A ROLE OF CALCIUM SIGNALING GENES IN HETEROKARYON INCOMPATIBILITY IN NEUROSPORA CRASSA

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Abstract

We have studied the Ca2+ signaling knockout mutants for their role in mating-type-associated heterokaryon incompatibility in Neurospora crassa. The found results showed on heterokaryons homokaryosis for ∆NCU05225, ∆NCU06366, ∆NCU06650, ∆NCU07075, and ∆NCU07966 Ca2+ signaling knockout mutants (Neurospora crassa unit number, NCU) displayed heterokaryon het compatibility; however heterokaryons heterokaryosis for ∆NCU05225, ∆NCU063665, ∆NCU06650, ∆NCU07075, and ∆NCU07966 mutants displayed het incompatibility like the wild-type control. In addition to that Two Ca2+ signaling knockout mutants ∆NCU02283, and ∆NCU09655 were tested for mating-type-associated heterokaryon incompatibility; these results showed, heterokaryons homokaryosis and heterokaryons heterokaryosis for ∆NCU02283, ∆NCU09655 mutants displayed het incompatibility. Cell death and hyphal compartmentation due to mating type associated incompatibility were confirmed by uptake of vital dye Evan’s blue. Thus, these results of ∆NCU05225, NCU06366, NCU06650, NCU07075, and NCU07966 Ca2+ signaling gene products could play a role in mating-type-associated heterokaryon incompatibility in N. crassa. In this article, we are reporting initially screened Ca2+ signaling gene deletion mutants of these five acts as recessive suppressors of mating type associated vegetative incompatibility in N. crassa.

Keywords: Ca2+ signaling genes; Neurospora crassa unit number (NCU); heterokaryon incompatibility; Neurospora crassa; non-self-recognition; vegetative incompatibility.

Introduction

A cell containing different nuclear types in the same cytoplasm is called heterokaryon. Heterokaryon incompatibility refers to a condition when two nuclei of different genotypes cannot co-exist together in the same cytoplasm (Fig.1) (Staben and Yanofsky, 1990; Glass et al., 1988, Debets and Griffiths, 1998; Worrall, 1997, Caten, 1972; Debets et al., 1994; VanDiepeningen et al., 1998). In N. crassa, both sexual and vegetative heterokaryon systems are present, sexual recognition is restricted by the mating type locus, whereas vegetative het incompatibility is genetically controlled by particular loci termed as het (for heterokaryon incompatibility) or vic (vegetative heterokaryon incompatibility) (Glass et al., 1988; Ferreira, et al., 1998). When two fungal individuals of distinct het alleles come together, the consequential heterokaryotic cells are quickly damaged or strictly inhibited in their growth (Glass, 2006 and Marek et al., 2003; Saupe, 2000) (Fig.1). This is analogous to histocompatibility system in invertebrates and major histo compatibility (MHC) in mammals (Xiang, 2002; Glass and Kaneko, 2003; Xiang, 2004). N. crassa has two mating (mat) types mat A and mat a. The coexistence of mat A and mat a during the vegetative phase is lethal; however, coexistence of both mating types is necessary for the sexual development (Saupe, 2000; Garnjobst et al., 1956, Wu, 2001; Sarkar et al., 2002). These two mating (mat) types mat A and mat a, alleles are differ in sequences although they occupy the same loci in different strains, and termed as ‘idiomorph’ (Glass et al., 1988). The A idiomorph is 5301 base pairs that consists of mat A-1, mat A-2 and mat A-3 genes; in contrast, a idiomorph is 3235 base pair and contains only one ORF, mat a-1. The total number of het loci in N. crassa is 11, five of them the mating locus, het-c, -d, -e, and i, they have been recognized using enforced heterokaryons between nearly isogenic strains. Among them, three have been characterized at the molecular level, i.e. the mating type mat (mat A and mat a) locus, het-c and het-6 (Glass et al., 1988; Xiang, 2002; Glass and Kaneko, 2003; Xiang, 2004; Saupe, 2000; Wu, 2001; Sarkar et al., 2002).

A non-self-recognition process called heterokaryon incompatibility (het) that operates during the vegetative and sexual phases of the filamentous in N. crassa (Glass et al., 1988; Ferreira et al., 1998; Garnjobst et al., 1956).
Heterokaryon Incompatibility in Other Filamentous Fungi

The fungal mating types which are tremendously dissimilar from each other, and do not show homology between strains of the opposite sex (as opposed to the allelic relationship in most polymorphic systems). The variance between dissimilar allelic forms of a *het* gene is generally widespread, but single-amino-acid differences can be sufficient to trigger incompatibility (Newmeyer, 1970; Glass et al., 1988; Deleu, 1993). The *mat A-1* gene, encode a polypeptide of 288 amino acids region containing amino acids 90-104 has significant similarity to the MAT α1 polypeptide of *Saccharomyces cerevisiae* (Glass et al., 1990), this is required for the expression of the *het* incompatibility and sexual functions. The *mat a-1* encodes a 382 amino acids residues MAT a-1 polypeptide consisting of an HMG domain, and a separate region from amino acid residues 216-220 that confers vegetative incompatibility (vic) in *N. crassa* (Staben and Yanofsky, 1990; Glass et al., 1990; Philley and Staben, 1994). In *Podospora anserine* a having two *het* loci, *het-s* and *het-e* are functional similar to the that of *het-c* and *het-6* of *N. crassa*, and *vib-l* (vegetative incompatibility block-1) of *Aspergillus nidulans*; but all of these genes are not homologous to each other (Ferreira et al., 1998; Sauge, 2000; Sarkar et al., 2002). The distinguish mechanism of *het* incompatibility reconcile by allele of differences at the *het-c* loci of *N. crassa* that inhibits phenotypic aspects of *het-c* vegetative incompatibility (Xiang, 2002; Glass and Kaneko, 2003; Xiang, 2004). The molecular description of *het loci* and *het* genes participating in the incompatibility effect has been achieved for two ascomycete’s *N. crassa* and *P. anserine* (Smith et al., 2000; Dementhnon et al., 2003; Xiang, 2002; Xiang, 2004; Glass and Kaneko, 2003; Sauge, 2000).

Biological Significance of Heterokaryon Incompatibility

The biological significance of heterokaryon incompatibility, two different views was explained by subject. First, it has been proposed that heterokaryon incompatibility genes continue living limit nutrient situation, and heterokaryon development between different individuals (Debets, 1994; Glass et al., 2004; Xiang, 2004). Second, the heterokaryotic cells strength boundary to the horizontal gene transfer of cytoplasmic infectious fundamentals elements such as senescence of plasmids, mycoviruses, transposes (Anagnostakis, 1977, 1983; Baidyaroy et al., 2000; Biella et al., 2002; Caten, 1972; Debets et al., 1994; Van Diepening et al., 1998; Hartil et al., 1975; Hickey et al., 2002; Glass et al., 2004). On the other hand it has been proposed that mating type associated heterokaryon incompatibility might be maintaining out breeding by preventing the construction of different mating types of heterokaryons between siblings of the identical crosses (Debets and Griffiths, 1998; Worrall, 1997).

