RESEARCH ARTICLE

Molecular analysis of intragenic recombination at the tryptophan synthetase locus in *Neurospora crassa*

A. WIEST[1], D. BARCHERS[1,2], M. EATON[1,3], R. HENDERSON[1,4], R. SCHNITTKER[1] and K. MCCLUSKEY[1]*

[1]*Fungal Genetics Stock Center, University of Missouri, Kansas City, MO 64110, USA*
[2]*Truman High School, 3301 S. Noland Road, Independence, MO 64055, USA*
[3]*Blue Valley North High School, 12200 Lamar Avenue, Overland Park, KS 66209, USA*
[4]*Blue Springs North High School, 2000 NW Ashton Drive, Blue Springs, MO 64015, USA*

Abstract

Fifteen different classically generated and mapped mutations at the tryptophan synthetase locus in *Neurospora crassa* have been characterized to the level of the primary sequence of the gene. This sequence analysis has demonstrated that intragenic recombination is accurate to order mutations within one open reading frame. While classic genetic analysis correctly ordered the mutations, the position of mutations characterized by gene sequence analysis was more accurate. A leaky mutation was found to have a wild-type primary sequence. The presence of unique polymorphisms in the primary sequence of the *trp-3* gene from strain 861 confirms that it has a unique history relative to the other strains studied. Most strains that were previously shown to be immunologically nonreactive with antibody preparations raised against tryptophan synthetase protein were shown to have nonsense mutations. This work defines 14 alleles of the *N. crassa trp-3* gene.

[Wiest A., Barchers D., Eaton M., Henderson R., Schnittker R. and McCluskey K. 2013 Molecular analysis of intragenic recombination at the tryptophan synthetase locus in *Neurospora crassa*. *J. Genet.* **92**, 523–528]

Introduction

For many decades *Neurospora crassa* was used in pioneering investigations of the nature of the gene and this work led to the description of the one-gene, one-enzyme hypothesis (Beadle and Tatum 1941). Because of the relative simplicity in generating auxotrophic mutations in *Neurospora*, it quickly became a preferred organism for research into questions as diverse as the chemical nature of the gene (Loring and Pierce 1944), the indivisibility of the gene (Bonner 1956), and on the directionality of gene conversion (Case and Giles 1958). While these studies were foundational to the understanding of gene structure and function, it was many years before a complete understanding of the underlying molecular events leading to gene conversion could be elucidated. These studies naturally led to *Neurospora* being an important model for studies of meiotic recombination (Case and Giles 1958; Catcheside *et al.* 2013) and several loci associated with alterations in recombination frequency, using marker genes to assay the regions around *his-2*, *ad-3* and *nit-2* regions on linkage group I, have been characterized to be comprised of three main loci (Perkins *et al.* 2001). Recent investigations are considering the impact of the mis-match repair protein MSH-1 (Koh and Catcheside 2007) on the frequency and nature of crossingover in defined strains carrying specific defects in DNA repair, as well as characterizing crossover hot spots in the well characterized *cog-his-3* system (Yeadon *et al.* 2012). Similarly, related *Neurospora* species are providing unique insight into the role of recombination suppression on evolution of sex chromosomes (Whittle and Johannesson 2011) cementing the role of *Neurospora* as a leading model for modern studies of the molecular and evolutionary nature of recombination. This is clearly an area where fungi can provide unique insight as related species are known to display different strategies of genome organization with some species exhibiting reduced genome size and complexity while others have higher genome size with elevated levels of complexity and diversity (Cuomo *et al.* 2007; Coleman *et al.* 2009; Ma *et al.* 2010).

In *Neurospora*, a variety of analyses of tryptophan mutants demonstrated the location of the *trp-3* (tryptophan synthetase, EC# 4.2.1.20, NCU08409) locus on linkage group II (Bonner *et al.* 1952; Yanofsky and Bonner 1955) and characterized complementation groups among these mutants (Lacy and Bonner 1961). Because of the relative facility of generating and identifying mutants at this locus, intragenic

*For correspondence. E-mail: mccluskeyk@umkc.edu.

