A Prolactin-Inducible T Cell Gene Product Is Structurally Similar to the *Aspergillus nidulans* Nuclear Movement Protein NUDC

Shelli M. Morris, Paul Anaya, Xin Xiang, N. Ronald Morris, Gregory S. May, and Li-yuan Yu-Lee

Department of Cell Biology (S.M.M., P.A., G.S.M., L-y.Y-L.)
Department of Microbiology and Immunology (L-y.Y-L.)
Department of Medicine (L-y.Y-L.)
Baylor College of Medicine
Houston, Texas 77030

Department of Pharmacology (X.X., N.R.M.)
University of Medicine and Dentistry of New Jersey
Robert Wood Johnson Medical School
Piscataway, New Jersey 08854

Clone 15 (*c15*) was originally identified as a PRL-inducible gene in activated T cells. Sequence analysis of *c15* revealed that the last 94 amino acids of *c15* are 68% identical and 78% similar to the filamentous fungus *Aspergillus nidulans* nuclear movement protein NUDC. The identification of the mammalian (rat) *c15* protein suggests that the carboxy-terminal NUDC-like region has been conserved over evolution for an important structure and/or function. To determine whether *c15* is functionally analogous to NUDC, complementation studies were performed using the inducible/repressible pAL5 vector system. The results of the complementation experiments show that the full-length mammalian *c15* protein is not only capable of rescuing the nuclear movement defect of the *nudC3* mutants, but is also able to restore the expression of the downstream endogenous NUDF protein to near wild type levels. These results indicate that rat *c15* and fungal NUDC not only share similar structures, but also serve similar functions. Taken together, the structural and functional conservation between *c15* and NUDC is consistent with the notion that *c15* is the rat homolog of *nudC* and has therefore been given the name *RnudC*.

(Molecular Endocrinology 11: 229–236, 1997)

INTRODUCTION

When T cells come in contact with antigen-presenting cells or antigen-bearing target cells, engagement of
In simple eukaryotes such as fungi and yeast, nuclear movement is a MT-dependent process (10, 11). The main MT-dependent “motor” involved in centrosomal and nuclear positioning has been identified as cytoplasmic dynein. The evidence that cytoplasmic dynein is the main motor protein for nuclear positioning comes from the studies of the nudA, ro-1, and DYN1 genes, which encode the cytoplasmic dynein heavy chains of Aspergillus nidulans, Neurospora crassa, and Saccharomyces cerevisiae, respectively. Mutations in the nudA gene and the ro-1 gene cause a failure of nuclear movement through the germ tubes (12, 13). In *A. nidulans*, the dynein heavy chain has been localized to the tip of the growing germ tube (14). A mutation in the 8-kDa *A. nidulans* dynein light chain, encoded by the nudG gene, causes the heavy chain to be lost from the tip and also affects nuclear movement (15). Similarly, disruption of DYN1 causes a partial failure of nuclear segregation between mother and daughter cells in yeast. This failure to segregate is caused by a defect in the ability of mitotic nuclei to migrate to the correct orientation before anaphase (16, 17). Additionally, in yeast, MTs attached to the spindle pole body (SPB), the centrosome equivalent in lower eukaryotes, have been shown to be essential for the nuclear orientation process (18). Furthermore, several of the *N. crassa* ropy mutations have been shown to affect dynein, a complex that is required for the coupling of dynein to its cargo, and result in a nuclear movement defect (13, 19, 20). In yeast, mutations have also been identified that affect nuclear positioning (21–23). Together these data support a model in which SPB orientation and nuclear movement are mediated via an interaction between SPB MTs and a dynactin-associated, minus end-directed dynein motor located at the cell cortex.

In filamentous fungi, e.g. *A. nidulans* and *N. crassa*, nuclear movement is required for normal colony formation (13, 24). During the germination of conidia (asexual spores), the parental nucleus divides and the daughter nuclei move out in the germ tube of the developing germling. Temperature-sensitive mutations have been identified in *A. nidulans* that affect this initial stage of nuclear movement as well as *A. nidulans’* ability to maintain nuclei of additional nuclear divisions at a uniform distance from each other. Because of the observable defect in nuclear distribution, this class of mutants was called nud’s (24). In addition to nudA and nudG, two other genes have been identified, nudC and nudF (12, 19–26). The nudC gene encodes a 22-kDa protein of unknown function (25). The nudF gene encodes a 49-kDa protein that is similar to the human lissencephaly (LIS-1) protein (26). LIS-1 is involved in controlling neuronal migration in the cerebral cortex (27). Through *A. nidulans* studies, NUDC has been shown to posttranscriptionally regulate NUDF (26). Additionally, NUDF appears to be an upstream regulator of cytoplasmic dynein/dynactin function, as a newly discovered mutation in the cytoplasmic dynein heavy chain acts as a bypass suppressor of the nudF deletion (D. A. Willins and N. R. Morris, unpublished data). Therefore, through genetic studies, NUDC can be placed upstream of NUDF, and in turn both NUDC and NUDF can be placed upstream of dynein and dynactin.