Present Understanding of Mating Type-Associated Het Incompatibility in *N. Crassa*

*N. crassa* has two mating (*mat*) types, *mat A* and *mat a*, both mating types are essential for sexual development, however, coexistence during the vegetative phase is lethal, and therefore display incompatibility. The importance of mating type associated heterokaryon incompatibility, as anticipated for other *het* genes, the appearance of mating type allied incompatibility might be chance (Sarkar et al., 2002). The *tol* gene (*tol* gene, for tolerant) is a suppressor of mating-type-associated heterokaryon incompatibility in *N. crassa* (Sauge, 2000). The Ca²⁺-signaling genes regulates numerous processes secretion, sporulation, cytoskeletal organization, circadian rhythm, hyphal tip growth and hyphal branching in *N. crassa* (Fig. 1). Does Calcium signaling genes impact on mating-type-associated heterokaryon incompatibility in *N. crassa*? On this foundation, we designed the experiment by using auxotropic marker for testing for complementation answer on Ca²⁺-signaling genes in *N. crassa*; and we crossed with Ca²⁺-signaling knockout mutants with auxotropic marker *leu-3*, and *his-3* strains (Garnjobst, 1953; Adams et al., 1987; Coenen, 1994).

Materials and Methods

Strains and Growth Conditions

Growth and maintenance of *Neurospora crassa* strains on Vogel’s medium supplement with glucose were essentially as described in Davis and De Serres (1970). The Ca²⁺-signaling knockout mutants, genotypes indicate as a *Neurospora crassa* unit number (NCU) (Table 1, and Table 2).

Fig. 1: Mechanism of heterokaryon incompatibility. Adapted from Gale Wichmann et al., 2008.
Table 1: Ca²⁺-signaling genes encodes proteins in N. crassa

| S.N. | Ca²⁺-signaling genes | No. of amino acid | Encodes Name of Proteins |
|------|----------------------|-------------------|--------------------------|
| 1    | NCU02283             | 467               | calcium/calmodulin-     |
|      |                      |                   | dependent protein kinase|
| 2    | NCU05225             | 674               | mitochondrial NADH       |
|      |                      |                   | dehydrogenase           |
| 3    | NCU06366             | 505               | Ca₂⁺/H⁺ antiporter      |
| 4    | NCU06650             | 186               | Ca²⁺ and/or CaM binding|
|      |                      |                   | protein, a secretory    |
|      |                      |                   | phosholipase A2         |
| 5    | NCU07075             | 508               | CAX , Ca²⁺ /H⁺         |
|      |                      |                   | exchanger               |
| 6    | NCU07966             | 1110              | Calcium transporting    |
|      |                      |                   | ATPase 3, a cation -    |
|      |                      |                   | ATPase                  |
|      |                      |                   | plasma membrane zinc    |
|      |                      |                   | ion transporter,        |
|      |                      |                   | phosphatidylinositol-4,5-|
|      |                      |                   | bisphosphate            |
|      |                      |                   | phosphodiesterase       |
|      |                      |                   | gamma 2                 |
| 7    | NCU09655             | 598               | 625                      |

Note: Ca²⁺-signaling genes encode proteins in N. crassa, available in site (http://www.broadinstitute.org/annotation/genome/neurospora/)

Table 2: list of strains used in this study.

| S. No. | FGSC | Genotype       | Source  |
|--------|------|----------------|---------|
| 1      | 12448a| ΔNCU02283 a    | FGSC    |
| 2      | 1249A | ΔNCU02283 A    | FGSC    |
| 3      | 11405a| ΔNCU05225a     | FGSC    |
| 4      | 11406A| ΔNCU05225A     | FGSC    |
| 5      | 11407a| ΔNCU06366 a    | FGSC    |
| 6      | 114084| ΔNCU06366 a    | FGSC    |
| 7      | 11246a| ΔNCU06650 a    | FGSC    |
| 8      | 11247A| ΔNCU06650A     | FGSC    |
| 9      | 11248a| ΔNCU07075a     | FGSC    |
| 10     | 11249A| ΔNCU07075A     | FGSC    |
| 11     | 11409A| ΔNCU07966A     | FGSC    |
| 12     | 11410A| ΔNCU07966A     | FGSC    |
| 13     | 11271a| ΔNCU09655a     | FGSC    |
| 14     | 11272A| ΔNCU09655A     | FGSC    |
| 15     | 1321A | leu-3A         | FGSC    |
| 16     | 3740a | leu-3a         | FGSC    |
| 17     | 6103A | his-3A         | FGSC    |
| 18     | 9716a | his-3a         | FGSC    |

Table 3: List of Ca²⁺-signaling knockout mutants obtained intensity band by Southern hybridization

| S. No. | Gene | R. E | Pro | Wild type bands | Knockout bands |
|--------|------|------|-----|-----------------|----------------|
| 1      | NCU022 | 83A  | ol  | 5F -            | 10kb,9.5,4kb   |
| 2      | NCU022 | 83A  | ol  | 5F -            | 10kb,9.5,4kb   |
| 3      | NCU052 | 25A  | I   | 5F -            | 0.8kb,5.5kb    |
| 4      | NCU052 | 25a  | I   | 5F -            | 0.8kb,5.5kb    |
| 5      | NCU063 | 66A  | c I | 5F -            | 5kb,1.5kb,6kb  |
| 6      | NCU063 | 66A  | c I | 5F -            | 5kb,4kb,6kb    |
| 7      | NCU066 | 50A  | c I | 5F -            | 6kb,0.8kb,6.5kb|
| 8      | NCU070 | 75A  | 3F  | 1kb             | 5kb,1kb        |
| 9      | NCU070 | 75a  | 3F  | 1kb             | 5kb,1kb        |
| 10     | NCU079 | 66A  | 5F  | 9.5kb,8kb,6kb  | 10kb,8kb,8.7kb |
| 11     | NCU079 | 66a  | 5F  | 10kb,8kb,5kb   | 0.7kb          |
| 12     | NCU096 | 55A  | a I | 5F -            | 10kb,6kb,5.5kb |
| 13     | NCU096 | 55a  | a I | 5F -            | 10kb,6kb,5.5kb |

The strains were obtained from the Fungal Genetic Stock Centre (FGSC), Kansas City, Missouri, USA. In this study we used two auxotrophic marker strains leu-3 and his-3 (leu-3, which is deficient to isopropylmalate synthetase, isopropylmalate dehydrogenase and isopropylmalate isomerase; his-3, histidinol dehydrogenase, phosphoribosyl-ATP-pprophosphohydrolase and phosphoribosyl-AMP-cyclohydrase) respectively; two auxotrophic strains leu-3 and his-3 were supplemented in the media with leucine (mg/ml) and histidine(mg/ml) nutrition for their proper growth. The crosses were performed by on Synthetic Crossing media 1X (SCM), and Sorbose-glucose-fructose media 1X (FGS) were used for germinating progeny/ascospore. The antibiotic hygromycin used at a working concentration 220 μg/ml for selecting Ca²⁺-signaling knockout mutants strains form their colonies.

