S4-3

A Gene of *Neurospora crassa* that Encodes a Protein Containing TPR Motifs

Bheong-Uk Lee
Department of Biological Sciences, Kosin University, Busan 606-701, Korea

Abstract

Analysis of the *Neurospora crassa* genome data reveals at least 14 proteins that contain tetratricopeptide repeat (TPR) motifs. One of them shows over 60% homology with SSN6 of *Saccharomyces cerevisiae*, a global repressor that mediates repression of genes involved in various cellular processes. Sequence analysis of its cDNA shows that it encodes a putative 102kDa protein. Mutant strains generated by RIP (repeat induced point mutation) process show four distinctive patterns of vegetative growth at various rates. They are male-fertile, yet all female-sterile and produced little or no perithecium. These results indicate that this gene is pleiotropic and involved in several cellular processes of vegetative growth, conidiation and sexual cycle. It is designated *rcm-1* (regulation of conidiation and morphology).

Introduction

Neurospora crassa, a filamentous fungus, has three distinct sporulation pathway that lead to the production of two types of asexual spores, macroconidia, and microconidia, or sexual spores, ascospore (1).

In 1996, Yamashiro *et al.* reported the *rco-1* gene that affected many aspects of growth and development of *N. crassa* (2). RCO1 shows overall 46.3% homology with *S. cerevisiae* TUP1 that contains WD40 repeats. RCO1 also contains seven WD40 repeats whose sequences show averaging 68% identity to those of TUP1. TUP1 is known to be a component of the TUP1-SSN6 complex that mediates transcriptional repression of genes concerned with a variety of cellular processes including growth rate, differentiation and fertility (3). *S. cerevisiae* SSN6 is a member of TPR family. TPR proteins have been found from bacteria to mammals (4). TPR proteins generally contain 3-19 TPR motifs that form scaffolds to mediate protein-protein interactions.

In this study, 14 TPR proteins searched from Whitehead Institute *N. crassa* genome data (version 3) (5) were described and a protein that appears to be a homolog of *S. cerevisiae* SSN6 was further examined. When repeat-induced point mutation (RIP) was introduced into this gene, mutants show various morphological changes, different degrees of conidiation and female-sterility. This gene was designated *rcm-1* (regulation of conidiation and morphology).

Materials methods

Strains and general techniques

N. crassa handling techniques were mostly performed according to protocols described by Davis *et al.* (6). *N. crassa* OR-74 *A* strains was used as wild-type. To generate RIP, *N. crassa* RLM35-35 *his-3 inl a* containing an extracopy of the *rcm-1* gene at the *his* locus was mated with *N. crassa* OR-74 *A*. *N. crassa fl A* and *fl a* were used for mating-type tests.

Cloning and electroporation of the *rcm-1* gene

The *rcm*-1 genomic DNA was amplified with two primers. 5'-ATTGAATTCACGATGGTA-GGTTCCGTGTCCGAG-3' and 5'-ATTACGCGTGGTACACGGCAGATGATGTAATC-3'. The amplified DNA product was electroporated into the *N. crassa* RLM35-35 conidia. Condition for electroporation was 1.5 kVolt/mm, 600Ω and 25 μF (7). The *rcm-1* cDNA was cloned by RT-PCR.

Results and discussion

***N. crassa* proteins containing TPR motifs**

At present, 821 contigs (38,044,343 base pairs) have been assembled by *N. crassa* genome project and 10,082 putative genes have been found (5). 14 proteins that contain TPR motifs are summarized in Table 1. Generally TPR proteins possess 3-19 TPR motifs. The numbers of TPR motifs were ranged from 2, a protein homology with a *Mus musculus* protein kinase to 11, a protein homology with *S. cerevisiae* SKi3p (Table 1).

Phenotypes of *rcm-1* mutants

Four phenotypes are summarized in Table 2. Type A (Fig. 1B) showed a denser mycelial growth and failed to separate conidia (*csp* conidial separation). The pattern of mycelial growth looked like a *ropy* mutant. Its color was yellow, not orange. Type B (not shown) was similar with type A. It however overproduced melanin on SC agar. Type C (Fig. 1C) apparently showed slower growth and denser mycelia, yet produced conidia. Type D (Fig. 1D) showed extremely slower growth and fail to produce conidia. They are male-fertile, yet all female-sterile and produced little or no perithecium.

