Molecular Cloning of a Gene (cftp) Encoding the Cytoplasmic Filament Protein P59Nc and Its Genetic Relationship to the snowflake Locus of Neurospora crassa

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ABSTRACT

P59Nc is a 59-kD polypeptide associated with 8–10-nm diameter cellular filaments in normal Neurospora crassa strains. Abnormally sized and shaped bundles of these structures are present in N. crassa strains carrying mutations at the locus sn (snowflake). By using molecular cloning and restriction fragment length polymorphism (RFLP) segregation analysis strategies we show here that sn is not the genetic locus of P59Nc. Several P59Nc cDNAs were cloned from a N. crassa XGT11 library after immunoscreening with specific polyclonal anti-P59Nc antibodies. Additional longer cDNAs were obtained from a N. crassa cDNA-λZAP library. When used as probes in Southern blots of total DNA from wild-type strains, multicent-2 (a multiple mutant strain), and snowflake mutants, the P59Nc cDNAs revealed comparable patterns of hybridizing bands for all of the restriction enzymes tested. Analysis of segregation of Bell and Clal RFLPs, detected in the genomic region of the P59Nc gene (locus cftp; cellular filament polypeptide), among a set of strains designed for RFLP mapping, or among selected progeny of crosses involving a snowflake parent, respectively, indicate that (i) there is in N. crassa a single cftp locus positioned on the right arm of linkage group VII between the locus for and the proximal breakpoint of the translocation T(VII → I)5936; (ii) the sn mutations in the centromere region of chromosome I do not represent translocations of cftp; and (iii) the snowflake mutants possesses a normal copy of the P59Nc gene on their chromosomes VII. Taken together the results indicate that the aberrant in vivo arrangement of the P59Nc 8–10-nm filaments occurring in snowflake mutants are not due to alterations in the P59Nc gene.

MICROTUBULES (20–25 nm in diameter) and microfilaments (5–7 nm in diameter) are the most clearly defined elements from the filamentous fungi cellular matrix (McKerracher and Heath 1987). Other filamentous cytoplasmic structures observed in electron microscope studies of filamentous fungi cells remain poorly characterized (see Rosa, Peralta-Soler and Maccioni 1990). Recently, the isolation and characterization of bundles of 8–10-nm diameter filaments in the fungus N. crassa were described (Rosa et al. 1990). Similar filament bundles were first observed in electron microscope studies of N. crassa wild type, in the morphological mutant snowflake (Allen, Lowry and Sussman 1974), and in other filamentous fungi (Anderson and Zaccharian 1974; Gull 1975; Hoch and Howard 1980). However, their biochemical nature remains unsettled (see Rosa, Peralta-Soler and Maccioni 1990). The N. crassa 8–10-nm filaments are constituted of a polypeptide of 59 kD ("P59Nc"; Rosa et al. 1990), are profusely distributed in the cytoplasmic and nuclear compartments of the cell in either young or old mycelia (Rosa, Peralta-Soler and Maccioni 1990), and are abnormal in size and shape in the N. crassa snowflake mutant (Allen, Lowry and Sussman 1974). Rosa, Alvarez and Malondado (1990) proposed that the locus sn (snowflake) may be the genetic locus for the P59Nc gene or for a gene whose product is involved in the in vivo assembly of the 8–10-nm filaments. We report here the molecular cloning of the P59Nc gene. Besides, by performing genomic Southern blot and RFLP segregation analyses, we have studied both (i) if the locus sn on the centromere region of chromosome I includes the P59Nc gene and (ii) if the snowflake mutants possess alterations in the cftp locus.

MATERIALS AND METHODS

Strains, growth conditions and crosses: Escherichia coli K802 (Raleigh and Wilson 1986), Y1089 and Y1090 (Young and Davis 1983a), and BB4 (Short et al. 1988) were used for plasmid, λGT11 and λZAP propagation, respectively. N. crassa strains used in this work are listed in Table 1. The "Set1" include 38 (FGSC 4450–87) progeny individuals selected from the cross un-2; arg-5; thi-4; pyr-1;
TABLE 1