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**Test for Heterokaryon Incompatibility**

Strains with auxotrophic markers were cultured in Vogel’s medium with required supplements. Conidial suspension of two auxotrophic strains, shown in below, were mixed and placed on a Petridis containing Vogel’s glucose media without any supplement.

| S. No. | Heterokaryon type | het incompatibility |
|-------|-------------------|---------------------|
| 7     | (leu-3;ΔNCU05225a(93))+(his-3;ΔNCU05225 A(64)) | Yes |
| 8     | (leu-3;ΔNCU05225a(89))+(his-3;ΔNCU05225 A(64)) | Yes |
| 9     | (leu-3;ΔNCU06366a(13))+(his-3;ΔNCU06366 a(60)) | No |
| 10    | (leu-3;ΔNCU06366a(13))+(his-3;NCU06366 a(38)) | Yes |
| 11    | (leu-3;NCU06366a(14))+(his-3;NCU06366 a(38)) | Yes |
| 12    | (leu-3;NCU06650a(37))+(his-3;NCU06650a(17)) | No |
| 13    | (leu-3;NCU06650a(65))+(his-3;NCU06650 A(41)) | Yes |
| 14    | (leu-3;NCU06650a(66))+(his-3;ΔNCU06650 A(44)) | Yes |
| 15    | (leu-3;NCU06650a(66))+(his-3;NCU06650 A(41)) | Yes |
| 16    | (leu-3;ΔNCU07075 a(22))+(his-3;ΔNCU07075 A(19)) | No |
| 17    | (leu-3 ;ΔNCU07075a(22))+(his-3 :NCU07075 A(24)) | Yes |
| 18    | (leu-3 ;ΔNCU07075a(25))+(his-3 :NCU07075A(19)) | Yes |
| 19    | (leu-3 ;ΔNCU07075a(25))+(his-3 :NCU07075 A(24)) | Yes |
| 20    | (leu-3 ;ΔNCU07075a(44))+(his-3 :NCU07075a(34)) | No |
| 21    | (leu-3 ;ΔNCU07075a(31))+(his-3 :NCU07075a(34)) | Yes |
| 22    | (leu-3 ;ΔNCU07075a(44))+(his-3 :NCU07075a(8)) | Yes |
| 23    | (leu-3 ;ΔNCU07075a(31))+(his-3 :NCU07066 A(8)) | Yes |
| 24    | (leu-3 ;ΔNCU07075a(97))+(his-3 :NCU09655 A(70)) | Yes |
| 25    | (leu-3 ;ΔNCU09655a(96))+(his-3 :NCU09655 A(70)) | Yes |
| 26    | (leu-3 ;ΔNCU09655a(97))+(his-3 :NCU09655 A(78)) | Yes |
| 27    | (leu-3 ;ΔNCU09655a(96))+(his-3 :NCU09655 A(78)) | Yes |
| 28    | (leu-3 ;ΔNCU09655a(96))+(his-3 :NCU09655 A(78)) | Yes |

**Microscopic Analysis with Evan’s Blue Staining**

Sterile pieces of cellophane were spread on top of the surface of solid Vogel’s glucose agar medium. Heterokaryons were enforced by co-inoculating conidia of two strains grown on the cellophane for 2 days. The cellophane contain hyphae was peeled off from the surface of the medium and stained with 1% Evan’s Blue dye (GAFF, 1971; JACOBSON et al., 1998). The hyphae were stained for 15 to 25 minutes, and after that the cellophane was placed in a Buchner funnel containing a piece of filter paper pre-wetted with sterile water. Sterile water was then gently pipette over the cellophane and very weak vacuum was applied in order to clean off absorb dye. The cellophane was then sited on a glass slide, with 5% glycerol to avoid drying, and observed under the bright field phase contrast microscope (Jacobson et al., 1998; Wu and Glass 2001; Xiang, 2002; Glass, 2002).

**Table 4:** Test for heterokaryon incompatibility activity

| S. No. | Heterokaryon type |
|--------|-------------------|
| 1      | (leu-3;ΔNCU02283a(105))+(his-3;ΔNCU02283a(86)) |
| 2      | (leu-3;ΔNCU02283a(104))+(his-3;ΔNCU02283a(86)) |
| 3      | (leu-3;ΔNCU02283a(105))+(his-3;NCU02283a(85)) |
| 4      | (leu-3;NCU02283a(104))+(his-3;NCU02283a(85)) |
| 5      | (leu-3;ΔNCU05225a(93))+(his-3;ΔNCU05225a(63)) |
| 6      | (leu-3;NCU05225a(89))+(his-3;ΔNCU05225 A(63)) |
Results and Discussion