The rat Nb2 T cell line can be stimulated to proliferate by the addition of 1–10 ng/ml PRL to the cell culture medium (28). One of the PRL-inducible genes cloned from Nb2 T cells is clone 15 (c15) (29). The c15 gene encodes a 332-amino acid (aa) protein (45-kDa) in which the carboxy-terminal 94 aa (Gly 239 to Asn 332) are 68% identical to the carboxy-terminal portion of the *A. nidulans* nuclear movement protein NUDC (Gly 105 to Gly 198). This striking similarity suggests that the dynein/dynactin pathway that mediates nuclear movement in fungi may also be involved in re-orientation of the centrosomal MTs and Golgi in T cells, and that the c15 and nudC gene products may share similar functions. The present work was designed to test the hypothesis that c15 and NUDC are functionally related by determining whether the rat T cell c15 protein could complement the temperature sensitivity of the *A. nidulans* nudC3 mutation.

**RESULTS**

c15 Complementation Strategy and Constructs

The temperature-sensitive *A. nidulans* used in this study contain the pyrG89 mutation. These *A. nidulans* lack orotidine 5’-P decarboxylase, an enzyme required for the uracil and uridine synthetic pathway (30). Therefore, to grow, the pyrG89 *A. nidulans* require exogenous uracil and uridine in their growth media. The pAL5 vector was generated by cloning the *A. nidulans* histone H2A 3’-fragment polyadenylation site downstream of the pAL3 multicloning site (Fig. 1) (31, 32). The H2A 3’-fragment allows for more efficient processing and export of the mature mRNA from the nucleus and greater stability of the mRNA transcript. The pAL5 vector contains, as does the pAL3 parental vector, the alcA alcohol dehydrogenase I gene promoter, which is inducible or repressible based on the growth media and carbon source present. Transcription of the gene of interest is repressed when *A. nidulans* containing this construct are grown on rich glucose media (MAG or YAG), and expression is allowed when *A. nidulans* are grown on glycerol-containing media (32, 33). In addition, both pAL5 and pAL3 vectors contain the ampicillin resistance gene, to allow for selection in *E. coli*, and the *N. crassa* pyrG gene. The pyr4 gene encodes for orotidine 5’-P decarboxylase and is capable of complementing the pyrG89 mutation in *A. nidulans*, thereby allowing *A. nidulans* to grow in the absence of exogenous uracil and uridine (30).

Complementation studies were performed using the pAL5 vector system. The entire c15 open reading frame (ORF), encoding the full-length 332-aa protein, was generated through PCR and was cloned down-
stream of the \(\text{alcA}\) promoter (Fig. 1). \(\text{A. nidulans}\) containing the \(\text{nudC3}\) temperature-sensitive mutation were stably transformed with either the c15 vector or the pAL5 vector. After transformation, 11 c15 vector-transformed strains and 13 pAL5 vector-transformed strains were obtained and assayed for complementation of the \(\text{nudC3}\) mutation at the restrictive temperature (42°C), on the appropriate media (glycerol).

**c15 Complements nudC3 Mutants**

The \(\text{A. nidulans}\) \(\text{nudC3}\) mutant strain is temperature sensitive (Fig. 2A). At the permissive temperature, 32°C, the colonies are similar to the wild type strain as they are large, white, have undergone normal growth and differentiation, and are able to generate spores. However, at the restrictive temperature, 42°C, the \(\text{nudC3}\) colonies are much smaller than the wild type colonies grown at the restrictive temperature, brown in color, restricted in cellular growth, and unable to undergo normal differentiation to form conidia.

After transformation of the \(\text{nudC3}\) mutant strain, the transformants were tested for their ability to complement the temperature-sensitive \(\text{nudC3}\) mutation. All 11 c15 vector-transformed strains complemented the \(\text{nudC3}\) mutation at the restrictive temperature on glycerol media. In contrast, all 13 pAL5 vector-transformed strains failed to complement the \(\text{nudC3}\) mutation at 42°C on glycerol media. To illustrate the complementation phenotype, two representative transformants of each of the two constructs, c15 (designated \(\text{c15}^\text{a1}\) and \(\text{c15}^\text{a2}\)) and pAL5 (designated \(\text{pAL5}^\text{a1}\) and \(\text{pAL5}^\text{a2}\)), were grown on either MAG or glycerol minimal media at the permissive or restrictive temperatures (Fig. 2B). \(\text{A. nidulans}\) containing either construct grew well on either of the two different media at 32°C. However, when the transformants were grown at the restrictive temperature, only those on the glycerol minimal media containing the c15 construct grew. The c15 transformants grew in a similar manner on glycerol minimal media at either 32°C or 42°C. Therefore, the results of this experiment indicate that the rat c15 protein has the ability to complement the functions lost by the mutant \(\text{A. nidulans}\) NUDC protein and restore normal growth and differentiation.