Keywords. mutation; recombination; DNA sequence; primary metabolism; trp-3.

recombination was used to characterize the relationship among different alleles of *trp-3* and these studies showed that a gene was 'composed of a larger number of elementary units, arranged in a linear sequence' (Kaplan *et al.* 1964). At the same time, studies of the bifunctional tryptophan synthetase protein complex were being carried out in *Saccharomyces cerevisae* (Kakar 1963) and in *Escherichia coli* (Crawford and Yanofsky 1958). As technology has developed, different technologies have been brought to bear upon the variation of *Neurospora trp-3* mutants and few loci, except perhaps the *ad-3B* locus (De Serres and Kolmark 1958) have been subject to as great a scrutiny as *trp-3*. Subsequent studies aimed at understanding the relationship among *trp-3* mutations led to a preliminary molecular characterization of *trp-3* using DNA sequence (Burns and Yanofsky 1989). Additional *trp-3* alleles were characterized by sequence analysis of 398–582 base fragments corresponding to various regions of the *trp-3* gene in biochemically characterized mutants (A. M. Lacy, M. E. Case and W. S. Nelson, unpublished data, GenBank AF084880.1–AF084906.1). Only four of these alleles are present in strains formally accessioned in the Fungal Genetics Stock Center (FGSC) collection. The FGSC collection includes 82 *N. crassa* strains carrying *trp-3* mutations, and of these 55 carry only *trp-3* mutations. By way of contrast, the FGSC collection also include 28 strains where *trp-1* is the only marker, 12 strains with *trp-2* as the only marker, and two each of *trp-4* or *trp-5* strains. Among the strains with *trp-3* as their primary marker, there are 39 unique *trp-3* alleles represented among 82 strains carrying *trp-3* among other markers. Many *trp-3* alleles are represented by strains of both mating types. In addition to genetic characterization, immunological studies were used to associate mutations which had been mapped to different regions of the trp-3 protein with the presence of immunologically cross-reacting material using crude antibody raised against a protein fraction enriched for tryptophan synthetase activity (Suskind *et al.* 1955). This analysis included several of the strains that are the subject of the present study. Similar genetic and biochemical analyses were applied to tryptophan requiring mutants from related species and the FGSC holds trp mutants for six different species.

In recent years, a number of investigators have undertaken similar retrospective analyses of other mutants in *N. crassa*. These studies have included an analysis of the products of incomplete biochemical reactions leading to pigment formation in albino mutants (Diaz-Sanchez *et al.* 2011) as well as a description of the molecular characteristics of mutants at the *ad-8* locus (Wiest *et al.* 2012). In the former case, it was reported that both substitution and truncation mutants could produce alternate end products. The latter report did not investigate the biochemical impact of various mutants, but did correlate the molecular changes in the primary gene sequence with the genetically inferred locations of the specific lesions.

Presently, we describe the full characterization of the primary DNA sequence of 15 unique alleles at the *trp-3* locus in *N. crassa*. Most of the strains originate in the same mutagenesis project (Kaplan *et al.* 1964; Suyama *et al.* 1964). Strain 861 carries a *trp-3* locus that originated in an Emerson background. All of the other alleles originated in an Oak Ridge (St Lawrence) background (Suyama *et al.* 1964).

Materials and methods

Strains

Many strains carrying mutations at the *trp-3* (NCU08409) locus were deposited into the FGSC collection between 1960 and 1964. Strains FGSC 3538 and 4038 were deposited in 1979 and 1982, respectively. The strains used in the current project are described in table 1. Once deposited into the FGSC collection, strains are preserved by storing both as lyophilized spores and also on anhydrous silica gel (Raper and Alexander 1945; Perkins 1962). Of the stocks utilized in the present study, all were prepared upon original deposit of the strains (1961–1982) except three (FGSC 73, 1017 and 1036) which had been passaged once at the FGSC. Actively growing vegetative cultures were generated by inoculating a few grains of silica gel bearing conidia onto agar-solidified Vogel's minimal medium supplemented with tryptophan as described by DeMoss and Bonner (1959).