In primarily, we screened for heterokaryon incompatibility, heterokaryons homokaryosis and heterokaryons heterokaryosis of 20 Ca²⁺-signaling knockout mutants out of 48 merely in Yeast, Peptone and D-glucose (YPD) media, results showed het incompatibility. On this foundation, we crossed ∆NCU05225, ∆NCU06366, ∆NCU06650, ∆NCU07075, and ∆NCU07966 Ca²⁺-signaling knockout mutants with the opposite mating types of leu-3 and his-3 auxotrophic marker strains (Table 1 and Table 2). We found that the result heterokaryons homokaryosis for ∆NCU05225, ∆NCU06366, ∆NCU06650, ∆NCU07075, and ∆NCU07966 mutants overcomes het incompatibility, therefore display mating type associate het compatibility and form vigours conidia on Petidis (Fig. 2, Test: HO, Table 4). Whereas, heterokaryons heterokaryosis for ∆NCU05225, ∆NCU06366, ∆NCU06650, ∆NCU07075, and ∆NCU07966 mutants continue to showed mating type associated het incompatibility and showed killing of conidia on Petidis (Fig. 2, Test: HO, Test controls: C1 and C2; wild type control: C3; Table 4). These results indicate that the knockout mutants of Ca²⁺-signaling genes NCU05225, NCU06366, NCU06650, NCU07075, and NCU07966 suppressing the mating type associated het incompatibility in a recessive manner, and it showed genetically complementation in the heterokaryons of homokaryosis. The Ca²⁺-signaling genes NCU05225, NCU06366, NCU06650, NCU07075, and NCU07966 encode proteins mitochondrial NADH dehydrogenase, Ca²⁺/H⁺ antiporter, Ca²⁺ and/or CaM binding protein a secretory phospholipase A2, CAX Ca²⁺/H⁺ exchanger, and Calcium transporting ATPase 3 respectively (Table 1). We have also tested two additional Ca²⁺-signaling knockout mutants ∆NCU02283, ∆NCU09655. We found the results in both the cases heterokaryons of homokaryosis and heterokaryons heterokaryosis for ∆NCU02283, ∆NCU09655 mutants display mating type associated het incompatibility like the wild-type control (Fig. 3, a, Test: HO, test controls: C1 and C2; wild type control: C3). The Ca²⁺-signaling gene deletion mutants of these acts as recessive phenotypic expression of mating type associated het incompatibility. Therefore showed cell death on Petidis (Fig. 3, a, test: HO, test controls: C1 and C2, wild type control: C3). This programmed cell death was confirmed by staining with vital dye Evan’s Blue (Fig. 3 and Fig. 4, test: HO, test controls: C1 and C2, wild type control: C3). These Ca²⁺-signaling genes NCU02283 and NCU09655 encodes protein calcium/calmodulin-dependent protein kinase type I and plasma membrane zinc ion transporter, phosphatidylinositol-4, 5-bisphosphate phosphodiesterase gamma 2 respectively (Table 1).

Fig. 2: Ca²⁺-signaling knockout mutant strains display mating type associated het compatibility activity in N. crassa. Ho: [(leu-3; ∆NCU06650 a (37)) + (his-3; ∆NCU06650 A (17))]; C1: [(leu-3; ∆NCU06650 a (65)) + (his-3; NCU06650 A (41))]; C2: [(leu-3; NCU06650 a (66)) + (his-3; ∆NCU06650 A (44))]; C3: [(leu-3; NCU06650 a (66)) + (his-3; NCU06650 A (41))]. Here, heterokaryons homozygous for ∆NCU05225, ∆NCU06366, ∆NCU06650, ∆NCU07075, and ∆NCU07966 mutants display het compatibility, and C1, C2 and C3 heterokaryons heterozygous for ∆NCU05225, ∆NCU06366, ∆NCU06650, ∆NCU07075, and ∆NCU07966 mutants display het incompatibility.

Fig. 3: Microscopic analysis of cell death mediated by heterokaryon incompatible, using Evan’s blue staining. Ho: [(leu-3; ∆NCU06650 a (37)) + (his-3; ∆NCU06650 A (17))]; C1: [(leu-3; ∆NCU06650 a (65)) + (his-3; NCU06650 A (41))]; C2: [(leu-3; NCU06650 a (66)) + (his-3; ∆NCU06650 A (44))]; C3: [(leu-3; NCU06650 a (66)) + (his-3; NCU06650 A (41))]. Here, blue staining indicates killing due to mating type associated het incompatibility; dye retains the portion and remaining portion exclude. Heterokaryons homozygous for ∆NCU05225, ∆NCU06366, ∆NCU06650, ∆NCU07075, and ∆NCU07966 mutants display het compatibility, therefore dye exclude within the portion.
Fig. 4: Two Ca2+-signaling genes NCU02283, NCU09655 do not suppress mating type associated het incompatibility

(a). Mating type associated heterokaryon incompatibility was observed for Ho: [(leu-3; DNCU02283 a (105)) + (his-3; DNCU02283 A (86))]; C1: [(leu-3;NCU02283 a (104))+ (his-3; DNU02283 A (86))]; C2: [(leu-3; DNCU02283 a (105)) + (his-3; DNCU02283 A (85))]; C3: [(leu-3; NCU02283 a (104)) + (his-3; NCU02283 A (85))]; similarly for DNU09655. Here, both the cases heterokaryons homozygous and heterozygous for △NCU02283, △NCU09655 mutants display het incompatibility. b). Microscopic analysis of heterokaryon incompatibility, using Evan’s blue staining. Ho: [(leu-3; DNU02283 a (105)) + (his-3; DNU02283 A (86))]; C1: [(leu-3; NCU02283 a (104)) + (his-3; DNU02283 A (86))]; C2: [(leu-3; DNU02283 a (105)) + (his-3; DNU02283 A (85))]; C3: [(leu-3; NCU02283 a (104)) + (his-3; NCU02283 A (85))]; similarly for DNU09655. Here, blue staining indicates killing due to mating type associated het incompatibility.

The Ca2+-signaling knockout mutants were isolated from progeny by hph screening method, and we used strategy to test involvement of Ca2+-signaling genes in mating type of associate heterokaryons incompatibility. These Ca2+-signaling knockout mutants were confirmed by PCR (Fig. 5), using PCR primers (Table 5) and Southern hybridization methods (Fig. 5, 6). The Ca2+-signaling gene deletion mutants of these five acts as recessive suppressors of mating type associated het incompatibility; and result reveals their role in mutant’s recessive phenotypes of mating-type-associated heterokaryon incompatibility in N. crassa.

Table 5: List of Primers used in this study

| S.NO | Gene   | Primers                           |
|------|--------|-----------------------------------|
| 1    | NCU02283 | 5F' AGG AGA AGT CTG AGA AGA AGA  |
|      |        | 5R' CTA GAG GCA CCG TAC TAT CG    |
| 2    | NCU05225 | 5F' TGT GAT TCA GGA TGT GGA GGA  |
|      |        | 5R' GTT AGT GCA GCC AGT AAA GG    |
| 3    | NCU06366 | 5F' CGG TAC ACT TGG TAA AGA AGA  |
|      |        | 5R' AGT TGT AGA CAG GTA GGT GG    |
| 4    | NCU06650 | 5F' TAC CTT ACC CAC CAG TAA CG    |
|      |        | 5R' CCT TCT CTT CTA TGT GCC AG    |
| 5    | NCU07075 | 3F' GAG GGT TTC TGG TAG GGA GC    |
|      |        | 3R' GTA CCT CTA CCT AGC CCT GC    |
| 6    | NCU07966 | 5F' AAG TTG AGT GTT CGT CCT CC    |
|      |        | 5R' GGT TCT TCT CCT GGT CC        |
| 7    | NCU09655 | 5F' CTG ACA GAG ATC TTG GAA GC    |
|      |        | 5R' GAT GAC TGA TGA CTG TGA CC    |