**c15 Protein Is Expressed in Complemented A. nidulans**

To verify that the c15 protein was being expressed in the c15-transformed and -complemented \(\text{A. nidulans}\),
Western blot analysis of the total cellular proteins was performed using affinity-purified rabbit anti-c15-carboxy-peptide (c15-C) antibodies (S. M. Morris, in preparation). The expression of the 45-kDa c15 protein (arrow) was detected in the two c15-complemented strains tested (Fig. 3, lanes 2 and 3) and not in the control nudC3 strain (Fig. 3, lane 1) or those strains containing the pAL5 vector (Fig. 3, lanes 4 and 5). Additionally, the level of c15 protein expression in the two different c15-transformed strains varied. The c15\textsuperscript{5}1 strain (Fig. 3, lane 2) consistently expressed more c15 protein than did the c15\textsuperscript{5}2 strain (Fig. 3, lane 3). Furthermore, the c15-C antibodies specifically recognized a 22-kDa protein band (asterisk), corresponding to the mutant NUDC protein in all protein preparations. Interestingly, the overall levels of endogenous mutant NUDC in the c15-expressing strains was lower than the levels of mutant NUDC found in the nontransformed nudC3 strain or in the two pAL5-transformed strains. Both the 45-kDa and 22-kDa protein bands could be specifically competed by the addition of c15-C to the immunoblotting solutions (data not shown).

Nuclear Movement Phenotype of Complemented A. nidulans

To better characterize the complemented phenotype, the nuclei of the transformed A. nidulans were stained with 4,6-diamidino-2-phenylindole (DAPI) to determine whether or not normal nuclear migration was occurring. A. nidulans were grown for 18 h on coverslips in minimal media containing glycerol at either 32 C or 42 C. When A. nidulans containing the nudC3 mutation were grown at 32 C, their nuclei migrated normally into the germ tube and were maintained at equal distances from each other (Fig. 4A). In contrast, the nuclei of A. nidulans containing the nudC3 mutation, when grown at 42 C, divided but failed to migrate out into the germ tube (Fig. 4C). When nudC3 mutant A. nidulans were transformed with the c15 complementation construct and were grown at 42 C on glycerol minimal media, their phenotype closely resembled that of the nontransformed nudC3 mutants grown at the permissive temperature (32 C). The c15-complemented A. nidulans extended and elongated their germ tube (Fig. 4E), and their nuclei migrated into the germ tube of the developing germling.

To further quantitate the percentage of A. nidulans that moved their nuclei under the various growth con-
ditions, DAPI-stained germlings were counted and scored for their ability to move their nuclei (Fig. 4G). In nudC3 mutants, grown at 32°C in glycerol media, more than 95% of the germlings moved their nuclei. In contrast, when nudC3 mutants were grown at 42°C in glycerol media, only approximately 18% of the germlings counted showed any form of nuclear movement. When the c15-transformed A. nidulans were grown at 42°C in glycerol media, nearly 90% of the germlings moved their nuclei. Therefore, c15 is capable of restoring normal nuclear migration.

c15 Protein Expression Restores NUDF Expression

Endogenous levels of NUDF decrease when nudC3 mutants are grown at the restrictive temperature of 42°C. Studies involving the use of an exogenous promoter to induce nudF gene expression revealed a similar decrease in overall NUDF expression when this construct was transformed into nudC3 mutants that were grown at 42°C. These results suggest that NUDC regulates NUDF levels in a posttranscriptional manner (26). To determine whether or not the expression of c15 at 42°C in nudC3 mutants could rescue NUDF expression, Western blot analysis of total cellular proteins was performed using affinity-purified anti-NUDF antibodies. The expression of the 49-kDa NUDF protein was detected at normal levels in the wild type strain (Fig. 5, lane 1) and in the nudA4 mutant strain (Fig. 5, lane 2) grown at 42°C. However, the expression of the NUDF protein was greatly decreased in the nudF7 mutant strain and the nudC3 mutant strain (Fig. 5, lanes 3 and 4) grown at 42°C. When the c15 protein was expressed at 42°C, in the two c15-complemented strains tested, the expression of NUDF was restored (Fig. 5, lanes 5 and 6). The level to which NUDF protein was restored correlates well with the level of c15 protein expression observed in the two c15-transformed strains (Fig. 3). As a control for the transformation, two control pAL5 vector-containing strains were tested and the levels of NUDF were decreased to levels observed in the nudC3 parental strain (Fig. 5, lanes 7 and 8). The lower band is nonspecific (dot). Therefore, the results of this experiment show that c15 expression can rescue the expression of NUDF at the restrictive temperature.

