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RESEARCH ARTICLE

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Targeted disruption of a G protein α subunit gene results in reduced pathogenicity in *Fusarium oxysporum*

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Abstract The cloning of *fga1*, the gene encoding a G protein α subunit, was performed by standard PCR techniques and by screening a *Fusarium oxysporum* genomic library, using the PCR product as a probe. The full-length open reading frame spanned 1,059 nucleotides and the deduced primary structure of the protein (353 amino acid residues) showed high identity to those of G protein α_i family proteins from other filamentous fungi. Disruption of *fga1* had no effect on vegetative growth, but reduced the conidiation and pathogenicity of the fungus. Disruptants also showed a decreased level of intracellular cAMP and increased resistance to heat shock at 45 °C. These results suggest that the Gα subunit encoded by *fga1* is involved in a signal transduction pathway in *F. oxysporum* that controls conidiation, heat resistance and pathogenicity.

Keywords *Fusarium oxysporum* · G protein α subunit · Gene disruption · cAMP · Pathogenicity

Introduction

Fusarium oxysporum is a soil-borne plant pathogen that causes wilt disease in a variety of crops. The fungus enters the plant root directly, using penetration hyphae, and colonizes the cortex by intra- and intercellular growth (Rodríguez-Gálvez and Mendgen 1995). Once it reaches the vascular tissue, it spreads rapidly upwards through the xylem vessels, provoking characteristic wilt symptoms (Beckman 1987). To perform these processes correctly, the fungus must perceive chemical and physical signals from the host plant and respond with the appropriate metabolic and morphological changes required for disease development. Many of these responses have been shown to be dependent on conserved signal transduction pathways involving the activation of G proteins (Bölker 1998).

G proteins are heterotrimeric GTP-binding proteins composed of α, β and γ subunits (Neer 1995). They are involved in transducing signals from activated membrane receptors to a variety of intracellular targets (effectors) via a cascade of interacting proteins. Genes for G proteins, especially for the α subunit, have been cloned in many organisms, but their functions are known in detail mainly in mammalian systems. Sequence analysis of the genes has identified four major groups of mammalian Gα subunits, designated as $G\alpha_i$, $G\alpha_s$, $G\alpha_q$ and $G\alpha_{12}$, based on their sequence comparisons and functional similarities (Simon et al. 1991; Wilkie and Yokoyama 1994). $G\alpha_i$ family members are distinguished by the presence of potential sites for *N*-myristoylation (Buss et al. 1987) and ADP-ribosylation by pertussis toxin (Simon et al. 1991; West et al. 1985); and their functions include inhibition of adenylate cyclase, regulation of K^+ and Ca^{2+} channels and activation of retinal cGMP phosphodiesterase (Neer 1995).

Genes for the Gα subunit have also been isolated in various filamentous fungi. Sequencing analysis of these genes has shown that some of them have structural similarities to mammalian $G\alpha_i$ members: they have amino acid sequence homologous to those of mammalian $G\alpha_i$ and they contain consensus sites for *N*-myristoylation and ADP-ribosylation, characteristic of $G\alpha_i$ family members (Simon et al. 1991). $G\alpha_i$ genes in filamentous fungi include *fadA* from *Aspergillus nidulans* (Yu et al. 1996), *cpg1* from *Cryphonectria parasitica* (Gao and Nuss 1996), *magB* from *Magnaporthe grisea* (Liu and Dean 1997), *gna1* from *Neurospora crassa* (Ivey et al. 1996; Turner and Borkovich 1993), *gpa1* from *Ustilago maydis* (Regenfelder et al. 1997), *cga1* from

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Cochliobolus heterostrophus (Horwitz et al. 1999), *ctg1* from *Colletotrichum trifolii* (Truesdell et al. 2000) and *bcg1* from *Botrytis cinerea* (Gronover et al. 2001). The function of these $G\alpha_i$ subunits has been analyzed by targeted gene disruption. Disruption of *bcg1*, *magB*, *cpg1* and *ctg1* results in loss or reduction of pathogenicity, whereas disruption of *gpa1* and *cga1* has no effect on pathogenicity. Disruption of *gna1*, *cpg1* and *cga1* leads to abnormality of sexual cycle. Disruptants of all these genes, except *gpa1* and *cga1*, show reduced vegetative growth. These analyses suggest that $G\alpha_i$ family proteins in filamentous fungi are involved in a wide range of biological processes, including growth, development and pathogenicity.

F. oxysporum is an economically important plant pathogen with a worldwide distribution, but the mechanism of its infection and disease development is not yet well understood. The present paper reports the cloning and sequencing of the gene encoding the $G\alpha_i$ subunit of *F. oxysporum* f. sp. *cucumerinum*. Moreover, disruption of the $G\alpha_i$ gene and altered phenotypes, including pathogenicity in the gene disruptants, are described.