Predicted RCM1 sequence

The deduced amino acid sequence of RCM1 reveals that it is a protein of 935 amino acid residues with a predicted molecular mass of 102 kDa (Fig. 2). The predicted amino acid sequence of RCM1 shows overall homology with that of *S. cerevisiae* SSN6. Especially, 7 TPR motifs presented at the N-terminal segments of both RCM1 and SSN6 show more than 80% homology. This structural similarity suggests that RCM1 may serve as a mediator like SSN6. The predicted RCM1 sequence contains a proline-rich region (PRRs). PRRs are found in both prokaryotic and eukaryotic proteins. The function of most PRRs is unknown, yet some are believed to interact with proteins involved in essential cellular processes (8). The predicted *N. crassa* RCO1 sequence also contains PRRs unlike *S. cerevisiae* TUP1 that contains glutamine-rich regions (2).

On-going study

1. Study of *rcm-1* mutants using SEM
2. Study of the RCM1-RCO1 relationship *in vivo*

References

1. Davis, R.H. 2000. *Neurospora*: contributions of a model organism. Oxford University Press, New York.
2. Yamashiro, C.T., D.J. Ebbole, B.-U. Lee, R.E. Brown, C. Bourland, L. Madi, and C. Yanofsky. 1996. Mol. Cell. Biol. 16: 6218-6228.
3. Smith, R.L., and A. D. Johnson. 2000. TIBS 25:325-330.
4. Lamb, J. R., S. Tugendreich, and P. Hieter. 1995. TIBS 20:257-259.
5. *Neurospora* Sequencing Project. Whitehead Institute/MIT Center for Genome Research "assembly version 3" [www-genome.wi.mit.edu]
6. Davis, R.H., and F.J. de Serres. 1970. Methods Enzymol. 27A: 79-143.
7. Margolin, B.S., M. Freitag, and E.U. Selker. 1997. Fungal Genet. Newslett. 44:34-36.

Table 1. *Neurospora crassa* proteins containing TPR motifs.

TPR roteins	Homologous proteins/genes	Known Function
1	*Saccharamyces cerevisiae* Ctr9p	Required for efficient CLN2 transcription
2	*S. cerevisiae* Tfc4p	Component of the tRNA biogenesis machinery
3	*S. cerevisiae* Stilp	Heat shock protein
4	*S. cerevisiae* SSN6 (CYC8)	General repressor protein
5	*S. cerevisiae* Ski3p	dsRNA virus protection family member
6	*S. cerevisiae* Ynl313cp	Required for cell viability
7	*Schizosaccharomyces pombe*	Protein kinase inhibitor
8	*S pombe*	Probable n-terminal acetyltransferase 1
9	*Neurospora crassa* cut9	Related to anaphase control protein
10	*N. crassa* ppt1	Serine/threonine protein phosphatase
11	*N. crassa* tom70	Mitochondrial precursor protein import receptor
12	*Mus musculus*	Protein kinase
13	*Emericella midulans bimA*	Nuclear protein for the completion of mitosis
14	*Pichia pastoris* Pas8p	Peroximal targeting signal receptor

Table 2. Summary of the charateristics of *rcm-1* mutants

Mutant types	Linear growth rate (mm/h)	Conidiation[a]	Female fertility[b]
WT	3.08	+	+
Type A	0.81	*csp*	-
Type B	1.36	+	-
Type C	<0.1	*acon*	-
Type D	1.58	+	-

[a] +, normal conidiation; *acon*, aconidial; *csp*, conidial separation defective, [b] +, ertile; -, sterile,