DISCUSSION

The c15 gene was originally cloned as a PRL-responsive gene from rat Nb2 T cells (29). GenBank searches revealed that the c15 protein exhibits high similarity over its carboxy-terminal 94 aa to the A. nidulans nuclear movement protein encoded by the nudC gene. Based on the similarity that exists between c15 and NUDC, complementation studies were performed to determine whether c15 could functionally rescue temperature-sensitive A. nidulans nudC3 mutants. The results of our experiments show that the expression of the 45-kDa c15 protein in an A. nidulans nudC3 mutant strain can complement the temperature-sensitive growth and nuclear movement phenotypes, as well as restore depleted endogenous NUDF protein to near wild type levels. These results indicate that c15 functions like NUDC in A. nidulans and suggest that c15 may play a functional role in Nb2 T cells similar to the role played by NUDC in A.nidulans. It is interesting to note that the levels of endogenous NUDC in the c15-complemented strains are lower than the levels seen in the nudC3 strain or vector control, pAL5-transformed strains. Potentially, the expression of the transformed c15 protein may have the ability to feedback and down-regulate the expression of the endogenous mutant NUDC protein or in some way affect the stability of the mutant NUDC protein, thereby allowing the cell to maintain a “critical” overall level of NUDC/c15 expression.

The nudC3 allele of nudC, like other temperature-sensitive nud mutants (nudA, nudF, and nudG) grows very slowly at the restrictive temperature (12, 25, 26). The nuclei of these various nud mutants divide normally; however, nuclear migration through the germ tube is arrested. Biochemical studies have shown that the nudC3 mutation affects nuclear migration and growth rate by causing a decrease in the intracellular levels of NUDF, a protein that is essential for nuclear migration in A. nidulans (26). Mutations in the nudF gene that cause a reduction in NUDF protein level also inhibit nuclear movement. Previous work has shown that the effect of the nudC3 mutation on NUDF levels is posttranscriptional, and that NUDC and NUDF are not in complex with each other (26). Recently, a mutation in the A. nidulans cytoplasmic dynein heavy chain that acts as a bypass suppressor of the nudF deletion has been discovered (D. A. Willins and N. R. Morris, unpublished data). This finding suggests that NUDF may serve as an upstream regulator of dynein/
senger, by removing the acetyl group at the tor acetylhydrolase (PAFAH) (40). The PAFAH enzyme regulatory subunit of bovine brain platelet-activating fac-
migration with those involved in neuronal migration and
These observations potentially link the genes and pro-
to establish a new position for the cell body (38, 39).

The nudF gene encodes a 49-kDa WD-40 protein with a putative coiled-coil domain, similar to the β-subunit of heterotrimeric G proteins (26, 35, 36). However, NUDF most closely resembles (42% identity) the human lissen-
cephaly-1 gene (LiS-1) (27). Lissencephaly is a neuronal migration disease characterized by the inability of neu-
rions to migrate to their proper positions in the cerebral
(37). There is evidence to suggest that neurons may migrate by first extending a long process through which the nucleus and associated organelles then move to establish a new position for the cell body (38, 39). These observations potentially link the genes and pro-
tions involved in centrosomal positioning and nuclear migration with those involved in neuronal migration and brain development. LIS-1 is 99% identical to the 45-kDa regulatory subunit of bovine brain platelet-activating fac-
eter acetylhydrolase (PAFAH) (40). The PAFAH enzyme inactivates platelet-activating factor, a lipid second messenger, by removing the acetyl group at the sn-2 position (41, 42). The structural similarities that exist between PAFAH, LIS-1, and NUDF suggest that these proteins may be involved in PAFAH-associated functions. Thus, NUDF may play a role in regulating the activity or the targeting of the PAFAH complex in mammalian cells.