Genotypes and origins of N. crassa strains

<table>
<thead>
<tr>
<th>Strain/Genotype</th>
<th>Remarks</th>
<th>FGSC No.</th>
<th>Source</th>
<th>Referencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type 74-OR23-1A</td>
<td>“Oak Ridge”</td>
<td>987</td>
<td>FGSC</td>
<td>1</td>
</tr>
<tr>
<td>Mauriceville-1-c-A</td>
<td>“Exotic”</td>
<td>4416</td>
<td>FGSC</td>
<td>2</td>
</tr>
<tr>
<td>snC136 - a</td>
<td>snowflake</td>
<td>947</td>
<td>FGSC</td>
<td>1</td>
</tr>
<tr>
<td>snJL301 - a</td>
<td>snowflake</td>
<td>4338</td>
<td>FGSC</td>
<td>1</td>
</tr>
<tr>
<td>T(VII → I)3936 - a</td>
<td>Translocation VII → I</td>
<td>2105</td>
<td>FGSC</td>
<td>4</td>
</tr>
<tr>
<td>un-2::arg-5::thi-4::pyr-1::lys-1::inl::nic-3::ars - I-a</td>
<td>Multiple mutant strain</td>
<td>4488</td>
<td>FGSC</td>
<td>3</td>
</tr>
<tr>
<td>Set 1 (01-38)</td>
<td>RFLP mapping</td>
<td>4450-4487</td>
<td>FGSC</td>
<td>3</td>
</tr>
<tr>
<td>Set 2 (RT01-RT10)</td>
<td>RFLP mapping</td>
<td></td>
<td></td>
<td>This work</td>
</tr>
</tbody>
</table>

*1, Fungal Genetics Stock Center (FGSC) Catalog, Ed. 3 (1990); 2, METZENBERG et al. (1985); 3, METZENBERG et al. (1984); and 4, PERKINS et al. (1982).

RESULTS

Molecular cloning of P59Nc-cDNAs: A rabbit anti-P59Nc polyclonal antibody (ROSA et al. 1990) was used for immunoscreening of about 30,000 AGT11 clones (80% recombinants as judged from the percentage of Lac- phages in X-Gal plates) (not shown) of a mycelial cDNA N. crassa library (SACHS et al. 1986). Six positive signals were obtained in the primary screening (ANc1 to 6; see MATERIALS AND METHODS). A 250-bp cDNA present in λNc4 was subcloned into the plasmid Bluescript M13(+) to construct the plasmid pBE4 (Figure 1) and used as a probe to obtain several longer P59Nc cDNAs from a mycelial specific AZAP N. crassa library (ORBACH, SACHS and YANOFSKY 1990). This, and further additional screening of
The cfp Locus in Neurospora

A B

FIGURE 2.—RFLPs in the genomic region of the cfp locus. (A) Southern blot analyses of total DNAs from Mauriceville-1-c (M) or multicent-2 (O) strains digested with EcoRV (EV), EcoRI (EI), XbaI (XI), ClaI (CI), and Bel (BI), and hybridized with the 2.0 kb (pET2) P59Nc-cDNA probe. The short horizontal lines at the left indicate the position of molecular size standards (ADNA, HindIII digested). RFLPs are detected for the BclI, ClaI, EcoRI and XbaI enzymes. (B) Southern blot showing the segregation of the BclI RFLP (indicated by arrows and represented as M or O below the photographs) among 38 individuals (01, 02, 03, ..., 38, indicated above the photographs) of a selected progeny from the cross Mauriceville-1-c (M) × multicent-2 (O) (METSZENBERG et al. 1984). For details see text.

Characterization of genomic RFLPs in the region of the P59Nc gene and RFLP mapping of the cfp locus: The search for RFLPs in the genomic region of the cfp locus was carried out in two N. crassa strains having large differences in nucleotide sequences scattered in the genome. The strains are designed M (Mauriceville-1-c) and O (multicent-2) (Table 1; METZENBERG et al. 1984, 1985). RFLPs for BclI, ClaI and other enzymes were detected with the 2.0-kb cDNA, representing the entire coding region of the P59Nc gene, was used in the experiments reported below.

Characterization of genomic RFLPs in the region of the P59Nc gene and RFLP mapping of the cfp locus: The search for RFLPs in the genomic region of the cfp locus was carried out in two N. crassa strains having large differences in nucleotide sequences scattered in the genome. The strains are designed M (Mauriceville-1-c) and O (multicent-2) (Table 1; METZENBERG et al. 1984, 1985). RFLPs for BclI, ClaI and other enzymes were detected with the 2.0-kb P59Nc-cDNA (Figure 1) as probe (see Figure 2A; five examples among the enzymes tested are shown).
The recent isolation and characterization of P59Nc, the polypeptide of 59-kD constituent of the 8–10-nm diameter cytoplasmic filaments in N. crassa cells (Rosa...
The *CJp* Locus in *Neurospora*