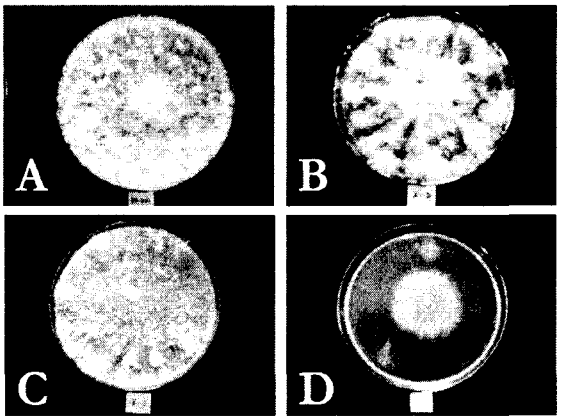

Figure 1. Growth patterns of *rcm-1* mutants

```
SSN6 MNPGGEQTIMEQPAQQQQQQQQQQQQQQQQAAVPQQPLDPLTQSTAETWLSIASLAETLGDGDRAAMAYDATLQFNPSSAKALTSLAHLYRSRDMFQRAAELYERALLVNPELSD
RCM1 M--ANHHPSPTMQMQMHHGPPGPPGPPPASIPSWNQQRQAFMSLTENVWIGIGSVSELMGNHNEALEAYERALAANPNSVTAMNAASLVLRTREDFPKASEYLQRILKIEPANGE
VWATLGHCYLMLDDLQRAYNAYQQALYHLSNPNVPKLWHGIGILYDRYGSLDYAEEAFAKVLELDPHFEKANEIYFRLGIIYKHQGKWSQALECFRYILPQPPAPLQEWDIVFQLGSVLES
AWGSLGHCFLMMEDLQQAYAAYQAALVNLPNPKEPRLWYGIGILYDRYGSLDHAEEAFSQVMAMDPNFDKAHEIYFRLGIIYKQQHKYQQSLDCFRYIVNSPPTPLTEEDIWFQIGHVHEQ
MGEWQGAKEAYEHVLAQNQHHAKVLQQLGCLYGMSNVQFYDPQKALDYLLKSLEADPSDATTWYHLGRVHMIRTDYTAAYDAFQQAVNRDSRNPIFWCSIGVLYYQISQYRDALDAYTRAI
QKDYDGAKQAYERVLQRDPKHAKVLQQLGWLHHQQSNSVASQEKAIEYLNQSVAADQTDAQSWYLLGRCYMQLQKYPKAYEAYQQAVYRDGRNPTFWCSIGVLYYQINQYRDALDAYSRAI
RLNPYISEVWYDLGTLYETCNNQLSDALDAYKQAARLDVNNVHIRERLEALTKQLENPGNINKSNGAPTNASPAPPPVILQPTLQPNDQGNPLNTRISAQSANATASMVQQQHPAQQTPIN
RLNPFISEVWYDLGTLYESCNNQISDALDAYQRAAELDPNNPHIKTRLQLLRSGQAN-------GGAPPGSVPMPTDIHPQTYNASGAVGPPGPQWAGSGSG--------------QPPHQ
SSATMYSNGASPQLQAQAQAQAQAQAQAQAQAQAQAQAQAQAQAQAQAQAQAQAQAQAHAQAQAQAQAQAQAQAQAQAQQQQQQQQQQQQQQQQQQQQQQQQQQQQQLQPLPRQQLQQK
PPQPMHNGGPGPGQGANSWGGRISDINPPPQPPNPYASGQDREPFRGPAPPLPRQPSPRQEQQMRPYQEARAPEPLRRGPTPPQAHYAPPPPPPPQQPHQPHPQQQLQQGP----QPTREG
GVSVQMLNPQQGQPYITQPTVIQAHQLQPFSTQAMEHPQSS-QLPPQQQQLQSVQHPQQLQGQPQAQAPQPLIQHNVEQNVLPQKRYMEGAIHTLVDAAVSSSTHTENNTKSPRQPTHAIP
GSGTRVRNPNYANPQNVVPSNSGPGPNGPPPPNAMMHFNNSPRTDGRPPHMHENRMPSPKSAYPQHQPPYPPHGEQGGPGGPE-----PGPPHPPQSGMAGEPPHQREHDPRP--------
TQAPATGITNAEPQVKKQKLNSPNSNINKLVNTATSIEENAKSEVSNQSPAVVESNTNNTSQEEKPVKANSIPSVIGAQEPPQEASPAEEATKAASVSPSTKPLNTEPESSSVQPTVSSE
---PSVGPKRMREWEDDREVKKPATEETRVR-----MDDHRHRRPSMTPPRMEPPYARRNSSEARRFDERRMEDSRRVEEQRRAEEQRRMEDMRRAEEQRHQNEGYHPSEAAHHPQSHSA
SSTTKANDQSTAETIELSTATVPAEASPVEDEVRQHSKEENGTTEASAPSTEEAEPAASRDAEKQQDETAATTITVIKPTLETMETVKEEAKMREEEQTSQEKSPQENTLPRENVVRQVE
PAHLPPMQQGSAPMQNLIHEQGHGPQQPVPGPQQQG---PGPAHQPAPEDRRMDHPPAQHPPIVNEPERAARKMDVDEDYDDSGEEDKKGGIIPGPSSGSGPAANESKNGASTSGSFNGI
EDENYDD
MGQKSESN
```

Figure 2. Alignment of SSN6 and RCM1. Underlined letters indicate TPR motifs.