Materials and methods

Strains, plasmids and culture conditions

F. oxysporum f. sp. *cucumerinum* strain F9 (To-Anun et al. 1995) was used in this experiment. Strain F9 was originally isolated from a diseased cucumber plant grown in a naturally infested field. For the extraction of nucleic acids, bud cells were inoculated in potato/sucrose (PS) medium at a concentration of 10^5 cells/ml and incubated at 26 °C on an orbital shaker (120 rpm). Bud cells are special cells produced when *F. oxysporum* is grown in a liquid medium with shaking. After 60 h incubation, mycelia were harvested by filtration through four layers of cheese cloth and washed with ice-cold water. Bud cells in the first filtrate were isolated by the second filtration with glass wool. *Escherichia coli* strain DH5α was used to propagate plasmids. Plasmid pUC18 was used to clone DNA fragments obtained in PCR experiments and to construct a genomic DNA library from *F. oxysporum*. Vector pGEM^R-T (Promega Corporation, USA) was used to clone DNA fragments obtained in RT-PCR experiments. Integrative transformation vector pSH75 (Shiotani and Tsuge 1995) was used for disruption of a fungal gene. pSH75 contains the *E. coli* hygromycin B phosphotransferase gene (*hph*) fused to the *A. nidulans trpC* promoter and terminator. For the construction of pSH75α, the 864-bp *Xho*I/*Eco*RV internal fragment of the putative Gα gene from *F. oxysporum* was blunt-ended using a DNA blunting kit (Takara, Japan) and inserted into the *Eco*RV site of pSH75. For isolation of conidia, fungi were grown on PS agar plates for 7–10 days. Conidia were then harvested using glass beads. Ten glass beads (3 mm diameter) and 5 ml of sterilized water were added to each plate and the plates were shaken gently for 5 min. The conidial suspension was then filtered through glass wool and used for the following experiments.

DNA and RNA manipulations

DNA was prepared from fungal mycelia according to Turgeon et al. (1987). Total RNA was isolated from fungal mycelia, using a Sepasol II RNA extraction kit (Nacalai Tesque, Japan). RNA was further purified using a Total RNA Safekit (BIO 101, USA) to eliminate DNA contamination. Genomic library screening, subcloning and other procedures, including agarose gel electrophoresis, Southern hybridization and colony hybridization, were performed according to standard protocols (Sambrook and Russell 2001). The hybridization signal was detected by the chemiluminescence reaction, using an AlkPhos direct labeling kit (Amersham Pharmacia Biotech, USA). All restriction enzymes, calf intestine phosphatase, T4 DNA ligase and Taq DNA polymerase were used as recommended by the manufacturer (Takara, Japan). The first PCR amplification of the *F. oxysporum* Gα gene was carried out using degenerate primers synthesized based on the amino acid sequences KWIHCF and FLNKKD, which are conserved in Gα proteins from several organisms (Choi et al. 1995; Strathmann et al. 1989). The sequences of these primers are: 5′-ATTCTAGAAART GGATICAYTGITT-3′ and 5′-ATTCTAGATCIACITTITTIAR RAA-3′, with R = A/G, Y = C/T and I = inosine. Each sequence contains a *Xba*I cleavage site at the 5′ end to facilitate the cloning of the PCR product. PCR was performed with the AmpliTaq Gold kit (Perkin Elmer, USA), as described by the supplier, using 0.5 μg of *F. oxysporum* genomic DNA as a template. This PCR product was used as a probe to screen the genomic library of *F. oxysporum*. For the construction of the genomic library, total *F. oxysporum* DNA was digested with *Xba*I and the fragments of approximately 3.0–5.0 kb were purified and inserted into the *Xba*I site of the plasmid pUC18. RT-PCR reactions were performed to obtain cDNA sequence information for the Gα gene and to analyze its transcript in gene disruption experiments. All reactions were carried out with an RNA-PCR Kit (Takara, Japan), using 0.5 μg of total RNA under conditions specified by the manufacturer. Primers used in RT-PCR were GA3 (5′-TCACAATGGGCTGCGGAATGA-3′) and GA4, (5′-CCTGCGTCTCTGTGGTCTTAT-3′), which correspond to N-terminal and C-terminal amino acid sequences, respectively, of the putative Gα protein in *F. oxysporum*. Sequencing reactions were performed using a DNA sequencing kit (Applied Biosystems, USA) and an automatic sequencer (ABI Prism 310; Applied Biosystems) at the Center for Gene Research, Ehime University. Online database comparisons were performed with the BLAST algorithm (Altschul et al. 1990). The sequence obtained in this study has been assigned GenBank accession no. AB072451.