**TABLE 2**

**P59Nc- RFLP types in *N. crassa* strains**

<table>
<thead>
<tr>
<th>RFLP for the restriction enzyme</th>
<th>Wild type</th>
<th>multicent-2</th>
<th>Mauriceville-1-c</th>
<th>snC136</th>
<th>snJL301</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>BamHI</em></td>
<td>I</td>
<td>I</td>
<td>III</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td><em>Cla I</em></td>
<td>I</td>
<td>II</td>
<td>I</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td><em>Eco RI</em></td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td><em>Pvu II</em></td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>

* RFLP types are arbitrarily defined as I ("Oak Ridge"), II ("No Oak Ridge"), and III ("exotic").
* RFLP types = "I": 4.48 kbp; "II": 3.98 kbp.
* RFLP types = "I": 0.59 kbp; "II": 0.91 kbp.
* RFLP types = "I": 1.03 kbp; "II": 3.75 kbp.
* RFLP type = "I": 3.44 kbp.

*et al. 1990; ROSA, PERALTA-SOLER and MACCIONI 1990*, opened the question about the cellular role(s) of these structures. Interestingly, it was found that the *N. crassa* morphological mutants *snowflake* showed a dramatic alteration in the *in vivo* array of the P59Nc 8–10-nm filaments (*et al.* 1990). We hypothesized that (i) the defect in *snowflake* could be related to a mutation in the P59Nc gene which modifies the properties of the polypeptide for *in vivo* supramolecular assembly, or (ii) the abnormal bundles of filaments observed in *snowflake* could be due to a mutation in a different gene whose product modifies the *in vivo* assembly-disassembly properties of the P59Nc 8–10-nm filaments (*et al.* 1990).

To distinguish between these possibilities we first cloned the P59Nc gene. In a second step, by using RFLP segregation analyses (*et al.* 1980; MEZETZENBERG et al. 1985), we mapped the *cfp* locus in wild-type and *sn* mutant strains. The study of the segregation of a *BclI* RFLP showed that the *cfp* locus is on the right arm of chromosome VII. Analysis of P59Nc *ClaI* and *BamHI* RFLPs, in strains partially duplicated for a distal fragment of the right arm of chromosome VII, indicated that the *cfp* locus is positioned at the left of the proximal breakpoint of the translocation *T(VII + I)*5936 roughly at about 5 map units of the locus for.

The mapping of the P59Nc gene to the linkage group VII strongly supported the notion that the *snowflake* locus (*sn*), on the centromere region of linkage group I, is not the genetic locus of P59Nc. RFLP mapping studies demonstrate that *sn* mutations did not represent a translocation of the P59Nc gene. Besides, the experiments indicate that the *snowflake* mutants possess a single, apparently normal, copy of the P59Nc gene at its normal locus on linkage group VII.

Taken together our results indicate that the aberrant bundles of 8–10-nm filaments observed in the *snowflake* mutants are not produced by alterations in the primary sequence of the P59Nc polypeptide. The possibility that the putative product of the *sn* locus is a post-translational modifier of the P59Nc polypeptide and/or of the *in vivo* properties of the P59Nc filaments to form bundles still remains.

**FIGURE 4.**—(A) P59Nc-*ClaI* RFLP types in DNAs from wild-type 74-OR23-1A ("Oak Ridge"), Mauriceville-1-c, multicent-2, or *snowflake* C136 and JL301, *N. crassa* strains. DNAs were digested with *ClaI* and hybridized, after Southern blotting, with the P59Nc-2.0-kb probe (PET2; see Figure 1). The arrows at the left indicate *ClaI* hybridizing fragments of 2.26, 0.91 and 0.59 kb, from top to bottom, respectively. (B) Southern blot showing the segregation of the P59Nc-*ClaI* RFLP among morphologically wild type (wt, 1–5) and *snowflake* (sn, 6–10) progeny strains selected at random from the cross Mauriceville-1-c × *snowflake* JL301.
We thank A. Kornblitt and H. N. Torres for the λGT11 and XZAP mycelial libraries; C. Yanofsky for the E. coli strain K802; F. Terenzi for the initial help with N. crassa crosses; M. Maunino for stimulating discussion along the course of this work and for sharing with us their unpublished results; Craig Wilson from the FGSC is acknowledged for his efforts to send strains and information relative to them. This work was supported by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), CONICOR (Consejo de Investigaciones Científicas y Técnicas de Cordoba) and FUNDACION ANTORCHAS.

LITERATURE CITED


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