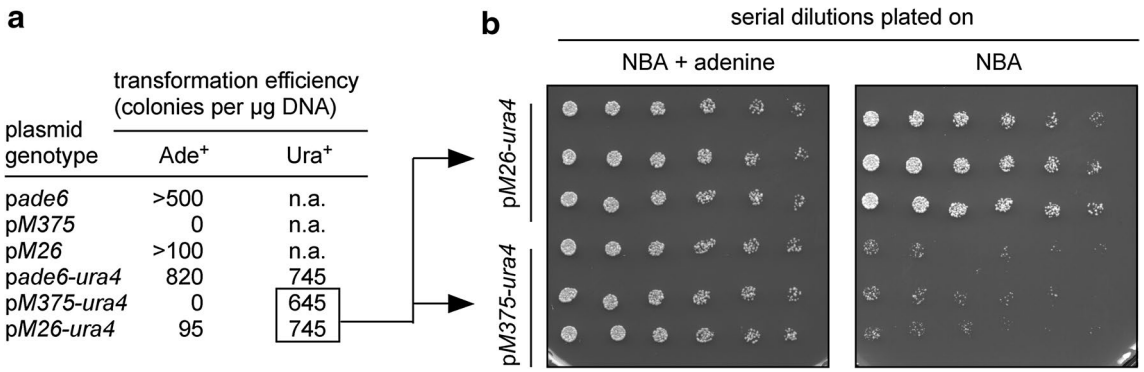

Fig. 3 Test for synthetic toxicity between *sup35-F592S* and *ade6-M375*. **a** Strains of genotype *sup35-F592S ade6-D1 ura4-D18* were transformed with plasmids p*ade6* (wild-type), p*M375*, p*M26*, p*ade6-ura4*, p*M375-ura4* and p*M26-ura4*. Data are transformation efficiencies when plated on media lacking adenine (Ade$^+$ colonies) or on media lacking uracil (Ura$^+$ colonies). *N.a.* not applicable. **b** Serial dilutions of Ura$^+$ transformants harboring p*M375-ura4* and p*M26-ura4* were plated on media without or with adenine. Note that while cells harboring both *sup35-F592S* and p*M375-ura4* are viable, they plate inefficiently in the absence of adenine

Nonsense codon suppression is due exclusively to the single amino acid substitution in the Sup35 protein (Sup35-F592S)

Sequence analyses of strains with and without the sup† phenotype indicated, but did not demonstrate at high resolution, that *sup35-F592S* is responsible for suppression (above). In addition, the differential suppressibility of reporter alleles suggested that additional loci might be involved. For example, extragenic modifiers in addition to *sup35-F592S* might affect the efficiencies of suppression for *ade6-M26* and *ade6-M375*. To test this possibility and to strengthen our linkage assignments, we crossed an *ade6-M26 sup35-F592S* strain (efficient suppression) to an *ade6-M375* strain (no suppressor). We then determined the segregation patterns of phenotypes and of genotypes at the *ade6* and *sup35* loci (Fig. 4).

We dissected tetrads and plated their spores on rich media with limiting amounts of adenine (YEA), which provides a colorimetric readout for phenotypes of *ade6* alleles (Gutz et al. 1974). All spores must inherit an *ade6* allele (*ade6-M375* or *ade6-M26*). Spores without suppression produce red colonies and those with suppression produce white colonies (Fig. 2). The phenotypes of 48 tetrads with four viable spores fell into three classes (Fig. 4) whose genotypes were determined by analyses of alleles at *ade6* and *sup35*:

- Parental ditype (PD, $n = 19$): 2 red (*ade6-M375*) and 2 white (*sup35-F592S ade6-M26*) colonies.
- Tetratype (TT, $n = 26$): 2 red (*ade6-M26* or *ade6-M375*), 1 small red (*sup35-F592S ade6-M375*), and 1 white (*sup35-F592S ade6-M26*) colonies.
- Non-parental ditype (NPD, $n = 3$): 2 red (*ade6-M26*) and 2 small red (*sup35-F592S ade6-M375*) colonies.