Cultural conditions and manipulations

Spore suspensions in sterile water were prepared and a small aliquot was transferred to liquid Vogel's minimal medium in a 50-mL plastic screw-cap test tube with a conical bottom. Cultures were incubated at ambient temperature (~20–22°C) with gentle agitation for 2–3 days after which mycelia were harvested using a wooden applicator stick and blotted dry on sterile paper towels.

DNA manipulations

DNA was extracted using the Zymo Research (Irvine, USA) Bacterial/Fungal DNA Preparation Kit and yield was quantified using a NanoDrop (Wilmington, USA) spectrophotometer. DNA yield was verified by submarine horizontal agarose gel electrophoresis according to standard protocols (Maniatis *et al.* 1982). Polymerase chain reaction (PCR) amplification (Mullis *et al.* 1986) of the *trp-3* open reading frame (ORF) was carried out on a Perkin Elmer (Waltham, USA) GeneAmp 2400 using the following protocol: 5 min at 72°C; 30 cycles of (30 s at 72°C followed by 20 s at 54°C and then 2 min and 45 s at 72°C); seven additional minutes at 72°C. Reactions were held at 4°C until subsequent analysis and DNA sequencing. Primers for amplification are listed in table 2. PCR reaction products were analysed by agarose gel electrophoresis as above. DNA sequencing was carried out on an Applied Biosystems 3100 Genetic Analyzer (Foster City, USA) at the University of Missouri-Kansas City Genomics Facility. Sequences analysed using Sequencher (Gene Codes Corporation, Ann Arbor, USA). Comparative

Table 1. *Neurospora crassa trp-3* mutant strains employed in the present study.

FGSC strain	Allele	Characteristics[a]	Mutagen[b]	Protein detected?[c]
73	c83 (td1)	1st report	UV	–
854	td3	Suppressor/ts	UV	+
856	td24	Similar to td3	UV	NR
859	td71	Slightly leaky or TS	X	+
861	td2	Allele specific suppressor	MC	+
1002	td48R	–	UV	+
1003	td96	Leaky	UV	+
1004	td97	Leaky	UV	+
1010	td101	–	UV	+
1017	td128	–	UV	–
1021	td133	Leaky	UV	–
1034	A78-149	Double mutant- td201+ UV	UV	NR
1036	A78-174	Double mutant- td201+ UV	UV	–
3538	td140	Amber suppressor	UV	–
4038	td37	Poor	X	–

[a]Characteristics are from the FGSC deposit sheets.
[b]Mutagens used are listed as follows: UV, ultra violet irradiation; X, X-ray irradiation; MC, methylcholanthrene.
[c]Protein was reported as 'Cross Reacting Material' in the 1963 description of these strains (Kaplan *et al.* 1964; Suyama *et al.* 1964). NR, status of strains not reported.

Table 2. Primers used for amplification of the *trp-3* locus.

Primer name	Bases	Sequence	Location[a]	Direction[b]
NCU08409-F20	18	TGC CGC CAG TGG TCA ATT	−380	F
NCU08409-F248	18	TGA CCA GCA ACC CAC TCA	−152	F
NCU08409-F714	20	GCG CAT AAC ACG TCT TTC TG	314	F
NCU08409-F1212	18	CTC AGG TTG GCG CCA TTG	812	F
NCU08409-F1715	20	TCC CAC AAG ATC AAC AAC GC	1315	F
NCU08409-F2226	17	TTG AGG CCG GTG GTG AC	1826	F
NCU08409-R122	18	TCG CCT TTG ACT CAC CCG	−295	R
NCU08409-R604	18	CCC TTC TCC ATG GCC AGG	205	R
NCU08409-R1124	18	GGT TGG CGT TGA GAG TGC	725	R
NCU08409-R1616	19	TCC AGG GGT AGT AAG AGC G	1217	R
NCU08409-R2104	18	CTG CTT GGT CTC GTT GCC	1705	R
NCU08409-R2610	18	ACA TCC TTG TCA CCG CGA	2211	R
NCU08409-R2836	18	TGG GAC AGT CAA GCA CCT	+162[c]	R