Fig. 5: PCR amplification of Ca2+-signaling genes to confirm the knockout mutants; 1) △NCU05225, 2)△NCU06366, 3)△NCU06650, 4)△NCU07075, 5)△NCU07966, 6)△NCU02283; and 7)△NCU09655. Here, ‘M’ is1kb (NEB) marker.
Discussion

The found results showed heterokaryons for homokaryosis ΔNCU05225, ΔNCU06366, ΔNCU06650, ΔNCU07075, and ΔNCU07966 of suppression of mating type associated het compatibility (Fig.3.1, HO, Table4); and heterokaryons for heterokaryosis ΔNCU05225, ΔNCU06366, ΔNCU06650, ΔNCU07075, and ΔNCU07966 mutants display het incompatibility like the wild-type control (Fig.3.1, test: HO, test controls: C1 and C2, wild type control: C3; Table4). That is, HO [(ko1; mat A) + (ko1; mat a)] are viable, whereas controls [(ko1+; mat A) + (ko1; mat a)] and [(ko1+; mat A) + (ko1+; mat a)] are inviable, likewise, for controls C1, C2, and C3 (Fig. 2, test: HO, test controls: C1 and C2, wild type control: C3; Table4).

Therefore NCU05225, NCU06366, NCU06650, NCU07075, and NCU07966 Ca2+-signaling genes have some significant role in het compatibility. An additionally, same approach, we tested two more Ca2+-signaling knockout mutants ΔNCU02283, ΔNCU09655 as control, for confirming of whether all calcium signaling genes showed heterokaryons compatibility (i.e., genetically complementation) or not. In this case two Ca2+-signaling knockout mutants ΔNCU02283, ΔNCU09655 showed results heterokaryosis and heterokaryosis ΔNCU02283, ΔNCU09655 displayed het incompatibility (vic) as like the wild-type control (Fig. 4a, test: HO, test controls: C1 and C2, wild type control: C3). The microscopic analysis of the heterokaryons involving ΔNCU05225, ΔNCU06366, ΔNCU06650, ΔNCU07075, and ΔNCU07966 mutants were confirmed by using the vital dye Evan’s blue, as well ΔNCU02283, ΔNCU09655 knockout mutants. Which revealed neither hyphal compartmentation nor cell death showed for the heterokaryon homokaryosis (Fig. 3, test: HO), whereas in heterokaryon heterokaryosis showed cell death. During the process of het incompatibility, some organelle modifications occurred like septal plugging, vacuolization, shrinkage of plasma membrane, organelle degradation, DNA fragmentation (Glass, 2006; Marek et al., 2003) (Fig. 1), and accumulation of lipid bodies are common microscopic features associated with het incompatibility. Blue staining retaining was indication of killing due to mating type associated het incompatibility (Fig.3, 2b, test: HO, test controls: C1 and C2, wild type control: C3), and live cells portion exclude the dye (Fig.3, test: HO). Both mating types are essential for sexual development, however, coexistence during the vegetative phase is lethal, and therefore display incompatibility (Fig. 1) (SAUPE, 2000; Garnjost, et al., 1956). The calcium signaling genes NCU05225 encode a 674aa residues of mitochondrial NADH dehydrogenase, NCU06366 encodes a 505aa residues of Ca2+/H+ antipporter, NCU06650 encode a of 186aa residues of novel Ca2+ and /or CaM binding protein, a secretory phospholipase A2, NCU07075 encode a of 508aa residues of CAX that is a Ca2+/H+ exchange, and NCU07966 encodes a 1110aa residues of calcium transporting ATPase 3 (Table 1) respectively. In additionally other two Ca2+-signaling genes encodes proteins like NCU02283 encode a 467aa residues of calcium/calmodulin-dependent protein kinase type I, and NCU09655 encodes plasma membrane zinc ion transporter(598aa) and phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma 2(625aa) (Table 1) respectively. This calcium signaling genes encode protein might be involved in heterokaryons incompatibility process and further needs to be studied at the molecular level in N. crassa. Therefore, the result indicates suppression of mating type associated het incompatibility is not common phenomenon for all the Ca2+-signaling knockout mutants in N. crassa.
Conclusion
Previous reports suggest that the nuclei from two different genotypic strains are incompatible within the same cytoplasm (Garnjobst et al., 1956; Saupé, 2000). In N. crassa at least 11 het loci exist. Five of them, the mating type locus and het-c, -d, -e and -I were originally identified using forced heterokaryons between nearly isogenic strains. Here, we reported five Ca²⁺-signaling knockout mutants ΔNCU05225, ΔNCU06366, ΔNCU06650, ΔNCU07075, and ΔNCU07966 play important role in the mating-type-associated het incompatibility in N. crassa(Fig.2, test: HO, test controls: C1 and C2, wild type control: C3), additionally tested two more Ca²⁺-signaling knockout mutants ΔNCU02283, ΔNCU09655; in both the condition like heterokaryons homokaryosis and heterokaryosis of ΔNCU02283, ΔNCU09655 mutants display het incompatibility like the wild-type control(Fig. 4a, test: HO, test controls: C1 and C2, wild type control: C3). In N. crassa heterokaryon compatibility is shown by strains with identical genotypic class of progeny, whereas different genotypes of progeny are het incompatible. But the five Ca²⁺-signaling knockout mutant strains are het compatible suggesting that the knockout Ca²⁺-signaling gene may interact with the genes in het domain that result in induced mating type-associated het incompatibility in N. crassa(Fig.1.1 sup.info). Therefore, the mutants of heterokaryons homokaryosis identical het loci specificity displayed development rate, normal conidiation, and aerial hyphae formation, suggesting that the mutation was compatibility (Fig.2, HO) and heterokaryotic homokaryosis auxotrophic mutant strains growth in limited nutrient condition. Whereas heterokaryons heterokaryosis different het loci specificity displayed decrease in the development rate like lack of growth, normal conidiation, and aerial hyphae formation, suggesting that the mutation was incompatibility (Fig. 2, and Fig. 4a, test: HO, test controls: C1 and C2, wild type control: C3).
During the time of experiment setup, we have taken more care in mixing of conidial cells from other conidial strains contamination, and we verified specific knockout mutants mating type as well. We reported here the involvement of Ca²⁺-signaling gene in heterokaryon incompatibility as a phenotypic expression in N. crassa not yet reported and further needs to be studied at the molecular level.

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Baidyaroy D, Glynn JM, Bertrand H (2000) Dynamics of asexual transmission of a mitochondrrial plasmid in Cryphonectria parasitica. *Curr. Genet.* 37: 257-267. DOI: 10.1007/s002940050527

Balguerie A (2003) Domain organization and structure-function relationship of the HET-s prion protein of Podosporaanserina. *EMBO J.* 22: 2071-2081. DOI: 10.1093/emboj/cdg213

Balguerie A (2004) The sequences appended to the amyloid core region of the HET-s prion protein determine higher-order aggregate organization in vivo. *J. Cell Sci.* 117: 2599-2610. DOI: 10.1242/jcs.01116

Barreau C, Iskandar M, Loubradou G, Levallois V and Begueret J (1998) The mod-A suppressor of nonallelic heterokaryon incompatibility in Podosporaanserina encodes a proline-rich polypeptide involved in female organ formation. *Genetics* 149: 915-926.