Centrosome orientation in T cells appears to be mediated by a force on centrosomal MTs just as nu-
clear movement is mediated by a force on SPB MTs (8, 11). We propose that c15 may play a role in the cen-
trosome reorientation that occurs when a T cell meets an antigen-presenting cell or antigen-bearing target cell (3, 43, 44). In this way, the Golgi apparatus and its associated secretory vesicles are reoriented, allowing transport of vesicles along the centrosomal MTs to-
the site of contact (3, 44). Although maximal c15 gene induction in T cells by PRL occurs at the G1/S transition (29), a constitutive level of c15 protein al-
ready exists (data not shown), which may participate in the rapid centrosome MTOC reorganization process. A similar kinetics of c15 induction was observed in IL-3-stimulated premyeloid cells which suggests that c15 induction by cytokines is part of an activation response (29). Furthermore, the c15 gene product could be involved in other cytoplasmic dynein-mediated functions other than moving centrosome and nuclei, e.g. vesicle migration or mitosis (45, 46), which occur later in the cell cycle as suggested by the kinetics of c15 induc-
in PRL-stimulated T cells (29). This additional func-
tional complexity is also suggested by the large differ-
ence in size between the 45-kDa c15 protein and the 22-kDa NUDF protein. The size difference is due to a much larger amino-terminal domain in the rat c15 protein, which contains basic and acidic motifs that may be involved in protein-protein interactions.

In summary, we have identified a mammalian (rat) gene from PRL-stimulated T cells, c15, that when ex-
pressed in A. nidulans nudC3 mutants can functionally rescue the nuclear movement defect. The fact that the rat c15 protein can replace the nuclear movement func-
tion of the A. nidulans nudC gene suggests that c15 and NUDF have similar functions. Because of the structural and functional similarities between c15 and NUDF, c15 is likely a mammalian homolog of nudC, and we have therefor named it RnuDc. Additionally, c15/RNUDC is widely represented throughout eukaryotes, as immuno-
reactive c15/RNUDC-like proteins have been detected in Drosophila (J. Cunniff and R. Warrior, personal commu-
nication), monkey, and man (data not shown).

Future studies will address the localization of c15/ RNUDC in the cell and its association with other pro-
teins. We are particularly interested in determining whether c15/RNUDC localizes to the centrosome MTOC or to the cortex in PRL-stimulated Nb2 T cells. These studies will help determine the role of c15/ RNUDC in the events leading to T cell activation, pro-
fiferation, and differentiation.

**MATERIALS AND METHODS**

**A. nidulans Strains and Growth Media**

The A. nidulans strains used were AO1 (nudC3; pabaA1; wa2; nicA2; pyrG89), XX21 (nudF7; pyrG89; ya2), X8B (nudA4; pyrG89; wa2; chaA1), GR5 (pyrG89; wa3; pyrA44), and SJ002 (pyrG89) (12, 25, 26). The temperature-sensitive nudC3, nudF7, and nudA4 mutants grow normally at the permissive temperature of 32°C, but are severely restricted in growth at the restrictive temperature of 42°C. Nontrans-
formed A. nidulans colonies were grown on Malt Extract Medium (MAG) [2% malt extract (Difco, Detroit, MI), 0.2% peptone, 1% dextrose, trace elements (1000 × solution: 40
mg/liter Na2B4O7·10H2O, 400 mg/liter CuSO4·5H2O, 800 mg/liter ferric citrate or chloride, 800 mg/liter MnSO4·H2O, 800 mg/liter NaMoO4·2H2O, 8 g/liter ZnSO4·7H2O, vitamins (500 × solution: 0.1% p-aminobenzoic acid, 0.1% niacin, 0.1% pyridoxine HCl, 0.1% riboflavin, 0.1% thiamine HCl, 0.1% choline HCl, 0.2% d-biotin), 2% agar] or Yeast Extract Medium (YAG) (0.5% yeast extract, 1% dextrose, 10 mM MgSO4, trace elements, vitamins, 1.5% agar) supplemented with 0.12% uridine and 0.11% uracil (UU) (United States Biochemical Corporation, Cleveland, OH) (24, 47). YAG without agar + UU was used for the growth of liquid cultures for transfections. After transformation, A. nidulans colonies were maintained on MAG or YAG plates lacking UU. For complementation experiments, transformed A. nidulans were additionally grown on supplemented minimal media plates [2% 50× salts (50× salts: 300 g/liter NaNO3, 26 g/liter KCl, 24.65 g/liter MgSO4·7H2O, 12 mM KPO4, pH 6.8, 2% trace ele-
ments, vitamins, 1.25% agar] containing 10 ml/liter glycerol (32). For protein isolation experiments, A. nidulans were
grown in liquid minimal media containing 10 ml/liter glycerol with or without UU.