[a]Position relative to the first codon.
[b]Forward is the same as the direction of transcription.
[c]Refers to the number of bases after the stop codon (TAA).

analysis was conducted using the alignment and gene discovery features at the National Center for Biotechnology Information (GenBank, www.ncbi.nlm.nih.gov/) as well as the European Bioinformatics Institute online sequence analysis resource (www.ebi.ac.uk/).

Results

Primary sequence

PCR primers were validated as being capable of amplifying the correct size fragment when DNA from the reference genome is used as the template. Similarly, the full-length product was amplified from genomic DNA from each of the 15 strains. The complete DNA sequence of the genomic locus corresponding to NCU08409 was determined for 15 mutant alleles at the *N. crassa trp-3* locus. The DNA sequence showed unique changes in 14 of the 15 mutants. Six of the genes contained nonsense mutations and eight of the genes contained mis-sense mutations (figure 1). There were no polymorphisms present among all of the strains indicating that this ORF is identical to the reference genome sequence. There were, however, 12 positions where the allele *td2* in strain 861 differs from both the reference sequence and all of the other alleles (figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/).

No changes relative to the wild-type sequence were detected in strain FGSC 1021 and this strain has been

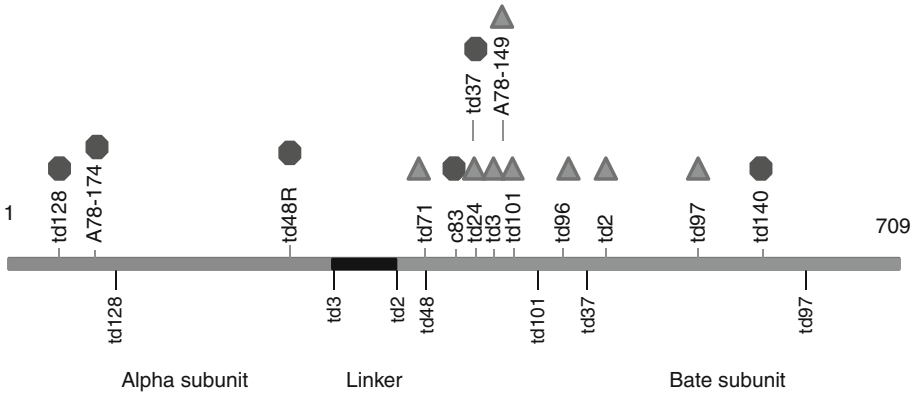

Figure 1. Mutations at the *N. crassa trp-3* locus. The locations of mutations determined by classical genetic analysis are shown below the line. The locations of mutations determined by molecular sequence analysis (the present study) are shown above the line. The mutations denoted with an octagon are nonsense mutations, while the mutations denoted with a triangle are missense mutations. The regions of the protein corresponding to alpha and beta subunits and the linker region are indicated by the shading of the boxed line.

variously described as leaky or reverted on FGSC documentation during deposit and quality control testing (K. McCluskey and A. Wiest, unpublished data).

Discussion

The *trp-3* locus in *N. crassa* is one of the most well studied loci from genetic (Kaplan *et al.* 1964), immunological (Matchett *et al.* 1987) and biochemical (Pratt and DeMoss 1988) perspectives. One outcome of this depth of research is that there are abundant unique well-characterized mutant strains carrying mutations at *trp-3* (McCluskey and Plamann 2004), as well as strains with *trp-3* mutations in the presence of second site suppressors (Yourno and Suskind 1964). The sequence of the *N. crassa trp-3* locus was among the first characterized and was shown to consist of an alpha and beta domain linked by a connector (Burns and Yanofsky 1989) and this structure is shared with other filamentous fungi including *Sordaria* (Nowrousian *et al.* 2010) and yeast (Zalkin and Yanofsky 1982) and other ascomycetes, although homology to genes in basidiomycete and zygomycete species (below ~60% amino acid identity) is comparable to homology to comparable genes in *Bacillus* or *Enterococcus* (GenBank, data not shown).