Beadle GW and Coomrad VL (1944) Heterokaryosis in Neurosporacrassa. *Genetics* 29: 291-308.

Biella S, Smith ML, Aist JR, Cortesi P, Milgroom MG (2002) Programmed cell death correlates with virus transmission in a filamentous fungus. *Proc. R. Soc. (London) - Ser. B: Biol. Sci* 269: 2269-2276.
Boye-Harnasch M and Cullin C (2006) A novel in vitro filter trap assay identifies tannic acid as an amyloid aggregation inducer for HET-s. J. Biotechnol. 125: 222-230. DOI: 10.1016/j.jbiotec.2006.03.006

Castro A, Lemos C, Falcao A, Glass NL and Videira A (2008) Increased resistance to complex I mutants to phytosphingosine-induced programmed cell death. J. Biol. Chem. 283: 19314-19321. DOI: 10.1074/jbc.M802112200

Caten CE (1972) Vegetative Incompatibility and Cytoplasmic Infection in Fungi. J. Gen. Microbiol. 72: 221-229. DOI: 10.1099/00221287-72-2-221

Coenen A, Debets AJM and Hoekstra RF (1994) Additive action of partial heterokaryon incompatibility (partial-het) genes in Aspergillus nidulans. Curr. Genet. 26: 233-237. DOI: 10.1007/BF00309553

Collins RA and Saville BJ (1990) Independent transfer of mitochondrial chromosomes and plasmids during unstable vegetative fusion in Neurospora. Nature 345: 177-179. DOI: 10.1038/345177a0

Coustou V (1999) Mutational analysis of the [Het-s] prion analog of Podosporaanerina. A short N-terminal peptide allows prion propagation. Genetics 153: 1629-1640

Coustou V, Deleu C, Saupe S and Begueret J (1997) The protein product of the het-s heterokaryon incompatibility gene of the fungus Podosporaanerina behaves as a prion analog. Proc. Natl. Acad. Sci. USA 94: 9773-9778. DOI: 10.1073/pnas.94.18.9773

Coustou-Linares V (2001) In vivo aggregation of the HET-s prion protein of the fungus Podosporaanerina. Mol. Microbiol. 42: 1325-35. DOI: 10.1046/j.1365-2958.2001.02707.x

Dalstra HJ (2003) Sexual transmission of the [Het-s] prion leads to meiotic drive in Podosporaanerina. Proc. Natl. Acad. Sci. USA 100: 6616-6621. DOI: 10.1073/pnas.1030058100

Dalstra HJ (2005) Non-mendelian inheritance of the HET-s prion or HET-s prion domains determines the het-S spore killing system in Podosporaanerina. Fungal Genet. Biol. 42: 836-47. DOI: 10.1016/j.fgb.2005.05.004

Davis RH and De Serres FJ (1970) Genetic and microbiological research techniques for Neurospora crassa. Methods Enzymol. 17A: 79-143. DOI: 10.1016/0076-6879(71)17168-6

Debets AJM and Griffiths AJF (1998) Polymorphism of het-genes prevents resource plundering in Neurospora crassa. Mycol. Res. 102: 1343-1349. DOI: 10.1017/S095375629800639X

Debets F, Yang X and Griffiths AJ (1994) Vegetative incompatibility in Neurospora: its effect on horizontal transfer of mitochondrial plasmids and senescence in natural populations.Curr. Genet. 26: 113-119. DOI: 10.1007/BF00313797

Deleu C (1993) A single amino acid difference is sufficient to elicit vegetative incompatibility in the fungus Podospora anserina. Genetics 135: 45-52.

Deleu C, Turcq B and Begueret J (1990) Repa, a repetitive and dispersed DNA sequence of the filamentous fungus Podosporaanerina. Nucleic Acids Res 18: 4901-4903. DOI: 10.1093/nar/18.16.4901

Demethon K, Iyer G and Glass NL (2006) VIB-1 is required for expression of genes necessary for PCD in Neurospora. Eukaryot Cell 5: 2161-2173. DOI: 10.1128/EC.00253-06

Demethon K, Paoletti M, Pinan-Lucarre B, Loubradou-Bourges N, Sabourin M and Saupe SJ (2003) Rapamycin mimics the incompatibility reaction in the fungus Podospora anserina. Eukaryot. Cell 2: 2-246. DOI: 10.1128/EC.2.2.238-246.2003

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17: 368-376. DOI: 10.1007/BF01734359

Ferreira ADV, An Z, Metzenberg RL and Glass NL (1998) Characterization of mat A-2, mat A-3 and mat-A mating-type mutants of Neurospora crassa. Genetics 148: 1069-1079.

Ferreira ADV, Saupe S and Glass NL (1996) Transcriptional analysis of the mat A idiomorph of Neurospora crassa identifies two genes in addition to mat A-1. Mol. Gen. Genet. 250: 767-774. DOI: 10.1007/BF02172989

Fleissner A, Diamond S and Glass NL (2009) The Saccharomyces cerevisiae PRM1 homolog in Neurospora crassa is involved in vegetative and sexual cell fusion events, but also has post-fertilization functions. Genetics 181: 497-510. DOI: 10.1534/genetics.108.096149

Fleissner A, Leeder AC, Roca MG, Read ND and Glass NL (2009) Oscillatory recruitment of signaling proteins to cell tips promotes coordinated behavior during cell fusion. Proc. Natl. Acad. Sci. USA 106: 19387-19392. DOI: 10.1073/pnas.0907039106

Fleissner A, Sarkar S, Jacobson DJ, Roca MG, Read ND and Glass NL (2005) The so locus Is Required for Vegetative Cell Fusion and Postfertilization Events

This paper can be downloaded online at http://ijasbt.org & http://nepiol.info/index.php/IASBT
in Neurospora crassa. Eukaryot Cell 4: 920-430. DOI: 10.1128/EC.4.5.920-930.2005

Fleissner A, Simonin AR and Glass NL (2008) Cell fusion in the filamentous fungus, Neurospora crassa. Methods Mol. Biol. 475: 21-38. DOI: 10.1007/978-1-59745-250-2_2

Galagan JE (2003) The genome sequence of the filamentous fungus Neurosporacassa. Nature 422: 859-68. DOI: 10.1038/nature01554