Fungal transformation and gene disruption

Protoplasts of *F. oxysporum* were prepared and transformed with plasmid DNA, according to the method described by Kistler and Benny (1988). Germinated bud cells were suspended in a buffer containing 10 mM Na_2HPO_4 (pH 5.8), 1.2 M $MgSO_4$ and 20 mg Novozyme 234/ml. After incubation at 28 °C for 1 h, the resulting protoplasts were isolated by centrifugation at 1,600 *g* for 10 min. Transformation was conducted with 2×10^7 protoplasts and 20 μg plasmid DNA/ml. Following transformation, the protoplasts were mixed with 0.7% sorbitol soft agar containing 100 μg hygromycin B/ml and overlaid onto sorbitol agar plates [1.2 M sorbitol, 10 mM Tris/HCl (pH 8.0), 1.3% agar]. Hygromycin-resistant transformants were identified after incubation for 4 days.

Phenotypic characterization of *fga1* disruptants

The germination frequency of the conidia was analyzed microscopically after 8 h incubation at 26 °C. Fungal conidia were suspended in PS media, placed on a slide glass and incubated under 100% humidity. Conidia with double the original length were counted as germinated. For the analysis of growth on solid medium, 5 μl conidial suspension containing 10^5 conidia/ml were placed at the center of PS agar plates and incubated at 26 °C for 6 days. For analyzing conidiation, conidia were harvested from a 6-day-old fungal colony, suspended in sterilized water and counted, using a microscope. In order to analyze the effect of heat shock on the survival of the fungus, bud cells were suspended in sterile water (10^3 cells/ml) and incubated at 26 °C, 43 °C or 45 °C for 1 h. Then, 0.1 ml of the cell suspension was plated onto PS plates and incubated at 26 °C for 2 days. Data presented in the results represent mean ± SD for ten replicates from two independent experiments. For statistical analysis, the results were subjected to Student's *t* test.

Pathogenicity tests were performed by the root-dip method (Wellman 1939), using the cucumber plant (*Cucumis sativus* L. cv. Suyo) as a host for the fungus. The roots of 4-day-old cucumber seedlings were gently washed with water and dipped in bud-cell suspension (10^5 cell/ml) for 15 s. The seedlings were then transplanted to pots filled with sterilized soil and cultivated in a growth cabinet under a 12 h light/dark photoperiod at 26 °C. Disease symptoms were rated for 5 weeks after inoculation as follows: 0 = death, 1 = wilting, 2 = yellowing, 3 = no symptoms. For each fungal strain, 5–20 plants were used at one time; and the tests were repeated many times as independent experiments, with similar results. The results shown represent data from two of these experiments chosen at random.

cAMP assay

To measure the in vivo cAMP levels of the fungus, Czapek plates (2% sucrose) overlaid with cellophane were inoculated with conidial suspension (10^3 conidia/plate). The plates were incubated at 26 °C for 8 days. Mycelial pads were scraped from the plates, frozen in liquid nitrogen and ground to a fine powder. Powdered mycelia were suspended in distilled water and the protein was precipitated by adding an equal volume of 20% tricarboxylic acid (TCA). After centrifugation, the supernatant was extracted with ether five times to remove TCA and then lyophilized. The lyophilized sample was dissolved in phosphate buffer (10 mM, pH 7.4) and used for cAMP assay. The assay was carried out using a cAMP enzymeimmunoassay system (Amersham Pharmacia Biotech, USA). Before protein precipitation with TCA, 100 µl of supernatant were removed for protein assay. The protein assay was performed using a Bio-Rad protein assay kit with bovine serum albumin as a standard. Values represent mean ± SD for six replicates from two independent experiments.

Results

Cloning and characterization of *fga1*

PCR amplification of the gene for the Gα homologue from *F. oxysporum* was carried out using degenerate oligonucleotide primers. Using these primers, a single PCR product of approximately 200 bp was obtained. The amino acid sequence deduced from nucleotide sequence of this product revealed 93% identity with *cpg1* from *Cryphonectria parasitica*, *ctg1* from *Colletotrichum trifolii* and *gna1* from *N. crassa*. This PCR product was used as a hybridization probe in Southern blotting analysis and screening of the *F. oxysporum* genomic library.

Total genomic DNA from *F. oxysporum* was digested with four restriction enzymes and subjected to Southern blot hybridization, using the 200-bp PCR product as a probe. A single band was observed in all samples digested with each enzyme (data not shown), suggesting that the Gα gene is present as a single copy in the genome of *F. oxysporum*. One of these enzymes, *Xba*I, showed a hybridized band of approximately 4 kb; and this enzyme was used to construct a genomic library.