The strains sequenced in the present study were distinct in that they were generated by UV, X-ray, or chemical mutagenesis followed by purification and characterization by genetic crossing (table 1). Perhaps because so few strains were studied, there was no association between the type of mutagen and the characteristics of the DNA sequence lesion. Similarly, most of the strains employed in the present study were characterized immunologically (Suskind *et al.* 1955) and the correlation between the immunological reactivity and the ability to form a full-length polypeptide is almost absolute. Five of the six proteins which are terminated prematurely at the site of a nonsense mutation were characterized as immunologically nonreactive while seven of the eight proteins with missense mutations retain their immunological reactivity (table 1; figure 1). The one strain which contains a nonsense mutation yet still produces immunologically cross reactive material is FGSC 1002 which produces a 235 residue polypeptide.

The progenitor strain (TD 78) to two of the strains characterized as having multiple mutations was previously shown to have a mutation in a substrate binding site (GenBank AF084887.1) and this mutation was detected in multiple-mutant strains FGSC 1034 and FGSC 1036. They both share the replacement of T 523 with a C and this was presumably indicative of their shared origin in strain A78 (figure 1 in electronic supplementary material). Moreover, the 582 base region of sequence covered in the otherwise unpublished GenBank data for the progenitor (GenBank AF084887) is identical among these three strains while this overlapping sequence has a number of polymorphisms between the mutations generated directly in the Oak Ridge lineage (e.g., FGSC 1002). None of the strains analysed in the present study contain mutations in the putative GCN4 consensus binding sequence upstream of the ATG (Burns and Yanofsky 1989), nor do any of the mutations map to the linker or connecting region. Interestingly this analysis includes more mutations in the beta domain than in the alpha domain and the present study can not address the question of whether this is a feature of the mutation, selection or mapping process, rather than just random sample error.

The present study used DNA sequence analysis to characterize mutant strains of *N. crassa* which are unable to synthesize tryptophan from indole. These mutant strains were previously characterized by analysis of intragenic recombination, by their immunological reactivity and by the presence of second site suppressors. Locations of the mutations in 14 of 15 strains were determined directly and the 15th strain was suspected to have either reverted or to have been

Table 3. Characteristics of mutants at the *N. crassa trp-3* locus.

Strain	Allele	Mutation	Mutated domain
73	c83 (td1)	A1021 deletion results in truncation at residue 379	B
854	td3	A1151G results in D384G	B
856	td24	G1147C results in E383Q	B
859	td71	G1613T results in G358V	B
861	td2	T1412C results in L470P	B
1002	td48R	G500 deletion results in truncation at residue 235	A
1003	td96a	T1367C results in L456P	B
1004	td97	A1618T results in S540C	B
1010	td101	C1169T results in S390F	B
1017	td128	A10 deletion results in truncation at residue 46	A
1021	td133	No mutation detected	–
1034	A78-149	T523C and C1169T result in Y175H and S390F	A and B
1036	A78-174	A10 deletion (among others) results in truncation at residue 58	A
3538	td140	C1792T results in truncation at residue 597	B
4038	td37	G740 deletion results in truncation at residue 379	B

contaminated (table 3). Since there was no heterogeneity in its *trp-3* sequence relative to the reference genome, it is likely that this strain is no longer authentic. There is strong correlation between prior immunological reactivity and the ability to synthesize a full-length polypeptide. The order of mutations as defined by intragenic recombination mapping is largely confirmed. One strain (FGSC 861) has sequence characteristics which confirm that it is unique among this group of strains. None of the 18 strains subject to whole genome analysis (McCluskey *et al.* 2011) had any polymorphisms at the *trp-3* locus emphasizing the importance of this gene and the value of well characterized mutant strains with defined genetic lesions. The value of these, and related, materials will increase as more information is available about classical mutations. The structural biological, enzymological and evolutionary biological implications of these types of studies promise a renewed interest in classical genetic materials.