Garnjobst L (1953) Genetic control of heterokaryosis in Neurosporacassa. Am. J. Bot. 40: 607-614. DOI: 10.2307/2438448

Garnjobst L (1955) Further analysis of genetic control of heterokaryosis in Neurosporacassa. Am. J. Bot. 42: 444-448. DOI: 10.2307/2438792

Garnjobst L and Wilson JF (1956) Heterokaryosis and protoplasmic incompatibility in Neurosporacassa. Proc. Natl. Acad. Sci. USA 42: 613-618. DOI: 10.1073/pnas.42.9.613

Glass NL and Kaneko I (2013) Fatal attraction: nonself recognition and heterokaryon incompatibility in filamentous fungi. Eukaryot Cell 1: 1-8. DOI: 10.1128/EC.2.1.1-8.2003

Glass NL and Kulda GA (1992) Mating-type and vegetative incompatibility in filamentous ascomycetes. Annu. Rev. Phytopathol. 30: 201-224. DOI: 10.1146/annurev.phyto.30.090192.001221

Glass NL and Lee L (1992) Isolation of Neurosporacassa Amating-type mutants by repeat-induced point (RIP) mutation. Genetics 132: 125-133.

Glass NL, Grotelueschen J and Metzenberg RL (1990) Neurospora crassa Amating-type region. Proc. Natl. Acad. Sci. USA 87: 4912-4916. DOI: 10.1073/pnas.87.13.4912

Glass NL, Grotelueschen J and Metzenberg RL (1990) Neurosporacassa A mating-type region. Proc. Natl. Acad. Sci. USA 87: 4912-4916. DOI: 10.1073/pnas.87.13.4912

Glass NL, Rasmussen C, Roca MG and Read ND (2004) Hyphal homing, fusion and mycelial interconnectedness. Trends Microbiol. 12: 135-41. DOI: 10.1016/j.tim.2004.01.007

Glass NL, Vollmer SJ, Staben C, Metzenberg RL and Yanofsky C (1988) DNAs of the two mating type alleles of Neurospora crassa are highly dissimilar. Science 241: 570-573. DOI: 10.1126/science.2840740

Hartl DL, Dempster ER and Brown SW (1975) Adaptive significance of vegetative incompatibility in Neurosporacassa. Genetics 81: 553-569.

Hickey PC, Jacobson DJ, Read ND and Glass NL (2002) Live-cell imaging of vegetative hyphal fusion in Neurosporacassa. Fungal Genet. Biol. 37: 109-119. DOI: 10.1016/S1087-1845(02)00035-X

Hughes AL and Yeager M (1998) Natural selection at major histocompatibility complex loci of vertebrates. Annu. Rev. Genet. 32: 415-435. DOI: 10.1146/annurev.genet.32.1.415

Hutchison E, Brown S, Tian C and Glass NL (2009) Transcriptional profiling and functional analysis of heterokaryon incompatibility in Neurosporacassa reveals that reactive oxygen species, but not metacaspases, are associated with programmed cell death. Microbiol 155: 3957-3970. DOI: 10.1099/mic.0.032284-0

Jacobson DJ, Beurkensand K and Klomparens L (1998) Microscopic and ultrastructural examination of vegetative incompatibility in partial diploids heterozygous athetloci in Neurospora crassa. Fungal Genet. Biol. 23: 45-56. DOI: 10.1006/fgb.1997.1020

Jones DT, Taylor WR and Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. Comp. Appl. Biosci. 8: 275-282. DOI: 10.1093/bioinformatics/8.3.275

Koichiro T, Daniel P, Nicholas P, Glen S, Masatoshi N and Sudhir K (2011) Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. 28: 2731-2739. DOI: 10.1093/molbev/msr121

Lew RR, Abbas Z, Anderca MI and Free SJ (2008) Phenotype of a mechanosensitive channel mutant, mid-1, in a filamentous fungus, Neurosporacassa. Eukaryot Cell 7: 647-655. DOI: 10.1128/EC.00411-07

Loubradou G, Bagueret J and Turcq B (1999) MOD photonics, a Galphaph subunit of the fungus Podosporamanserina, is involved in both regulation of development and vegetative incompatibility. Genetics 152: 519-28.

Maddelein ML (2002) Amyloid aggregates of the HET-s prion protein are infectious. Proc. Natl. Acad. Sci. USA 99: 7402-7407. DOI: 10.1073/pnas.072199199

Malato L (2007) Role of Hsp104 in the propagation and inheritance of the [Het-s] prion. Mol. Biol. Cell 18: 4803-4812. DOI: 10.1091/mbc.E07-07-0657
Marchler B (2009) CDD: specific functional annotation with the Conserved Domain Database. *Nucleic Acids Res.* **37**: D205-D2010. DOI: 10.1093/nar/gkn845

Marek SM, Wu J, Glass NL, Gilchrist DG and Bostock RM (2003) Nuclear DNA degradation during heterokaryon incompatibility in *Neurospora crassa*. *Fungal Genet Biol.* **40**: 126-137. DOI: 10.1016/S1087-1845(03)00086-0

Mathias S, Pena LA and Kolesnck RN (1998) Signal transduction of stress via ceramide. *Biochem. J.* **335**: 465-480. DOI: 10.1042/bj3350465

Metzenberg RL and Glass NL (1990) Mating type and mating strategies in Neurospora. *Bio. Essays* **12**: 53-59. DOI: 10.1002/bies.950120202

Micali OC and Smith ML (2005) A Nonself recognition gene complex in *Neurospora crassa*. *Genetics* **173**: 1991-2004. DOI: 10.1534/genetics.106.057562

Mylyk OM (1975) Heterokaryon incompatibility genes in Neurosporacrassa detected using duplication-producing chromosome rearrangements. *Genetics* **80**: 107-124.

Mylyk OM (1976) Heteromorphism for heterokaryon incompatibility genes in natural populations of *Neurospora crassa*. *Genetics* **83**: 275-84.

Nazabal A (2003) Conformational transition occurring upon amyloid aggregation of the HET-s prion protein of Podosporaanserina analyzed by hydrogen/deuterium exchange and mass spectrometry. *Biochemistry* **42**: 8852-8861. DOI: 10.1021/bi0344275

Nazabal A (2005) Probing the structure of the infectious amyloid form of the prion-forming domain of HET-s using high resolution hydrogen/deuterium exchange monitored by mass spectrometry. *J. Biol. Chem.* **280**: 13220-13228. DOI: 10.1074/jbc.M413185200

Nazabal A (2005a) High-resolution H/D exchange studies on the HET-s218-295 prion protein. *J. Mass Spectrom* **40**: 580-590. DOI: 10.1002/jms.819

Nazabal A and Schmitter JM (2006) Hydrogen-deuterium exchange analyzed by matrix-assisted laser desorption-ionization mass spectrometry and the HET-s prion model. *Methods Enzymol.* **413**: 167-181. DOI: 10.1016/S0076-6879(06)13009-8

Newmeyer D (1970) A suppressor of the heterokaryon-incompatibility associated with mating type in *Neurospora crassa*. *Can. J. Genet. Cytol.* **12**: 914-926. DOI: 10.1139/g70-115

Newmeyer D and Taylor CW (1967) A pericentric inversion in Neurospora, with unstable duplication progeny. *Genetics* **56**: 771-791.