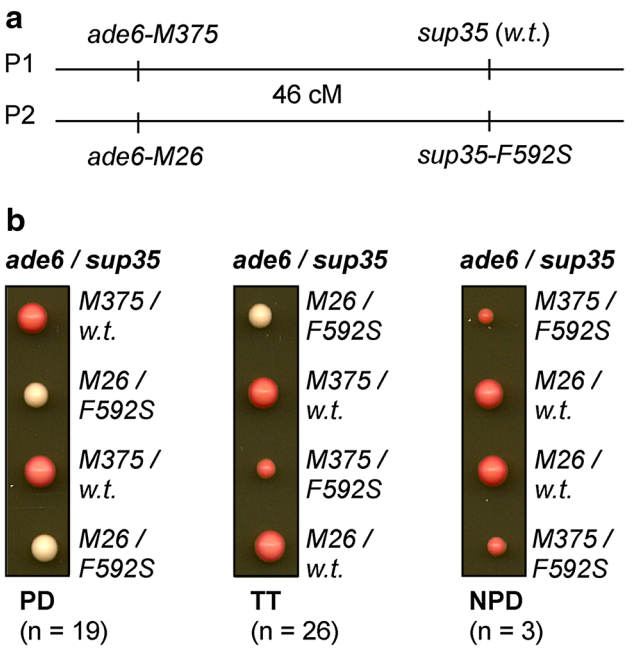

Fig. 4 The Sup35-F592S protein is the suppressor. **a** Diagram of genetic cross and summary of linkage mapping. **b** Tetrads were dissected and spores were plated on media with limiting adenine to provide a colorimetric readout (*white*, efficient suppression; *red*, inefficient or no suppression). Phenotypes of spores from representative parental ditype (PD), tetratype (TT) and non-parental ditype (NPD) tetrads are shown; totals of each class are below images. Genotypes inferred phenotypically were confirmed by additional phenotyping (e.g, Fig 2) and by direct analyses of alleles at the *ade6* and *sup35* loci. The tetrad data demonstrate inheritance of traits controlled by alleles of only two, weakly linked loci

These data support four important conclusions. First, the suppression and adenine phenotypes (and corresponding genotypes) are each inherited in a Mendelian fashion (2:2). Second, application of genetic mapping functions to the segregation data revealed that the *sup35* locus is weakly linked (46 cM) to the *ade6* locus. This is in good agreement with existing genetic and physical maps (Wood et al. 2012). Third, at the resolution of our tetrad (and all other) analyses the suppressor phenotype is inseparable from the *sup35-F592S* mutation, indicating that the Sup35-F592S protein is responsible for suppression. Fourth, all phenotypes were attributable unambiguously to the physical segregation of alleles at only two loci (*ade6* and *sup35*), demonstrating that there are no additional, unlinked modifiers.

Target allele-specific effects of on adenine auxotrophy/prototrophy map exclusively to *ade6*

The tetrad dissection data also revealed that the differential suppressibility of adenine auxotrophy for the *ade6-M375* and *ade6-M26* alleles (Figs. 2, 3) is intrinsic to (inseparable genetically from) the alleles themselves (Fig. 4). While spores of genotypes *sup35-F592S ade6-M375* and *sup35-F592S ade6-M26* each formed colonies when plated on media containing adenine, only the *sup35-F592S ade6-M26* cells continued to grow when subsequently patched to media lacking adenine. Similarly, when we plated random spore populations directly on dropout media, all 50 of the Ade^+ spore colonies tested were of genotype *sup35-F592S ade6-M26*. This occurred even though spores of genotype *sup35-F592S ade6-M375* were generated and were proficient for germination and colony formation when plated on nonselective media.

Discussion

We report that the *sup9-e/sup9-UGA* nonsense codon suppressor, known to involve mutation of the anticodon of a

serine tRNA (Willis et al. 1984), is due to mutation of the *spctrnaser.11* locus. Our findings on the nature and mechanisms of this suppressor are consistent with those described previously (Willis et al. 1984). Mutation of the $tRNA^{Ser.11}$ anticodon from 5′-UGA-3′ to 5′-UCA-3′ allows the tRNA to recognize a UGA (opal) stop codon and add a serine to the growing polypeptide chain.

We also report that a suppression phenotype attributed previously to an allele of *sup9* (Ponticelli et al. 1988) is actually due to a single amino acid substitution in the Sup35 protein, resulting from a single base pair substitution in the coding region of the *sup35* gene (Fig. 1). Sup35 (eRF3) is a broadly conserved subunit of the eukaryotic translation termination complex that catalyzes the release of the nascent polypeptide chain when the ribosome encounters a stop codon (Kisselev et al. 2003; Salas-Marco and Bedwell 2004; Alkalaeva et al. 2006). Our finding that Sup35-F592S is a nonsense codon suppressor is consistent with evidence that mutations in, or changes in the dosage of, Sup35 in other species can confer suppression [e.g, (Kushnirov et al. 1990; Wakem and Sherman 1990; Gagny and Silar 1998; Chao et al. 2003; Janzen and Geballe 2004; Liu et al. 2014)]. When the functions of Sup35 are compromised, the stop codons are no longer utilized efficiently and the ribosome can insert an amino acid into the nascent polypeptide. In the case of nonsense alleles within coding regions (e.g, *ade6-M375*), the "wrong" amino acid is almost always inserted (Fig. 1c) and then translation continues on to the normal stop codon, at which point it can either terminate or read through. The net effect is the production of some full-length protein with amino acid substitutions corresponding to the position of the nonsense allele.