Acknowledgements

The Fungal Genetics Stock Center is supported by grant 742713 from the US National Science Foundation. The authors would like to thank Dr S. E. Baker for comments on the manuscript.

References

Beadle G. W. and Tatum E. L. 1941 Genetic control of biochemical reactions in *Neurospora*. *Proc. Natl. Acad. Sci. USA* **27**, 499–506.

Bonner D. M. 1956 *The genetic unit dedicated to the memory of Professor Lewis J. Stadler*, pp. 163–170. Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor Laboratory Press, New York, USA.

Bonner D. M., Yanofsky C. and Partridge C. 1952 Incomplete genetic blocks in biochemical mutants of *Neurospora*. *Proc. Natl. Acad. Sci. USA* **38**, 25.

Burns D. M. and Yanofsky C. 1989 Nucleotide sequence of the *Neurospora crassa trp-3* gene encoding tryptophan synthetase and comparison of the *trp-3* polypeptide with its homologs in *Saccharomyces cerevisiae* and *Escherichia coli*. *J. Biol. Chem.* **264**, 3840–3848.

Case M. E. and Giles N. H. 1958 Evidence from tetrad analysis for both normal and aberrant recombination between allelic mutants in *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA* **44**, 378.

Catcheside D. E., Bowring F. J. and Yeadon P. J. 2013 Genetic Recombination in *Neurospora crassa*. In *Neurospora: genomics and molecular biology* (ed. K. McClusky and D. P. Kasbekar), pp. 85. Horizon Scientific Press, UK.

Coleman J. J., Rounsley S. D., Rodriguez-Carres M., Kuo A., Wasmann C. C., Grimwood J. *et al.* 2009 The genome of *Nectria haematococca*: contribution of supernumerary chromosomes to gene expansion. *PLoS Genet.* **5**, e1000618.

Crawford I. P. and Yanofsky C. 1958 On the separation of the tryptophan synthetase of *Escherichia coli* into two protein components. *Proc. Natl. Acad. Sci. USA* **44**, 1161.

Cuomo C. A., Güldener U., Xu J. R., Trail F., Turgeon B. G., Di Pietro A. *et al.* 2007 The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science* **317**, 1400–1402.

De Serres F. J. and Kolmark H. G. 1958 A direct method for determination of forward-mutation rates in *Neurospora crassa*. *Nature* **182**, 1249–1250.

DeMoss J. and Bonner D. 1959 Studies on normal and genetically altered tryptophan synthetase from *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA* **45**, 1405.

Diaz-Sanchez V., Estrada A. F., Trautmann D., Limon M. C., Al-Babili S. and Avalos J. 2011 Analysis of *al-2* mutations in *Neurospora*. *PLoS One* **6**, e21948.

Kakar S. N. 1963 Allelic recombination and its relation to recombination of outside markers in yeast. *Genetics* **48**, 957.

Kaplan S., Suyama Y. and Bonner D. M. 1964 Fine structure analysis at the *td* locus of *Neurospora crassa*. *Genetics* **49**, 145–158.

Koh L. Y. and Catcheside D. E. 2007 Mutation of *msh-2* in *Neurospora crassa* does not reduce the incidence of recombinants with multiple patches of donor chromosome sequence. *Fungal Genet. Biol.* **44**, 575–584.

Lacy A. M. and Bonner D. M. 1961 Complementation between alleles of the *td* locus in *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA* **47**, 72.

Loring H. S. and Pierce J. G. 1944 Pyrimidine nucleosides and nucleotides as growth factors for mutant strains of *Neurospora*. *J. Biol. Chem.* **153**, 61–69.