Complementation Vectors

The 100A plasmid, containing the entire rat c15 ORF (29), was used as a template to generate a 1.0-kb DNA fragment by PCR using Pfu polymerase (Stratagene, La Jolla, CA). The upstream primer contained a KpnI linker (lowercase): 5’-gcgggacccGATGGGAGGGAAGC-3’ (start codon underlined), and the downstream primer contained an XbaI linker (lowercase): 5’-gcgggacccCTAGTTGAATTTGGC-3’ (stop codon underlined). The KpnI/XbaI-digested PCR product, encoding c15, was cloned downstream of the aicA promoter in the pAL5 vector (32) and confirmed by sequencing. The c15 vector and pAL5 vector were used to transform A. nidulans containing the nudC3 mutation.

A. nidulans Transformation

Conidia (1 × 10⁸) were inoculated into 50 ml YAG without agar supplemented with UU. The conidia were allowed to germinate for about 5.5 h, or until the emerging germ tube was visible, at 32 C with shaking. The germinated conidia were harvested by centrifugation, resuspended in 40 ml lytic mix (20 ml Solution A (0.1 M citric acid, 0.8 M (NH₄)₂SO₄ pH 5.8 with KOH pellets), 20 ml Solution B (1% yeast extract, 2% sucrose, 40 mM glucose, trace elements, vitamins), 10 mM MgSO₄, 200 mg BSA (Sigma, St. Louis, MO), 100 mg Noverzyme 234 (Sigma), 125 μl gluconoridase (Sigma), and the cell wall was digested for 2 h at 32 C with shaking. The protoplasts were washed two times in Solution C (50 mM citric acid, pH 6.0, 0.4 M (NH₄)₂SO₄, 1% sucrose) and were resuspended in 1 ml Solution E (0.6 M KCl, 100 mM CaCl₂, 10 mM Tris-HCl, pH 7.5). Protoplasts (100 μl) were added to 6 μg plasmid DNA, followed by the addition of 50 μl Solution D (25% polyethylene glycol 8000, 100 mM CaCl₂, 0.6 M KCl, 10 mM Tris-HCl, pH 7.5), and incubated on ice for 20 min. Next, 1 ml Solution D was added to the protoplasts and allowed to incubate at room temperature for 30 min. Aliquots (200 μl) of the protoplast mixture were plated in 3 ml 45 C sucrose top agar (0.5% yeast extract, 20 mM glucose, 1 M sucrose, trace elements, vitamins, 1% agar) onto sucrose plates (0.5% yeast extract, 20 mM glucose, 0.2 M sucrose, trace elements, vitamins, 1.5% agar), and incubated at 32 C for 3-4 days.

Complementation of nudC3 Mutants with c15

To test for complementation, conidia containing either the c15 or pAL5 construct were streaked on either MAG or glyc-agar supplemented with UU. The conidia were allowed to germinate for about 5.5 h, or until the emerging germ tube was visible, at 32 C with shaking. The germinated conidia were harvested by centrifugation, resuspended in 40 ml lytic mix (20 ml Solution A (0.1 M citric acid, 0.8 M (NH₄)₂SO₄ pH 5.8 with KOH pellets), 20 ml Solution B (1% yeast extract, 2% sucrose, 40 mM glucose, trace elements, vitamins), 10 mM MgSO₄, 200 mg BSA (Sigma, St. Louis, MO), 100 mg Noverzyme 234 (Sigma), 125 μl gluconoridase (Sigma), and the cell wall was digested for 2 h at 32 C with shaking. The protoplasts were washed two times in Solution C (50 mM citric acid, pH 6.0, 0.4 M (NH₄)₂SO₄, 1% sucrose) and were resuspended in 1 ml Solution E (0.6 M KCl, 100 mM CaCl₂, 10 mM Tris-HCl, pH 7.5). Protoplasts (100 μl) were added to 6 μg plasmid DNA, followed by the addition of 50 μl Solution D (25% polyethylene glycol 8000, 100 mM CaCl₂, 0.6 M KCl, 10 mM Tris-HCl, pH 7.5), and incubated on ice for 20 min. Next, 1 ml Solution D was added to the protoplasts and allowed to incubate at room temperature for 30 min. Aliquots (200 μl) of the protoplast mixture were plated in 3 ml 45 C sucrose top agar (0.5% yeast extract, 20 mM glucose, 1 M sucrose, trace elements, vitamins, 1% agar) onto sucrose plates (0.5% yeast extract, 20 mM glucose, 0.2 M sucrose, trace elements, vitamins, 1.5% agar), and incubated at 32 C for 3-4 days.