The Sup35 protein of some organisms is capable of a conformational change and self-aggregation to form prions, an infectious amyloid state [extensive body of work reviewed by (Serio and Lindquist 1999; Uptain and Lindquist 2002; Tuite and Cox 2007; Prusiner 2013)]. The Sup35 aggregates are less soluble and less functional, and thereby cause an increased rate of read-through at stop codons (increased nonsense suppression). Notably, Sup35 proteins with the prion conformation are capable of triggering other, regularly folded Sup35 molecules to adopt the amyloid state, so the prions are "infectious proteins" that exhibit the remarkable property of non-Mendelian inheritance. This raises the intriguing possibility that the fission yeast Sup35-F592S protein adopts a prion-like state that compromises its normal functions in translation termination and thereby contributes to elevated suppression of nonsense codons.

However, we think that Sup35-F592S is unlikely to form prions for three reasons. First, the amino acid substitution (Fig. 1a) is outside of the domains necessary and sufficient for prion formation by Sup35 proteins (Liu et al. 2002; Bradley and Liebman 2004; Chang et al. 2008; Bateman and Wickner 2012). Second, we did not detect any evidence for an infectious state: crossing of *sup35* and *sup35-F592S* strains never produced any progeny of genotype *sup35* that displayed elevated suppression (Figs. 2, 4). Third, it was reported recently that the ability of Sup35 proteins to form prions varies widely among species and does not occur for the fission yeast protein (Edskes et al. 2014). Given that the *sup35* gene is essential (Kim et al. 2010), we infer that the Sup35-F592S protein is expressed and is hypomorphic for its functions in translation termination. In support of this idea, the amino acid substitution F592S affects a conservative residue within the GTP elongation factor Tu domain 3 (Fig. 1a).

In the course of our studies, and complicating our efforts to identify the gene responsible for the sup† phenotype, we discovered reporter allele-specific effects. Phenotypically, the opal nonsense codon of *ade6-M26* is efficiently suppressed by *sup35-F592S* and forms a basis for positive selection (prototrophy for adenine), but that of *ade6-M375* is at best weakly suppressed (Figs. 2, 3, 4). The *sup35-F592S ade6-M375* genotype is so severely hypomorphic that it cannot be used for the selection of spore colonies from genetic crosses or for the maintenance of plasmids bearing *ade6-M375*. Genetic mapping revealed that the differential suppressibility of adenine auxotrophy is intrinsic to the *ade6* alleles themselves (Fig. 4). Correspondingly, the differential suppression is neither attributable to nor unique to the Sup35 suppressor because it also occurs for a tRNA suppressor (Fig. 2).

The fact that identical stop codons at adjacent positions are differentially suppressed, and that this effect is intrinsic to the alleles themselves, revealed that the position or context in which the stop codon resides affects the efficiency of suppression. This property is not unique to fission yeast and it is known that the efficiency of translation termination is influenced by sequences surrounding stop codons [(Dalphin et al. 1997; Jacobs et al. 2009) and refs. therein]. The type and position of amino acid substitution can also affect the phenotype. The best-studied model systems, such as *E. coli* and *S. cerevisiae*, have revealed complex determinants extending at least six base pairs downstream of the stop codon itself [e.g, (Poole et al. 1998; Namy et al. 2001; Poole et al. 2003; Hatin et al. 2009)]. Our findings are consistent with such mechanisms operating in *S. pombe*, too.

In conclusion, we report that two different nonsense codon suppressors of fission yeast are due to mutations in the *spctrnaser.11* and *sup35* genes. The mechanisms of suppression involve, respectively, alteration of a serine tRNA anticodon such that it recognizes opal stop codons, and increased read-through of stop codons by attenuating the activity of a ubiquitous eukaryotic translation release factor. Secondarily, we report that local context-dependent

suppression discovered in other organisms also applies to fission yeast and we provide additional evidence that fission yeast Sup35 does not form prions.

Acknowledgments We thank Jürg Kohli and Gerald Smith for providing yeast strains and Jun Gao for assistance with plasmid constructions. This work was supported by a grant from the National Institutes of Health (GM081766) and bridging funds from the University of Arkansas for Medical Sciences College of Medicine. Core facilities were supported in part by a National Institutes of Health Translational Research Institute grant (TR000039).

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