Ma L. J., van der Does H. C., Borkovich K. A., Coleman J. J., Daboussi M. J., Di Pietro A. *et al.* 2010 Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* **464**, 367–373.

Maniatis T., Fritsch E. F. and Sambrook J. 1982 *Molecular cloning: a laboratory manual*. Cold Spring Harbor, New York, USA.

Matchett W. H., Lacy A. M. and DeMoss J. A. 1987 Orientation of enzymatic domains in tryptophan synthase of *Neurospora crassa*: an immunoblot analysis of TRP3 mutant products. *Mol. Gen. Genet.* **208**, 398–407.

McCluskey K. and Plamann M. 2004 10th Fungal genetics stock center catalogue of strains. *Fungal Genet. Newslett.* **51S**, 1–208.

McCluskey K., Wiest A., Grigoriev I. V., Lipzen A., Martin J., Schackwitz W. and Baker S. E. 2011 Rediscovery by whole genome sequencing: classical mutations and genome polymorphisms in *Neurospora crassa*. *G3* **1**, 303–316.

Mullis K., Faloona F., Scharf S., Saiki R., Horn G. and Erlich H. 1986 Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring. Harb. Symp. Quant. Biol.* **1**, 263–273.

Nowrousian M., Stajich J. E., Chu M., Engh I., Espagne E., Halliday K. *et al.* 2010 De novo assembly of a 40 Mb eukaryotic genome from short sequence reads: *Sordaria macrospora*, a model organism for fungal morphogenesis. *PLoS Genet.* **6**, e1000891.

Perkins D. D. 1962 Preservation of *Neurospora* stock cultures with anhydrous silica gel. *Can. J. Microbiol.* **8**, 591–594.

Perkins D. D., Radford A. and Sachs M. S. 2001 *The Neurospora compendium: chromosomal loci*. Academic Press, San Diego, USA.

Pratt M. L. and DeMoss J. 1988 *Neurospora* tryptophan synthase. Characterization of the pyridoxal phosphate binding site. *J. Biol. Chem.* **263**, 6872–6876.

Raper K. B. and Alexander D. F. 1945 Preservation of molds by the lyophil process. *Mycologia* **37**, 499–525.

Suskind S. R., Yanofsky C. and Bonner D. M. 1955 Allelic strains of *Neurospora* lacking tryptophan synthetase: a preliminary immunochemical characterization. *Proc. Natl. Acad. Sci. USA* **41**, 577–582.

Suyama Y., Lacy A. M. and Bonner D. M. 1964 A genetic map of the *Td* locus of *Neurospora crassa*. *Genetics* **49**, 135–144.

Whittle C. A. and Johannesson H. 2011 Evidence of the accumulation of allele-specific non-synonymous substitutions in the young region of recombination suppression within the mating-type chromosomes of *Neurospora tetrasperma*. *Heredity* **107**, 305–314.

Wiest A., McCarthy A. J., Schnittker R. and McCluskey K. 2012 Molecular analysis of mutants of the *Neurospora* adenylosuccinate synthetase locus. *J. Genet.* **91**, 199–204.

Yanofsky C. and Bonner D. M. 1955 Gene interaction in tryptophan synthetase formation. *Genetics* **40**, 761.

Yeadon P., Bowring F. and Catcheside D. 2012 A crossover hotspot near *his-3* in *Neurospora crassa* is a preferential recombination termination site. *Mol. Genet. Genomics* **287**, 155–165.

Yourno J. D. and Suskind S. R. 1964 Suppressor gene action in the tryptophan synthetase system of *Neurospora crassa*. II. biochemical studies. *Genetics* **50**, 817.

Zalkin H. and Yanofsky C. 1982 Yeast gene *TRP5*: structure, function, regulation. *J. Biol. Chem.* **257**, 1491–1500.

Received 16 July 2013; accepted 6 August 2013
Published on the Web: 12 December 2013