Protein Preparation and Western Blot Analysis

To prepare total cellular proteins, 5 × 10⁸ conidia were inoculated into 50 ml supplemented minimal media containing 10 ml/liter glycerol and incubated for 42 h at 42 C with shaking. The mycelia were harvested by centrifugation, washed in ice-cold H2O, collected by filtration through cheesecloth, and pressed dry. The mycelia were ground in a Tenbroek homogenizer (Fisher Scientific, Pittsburgh, PA) in 1–2 ml extraction buffer [50 mM Tris-HCl, pH 7.4, 200 mM NaCl, 5 mM EDTA, 5 mM benzamidine, 1 mM phenylmethyl-sulfonyl fluoride, 10 μg/ml each of soybean trypsin inhibitor, aprotinin, leupeptin, N-tosyl-l-phenylalanine chloromethyl ketone (Sigma), and insoluble proteins were removed by centrifugation at 14,000 rpm for 5 min at 4 C. Protein concentration was determined by Bradford assay (Bio-Rad, Richmond, CA) using BSA as a standard. The cellular proteins were stored at −20 C.

For Western blotting, 50 μg total cellular proteins were analyzed by 12% SDS-PAGE and transferred to Immobilon P (Millipore, Bedford, MA) or nitrocellulose (Bio-Rad) as previously described (29). To determine c15 expression, the blot was blocked with 5% nonfat milk, 0.2% Tween 20 (Sigma) in Tris-buffered saline, and affinity-purified rabbit anti-c15-C antibodies (S. M. Morris, in preparation) were applied in a 1:500 dilution, followed by the addition of donkey anti-rabbit IgG antibodies conjugated to horseradish peroxidase (Amer- sham, Arlington Heights, IL) in a 1:2000 dilution. c15 proteins were detected with the enhanced chemiluminescence system as suggested by the manufacturer (Amersham). To detect NUDF, affinity-purified rabbit anti-NUDF antibodies were used at a 1:100 dilution, followed by goat anti-rabbit IgG antibodies coupled to alkaline phosphatase in a 1:2000 dilution, and developed with 5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt and p-nitro blue tetrazolium chloride (26).

DAPI Staining of Nuclei

To stain the nuclei of the developing germlings, 5 × 10⁸ conidia were inoculated into Petri dishes containing sterile coverslips and 25 ml supplemented liquid minimal media with 10 ml/liter glycerol and were incubated at either 32 C or 42 C for 18 h. The coverslips with the attached germlings were rinsed in H2O and placed in methanol at −20 C for 10 min, rinsed well with H2O, and placed in acetone at −20 C for 10 min. The coverslips were rinsed again and placed in a 50 ng/ml DAPI (Sigma) solution for 10 min. After a final rinse, the coverslips were mounted in ProLong Antifade (Molecular Probes, Inc., Eugene, OR) and viewed at 1000× using the Zeiss Axiohot system (Zeiss, Jena, Germany) (Baylor Integrated Microscopy Core, Baylor College of Medicine, Houston, TX).

Acknowledgments

We thank Dr. Stephen A. Osmani for the A. nidulans nudC3 strain and Dr. Sophia Tsai for her critical comments on this manuscript.

Received September 9, 1996. Re-revision received November 19, 1996. Accepted November 21, 1996.

Address requests for reprints to: Dr. Li-yuan Yu-Lee, Department of Medicine, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030.

This work was supported by grants from the American Cancer Society (BE-494) (to L.-y., Y.-L.) and The Linda and Ronald Finger Lupus Research Center (to S. M. M.)

REFERENCES

1. Lenschow DJ, Walunas TL, Bluestone JA 1996 CD28/B7 system of T cell costimulation. Annu Rev Immunol 14:233–258
2. Cantrell D 1996 T cell antigen receptor signal transduction pathways. Annu Rev Immunol 14:259–274
3. Kupfer A, Singer SJ 1989 Cell biology of cytotoxic and helper T cell functions: immunofluorescence microscopic studies of single cells and cell couples. Annu Rev Immunol 7:309–337
4. Euteneuer U, Schliwa M 1992 Mechanism of centrosome positioning during the wound response in BSC-1 cells. J Cell Biol 116:1157–1166
15. Beckwith SM, Morris NR, Cytoplasmic dynein is missing

