Identification of the *Neurospora crassa* mutation *un-10* as a point mutation in a gene encoding eukaryotic translation initiation factor 3, subunit B.

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The *Neurospora crassa* temperature-sensitive mutant known as *un-10* has been shown by a map-based complementation approach to be a single nucleotide change in the open reading frame of the eukaryotic translation initiation factor 3b (NCU02208.3).

Inoue and Ishikawa defined a set of non-remediable, temperature-sensitive "unknown" mutants in *Neurospora crassa* (Inoue and Ishikawa,1970). To this day, the actual gene altered in many of these "unknown" mutants has not been determined. In order to add value to the Fungal Genetics Stock Center collection, we continue to define the genetic defects associated with these temperature-sensitive mutations (McCluskey et al., 2007, Wiest et al., 2008).

Using a complementation-based approach, we have identified the mutation in *un-10* as a missense mutation in the eIF3b open reading frame. Building on the demonstration by T. Schmidhauser, that cosmids from the pSV50 cosmid library (Vollmer and Yanofsky, 1986) complement the *un-10* mutation in strain FGSC 2342 (Wilson, 1990), we had cosmids 10E12, 11D2, 16C5, and 23C1 end-sequenced. Based on this sequence data, the mutation in FGSC 2342 was predicted to be on contig 10 between bases 68,000 and 92,000 (Galagan, et al, 2003). We selected overlapping cosmids spanning this region and tested their ability to complement the *un-10* mutation in FGSC 2342 using electroporation-based transformation (Margolin et. al, 2000; Table 1).

Cosmid ID	Colonies at 37ºC (per ug DNA)	Hyg^R Colonies at 24ºC (per ug DNA)
pLorist6xh 25D10	21	11
pLorist6xh 66B1	<1	7
pLorist6xh 75A9	56	18
pLorist6xh 107D10	24	7
pSV50 10E12	<1	ND[a]
pSV50 11D2	0	ND
pSV50 23C1	0	ND
No DNA	0	ND

Table 1. Identification of cosmids that complement *un-10*
[a.] Not Done. The pSV50 does not encode hygromycin resistance.

Complementation was successful with cosmids 25:D10, 75:A9 and 107:D10 but not 66:B1. There were four open reading frames in the region common to these overlapping cosmid clones: NCU02205.3, NCU02206.3, NCU02207.3 and NCU02208.3. We amplified copies of the genomic DNA for these open reading frames and used them to transform strain 2342 (Table 2). Only PCR product from NCU02208.3 complemented the *un-10* mutation.

PCR Product	Colonies at 37ºC (per ug DNA)[a]	
NCU02205	0	0
NCU02206	0	<1[b]
NCU02207	0	<1
NCU02208	10	13
No DNA	0	0

Table 2. Identification of PCR products that complement *un-10*
[a]Data from two different replicates
[b]Fewer than one transformant colony per microgram of DNA

DNA sequence obtained directly from PCR amplified genomic DNA from strain 2342 showed a single T to C transition at position 1411, resulting in a tryptophan to arginine change in amino acid residue 471. This tryptophan residue is conserved among most fungi (Figure 1) and even higher eukaryotes. The orthologous gene in *Saccharomyces cerevisae*, PRT1, has alleles which confer a temperature-sensitive phenotype (Hanic-Joyce et al, 1987). None of these corresponds to the mutation

in Neurospora (Evans et al., 1995).

Figure 1. Alignment of the amino acid sequence from eIF3b among sequenced fungi. Neurospora sequence is shown from amino acid 452 to 493. Position 471is highlighted in white.

The demonstration that *un-10* is in the *eIF3b* gene adds value to the strains carrying this mutation. The ability to study both the interactions of subunits of the initiation factor 3 complex and the ability to use a temperature-sensitive mutation to control protein production are significant benefits of the identification of this mutation. Further, the high degree of conservation of the tryptophan residue at position 471 opens the possibility that this mutation can generate a temperature-sensitive growth phenotype when introduced into other organisms.

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