Perkins DD (1975) The use of duplication-generating rearrangements for studying heterokaryon incompatibility genes in *Neurospora. Genetics* **80**: 87-105.

Perkins DD, Radford A, Newmeyer D and Bjorkman M (1982) Chromosomal Loci of *Neurospora crassa*. *Microbiol. Rev.* **46**: 426-570.

Ren J, Wen L, Gao X, Jin C, Xue Y and Yao X (2009) DOG 1.0: illuminator of protein domain structures. *Cell Res.* **19**: 271-273. DOI: 10.1038/cr.2009.6

Ritter C (2005) Correlation of structural elements and infectivity of the HET-s prion. *Nature. 435*: 844-848. DOI: 10.1038/nature03793

Sabate R (2007) Prion and non-prion amyloids of the HET-s prion forming domain. *J. Mol. Biol.* **370**: 768-783. DOI: 10.1016/j.jmb.2007.05.014

Sabate R (2008) On the binding of Thioflavin-T to HET-s amyloid fibrils assembled at pH 2. *J. Struct. Biol.* **162**: 387-396. DOI: 10.1016/j.jsb.2008.02.002

Sarkar S, Iyer G, Wu J and Glass NL (2002) Nonself recognition is mediated by HET-C heterocomplex formation during vegetative incompatibility. *EMBO J.* **18**: 4841-4850. DOI: 10.1093/emboj/cdf479

Sauf S, Descamps C, Turcq B and Begueret J (1994) Inactivation of the Podosporaanserina vegetative incompatibility locus het-c, whose product resembles a glycolipid transfer protein, drastically impairs ascospore production. *Proc. Natl. Acad. Sci. USA* **91**: 5927-5931. DOI: 10.1073/pnas.91.13.5927

Sauf SJ, Kuldaug GA, Smith ML and Glass NL (1996) The product of the het-C heterokaryon incompatibility gene of Neurosporacrassa has characteristics of a glycine-rich cell wall protein. *Genetics* **143**: 1589-1600.

Shiu PKT and Glass NL (1999) Molecular characterization of tol, a mediator of mating-type-associated vegetative incompatibility in *Neurospora crassa*. *Genetics* **151**: 545-555.

Siemer AB (2006) 3C, 15N resonance assignment of parts of the HET-s prion protein in its amyloid form. *J. Biomol. NMR* **34**: 75-87. DOI: 10.1007/s10585-005-5582-7

Smith ML, Hubbard SP, Jacobson DJ, Micali OC and Glass NL (2000a) An osmotic-remedial, temperature-sensitive mutation in allosteric activity site of ribonucleotidereductase in *Neurospora crassa*. *Mol.
Smith ML, Micali OC, Hubbard SP, Mir-Rashed N, Jacobson DJ and Glass NL (2000) Vegetative incompatibility activity in the het-6 region of Neurospora crassa is mediated by two linked genes. Genetics 155: 1095-1104.

Staben C and Yanofsky C (1990) Neurospora crassa a mating-type region. Proc. Natl. Acad. Sci. USA 87: 4917-4921. DOI: 10.1073/pnas.87.13.4917

Sven SJ (2000) Molecular Genetics of Heterokaryon Incompatibility in Filamentous Ascomycetes. Microbiol. Mol. Biol. Reviews 64: 489-502. DOI: 10.1128/MMBR.64.3.489-502.2000

Taneja V (2007) A non-Q/N-rich prion domain of a foreign prion, [Het-s], can propagate as a prion in yeast. Mol. Cell 27: 67-77. DOI: 10.1016/j.molcel.2007.05.027

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25: 4876-4882. DOI: 10.1093/nar/25.24.4876

Turcq B and Denayrolles M (1990) Isolation of two allelic incompatibility genes s and S of the fungus Podospora anserina. Curr. Genet. 17: 297-303. DOI: 10.1007/BF00314876

Van Diepeningen AD and Hoekstra RF (1997) Heterokaryon incompatibility blocks virus transfer among natural isolates of black Aspergilli. Curr. Genet 32: 209-217. DOI: 10.1007/s002940050268

Vellani TS, Griffiths AJF and Glass NL (1994) New mutations that suppress mating-type associated incompatibility in Neurospora crassa. Genome 37: 249-255. DOI: 10.1139/g94-035

Vogel HJ (1964) Distribution of lysine pathways among fungi: evolutionary implications. Am. Nat. 98: 435-446. DOI: 10.1086/282338

Wasmer C (2008) Amyloid fibrils of the HET-s (218-289) prion form a beta solenoid with a triangular hydrophobic core. Science 319: 1523-1526. DOI: 10.1126/science.1151839

Worrall JJ (1997) Somatic incompatibility in basidiomycetes. Mycologia. 89: 24-36. DOI: 10.2307/3761169

Wu J and Glass NL (2001) Identification of specificity determinants and the generation of alleles with novel specificity at the het-c heterokaryon incompatibility locus of Neurospora crassa. Mol. Cell Biol. 21: 1045-1057. DOI: 10.1128/MCB.21.4.1045-1057.2001

Xiang Q and Glass NL (2002) Identification of vib-1, a locus involved in vegetative incompatibility mediated by het-c in Neurospora crassa. Genetics 162: 89-101.

Xiang Q and Glass NL (2004) Chromosome rearrangements in isolates that escape from het-c heterokaryon incompatibility in Neurospora crassa. Curr. Genet. 44: 329-332. DOI: 10.1007/s00294-003-0451-y

Xiang Q and Glass NL (2004) The control of mating type heterokaryon incompatibility by vib-1, a locus involved in het-c heterokaryon incompatibility in Neurospora crassa. Fungal Genet Biol. 41: 4-11076. DOI: 10.1016/j.fgb.2004.07.006

Xiang Q, Rasmussen C and Glass NL (2002) The ham-2 locus, encoding a putative transmembrane protein, is involved in hyphal fusion in Neurospora crassa. Genetics 160: 169-180.

Yamazaki T (2008) Hydration effects on the HET-s prion and amyloid-beta fibrillous aggregates, studied with three-dimensional molecular theory of salvation. Biophys. J. 95: 4540-4548. DOI: 10.1529/biophysj.107.123000

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