12. Xiang X, Beckwith SM, Morris NR 1994 Cytoplasmic

14. Xiang X, Roghi C, Morris NR 1995 Characterization and

13. Plamann M, Minke PF, Tinsley JH, Bruno KS 1994 Cy-

10. Oakley BR, Morris NR 1980 Nuclear movement is

21. Schatz PJ, Solomon F, Botstein D 1988 Isolation and

236

MOL ENDO · 1997 Vol 11 No. 2

9. Freeman M, Nusslein-Volhard C, Glover DM 1986 The

8. Raff JW, Glover DM 1989 Centrosomes, and not nuclei,

7. Zolakar M, Erk I 1976 Division and migration of nuclei

6. Hyman AA, White JG 1987 Determination of cell division

5. Hyman AA 1989 Centrosome movement in the early divide-

visions of Caenorhabditis elegans: a cortical site deter-

1. Oka Y, Shiozawa Y 1999 Drosophila microtubule-asso-

26. Xiang X, Osmani AH, Osmani SA, Xin M, Morris NR 1995

NuF, a nuclear migration gene in Aspergillus nidulans, is

similar to the human KIAA1956 gene required for neuronal

migration. Mol Biol Cell 6:297–310

27. Reiner O, Carrozzo R, Shen Y, Wehnert M, Faustinella F,

Dobyns WB, Caskey CT, Ledbetter DH 1993 Isolation of a

Miller-Dieker lissencephaly gene containing G protein β-

subunit-like repeats. Nature 364:717–721

28. Yu-Lee L-Y, Hrachovy JA, Stevens AM, Schwarz LA 1990

Interferon-regulatory factor 1 is an immediate-early gene

under transcriptional regulation by prolactin in Nb2 T

cells. Mol Cell Biol 10:3087–3094

29. Axtell SM, Truong TM, O’Neal KD, Yu-Lee L-y 1995

Characterization of a prolactin-inducible gene, clone 15,
in t cells. Mol Endocrinol 9:312–318

30. Balance DJ, Buxton FP, Turner G 1983 Transformation of

Aspergillus nidulans by the orotidine-5’-phosphate de-

carboxylase gene of Neurospora crassa. Biochem Bio-

phys Res Commun 112:284–289

31. May GS, Morris NR 1987 The unique histone H2A gene of

Aspergillus nidulans contains three introns. Gene 58:59–66

32. Waring RB, May GS, Morris NR 1989 Characterization of

a nuclear migration mutant in Aspergillus nidulans Cell 19:255–262

33. Pateman JA, Doy CH, Olsen JE, Norris U, Creaser EH,

34. Vallee R 1993 Molecular analysis of the microtubule mo-

tor dynein. Proc Natl Acad Sci USA 90:8769–8772

35. Neer EJ, Schmidt CJ, Nambudripad R, Smith TF 1994

Cytoplasmic dynein is required for normal nuclear segrega-

tion. Mol Biol Cell 7:731–742

36. Birnbaumer L 1990 G proteins in signal transduction.

Ann Rev Pharmacol Toxicol 30:675–705

37. Dobyns WB, Elias ER, Newlin AC, Pagon RA, Ledbetter

DH 1992 Casual heterogeneity in isolated lissencephaly. Neuro-

logy 42:1375–1388

38. Morest DK 1970 A study of neurogenesis in the forebrain

of opossum pouch young. Z Anat Entwick-Gesch 130:265–305

39. Book KJ, Morest DK 1990 Migration of neuroblasts by

perikaryal translocation: role of cellular elongation and

axonal outgrowth in the acoustic nuclei of the chick

embryo medulla. J Comp Neurol 297:55–76

40. Hattori M, Adachi H, Tsujimoto M, Arai H, Inoue K 1994

Miller-Dieker lissencephaly gene encodes a subunit of

brain platelet-activating factor. Trends Genet 130:216–218

41. Liscovitch M, Cantley LC 1994 Rapid second messen-

gers. Cell 77:329–334

42. Hattori M, Arai H, Inoue K 1993 Purification and charac-

terization of bovine brain platelet-activating factor acetyl-

hydrolase. J Biol Chem 268:18748–18753

43. Kupfer A, Dennert G 1984 Reorientation of the microtu-

bule-organizing center and the Golgi apparatus in cloned

cytotoxic lymphocytes triggered by binding to lysable

target cells. J Biol Chem 133:2762–2766

44. Kupfer A, Swain SL, Janeway CA, Singer SJ 1986 The

specific direct interaction of helper T cells and antigens-

presenting B cells. Proc Natl Acad Sci USA 83: 6080–6085

45. Lin SX, Ferro KL, Collins CA 1994 Cytoplasmic dynein

undergoes intracellular redistribution concomitant with

phosphorylation of the heavy chain in response to serum

starvation and okadaic acid. J Cell Biol 127:1009–1019

46. Vaisberg EA, Koonce MP, McIntosh JR 1993 Cytoplas-

mic dynein is required for normal nuclear segregation in

filamentous fungi. J Cell Biol 127:139–149

47. Cove DJ 1977 The genetics and physiology of Aspergillus

nidulans. In: Smith JE, Pateman JA (eds) The Genetics of

Aspergillus nidulans. Academic Press, London, pp 81–95

Downloaded from https://academic.oup.com/mend/article-abstract/11/2/229/2754062
by guest

on 27 July 2018