The *F. oxysporum* genomic library was screened for the presence of the Gα gene by colony hybridization using the 200-bp PCR product as a probe. Two positive clones were obtained out of 3,000 clones analyzed, both of which contained a 4.2-kb *Xba*I fragment. The full sequence of this fragment contained a putative ORF homologous to Gα from other fungi. RT-PCR was then performed, using oligonucleotide primers corresponding to both ends of the ORF. These experiments showed that the ORF consisted of 1,059 nucleotides and coded for a putative protein of 353 amino acid residues with a molecular mass of 41,024 Da, as shown in Fig. 1. Three introns were inserted within this ORF. Analysis of the deduced primary sequence of the protein showed a high degree of identity with $G\alpha_i$ subunits from other fungi: 99% with *cpg1* from *Cryphonectria parasitica* (Gao and Nuss 1996) and with *ctg1* from *Collectotrichum trifolii* (Truesdell et al. 2000), 98% with *magB* from *M. grisea* (Liu and Dean 1997) and 97% with *gna1* from *N. crassa* (Turner and Borkovich 1993). Based on these similarities, it was concluded that the cloned gene, designated as *fga1*, encodes the homologue of $G\alpha_i$ subunits reported in other fungi.

The predicted amino acid sequence for the gene product of *fga1* contains conserved domains in $G\alpha_i$ proteins (Fig. 1). These include the N-terminal myristoylation site (MGXXXS; Buss et al. 1987) and the C-terminal ADP-ribosylation site by pertussis toxin (CXXX; Simon et al. 1991; West et al. 1985). *fga1*-encoded protein also contains sequence motifs thought to be involved in GTP-binding and hydrolysis (Simon et al. 1991).

Disruption of *F. oxysporum fga1* gene

For disruption of the *fga1* gene, pSH75α containing a 854-bp internal fragment from the *fga1* gene was transformed into *F. oxysporum* and hygromycin

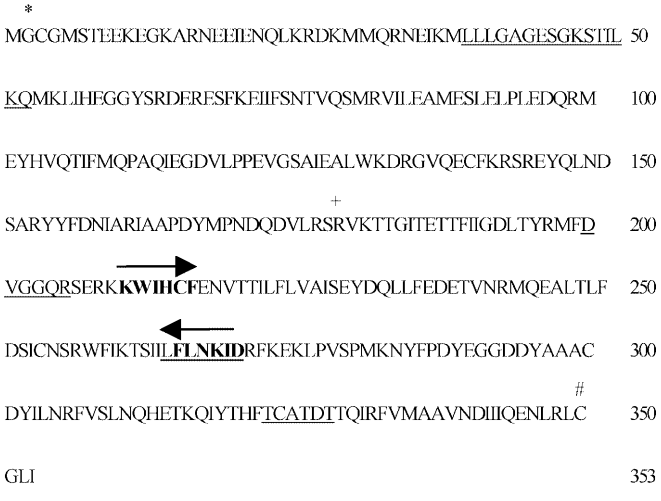

Fig. 1. Deduced amino acid sequence for the G protein α subunit encoded by *fga1*. Potential sites for *N*-myristoylation (*, G2), ADP-ribosylation by cholera toxin (+, R178) and pertussis toxin (#, C350) are *indicated*. Sequences involved in interaction with guanine nucleotide are *underlined*. Amino acid sequences used to design degenerate oligonucleotide primers for PCR amplification are *in bold* and *marked with arrows*

B-resistant transformants were selected (Fig. 2a). Disruption of *fga1* was confirmed by Southern hybridization analysis. Total DNA from the wild-type strain and the transformants was digested with *Xba*I and subjected to Southern analysis, using the 854-bp fragment as a probe. As shown in Fig. 2b, the *Xba*I-digested genomic DNA from the wild-type strain revealed a single band of 4.2 kb. In contrast, two bands of 6.6 kb and 4.4 kb were observed in the two transformants, designated as G109 and G204. These results indicate that *fga1* is disrupted via a single crossover event, due to homologous recombination in these transformants.

Absence of *fga1* transcript in the transformants was confirmed by RT-PCR analysis, using primers designed on the 5′ end and 3′ end sequences of *fga1*. As shown in Fig. 2c, a band of approximately 1.1 kb corresponding to a full transcript of *fga1* was produced in the wild-type strain. However, no such band was observed in the two transformants. These results indicate that *fga1* is disrupted and intact *fga1* transcript is absent in the transformants. In Fig. 2c, RT-PCR analysis for *fgb1*, the gene for a G protein β subunit cloned in this laboratory (unpublished data) in *F. oxysporum*, is also shown. The transcript for the β subunit was detected in both the wild-type strain and *fga1* disruptants, suggesting that expression of *fgb1* is not affected by the disruption of *fga1*.

Phenotypic characterization of *fga1* disruptants

The effect of *fga1* disruption on the growth and development of the fungus was analyzed. Vegetative growth of the disruptants was evaluated on the basis of colony diameter on agar media. As shown in Fig. 3a, the diameter of colonies of the wild-type strain and the disruptants was almost the same after 6 days incubation, suggesting that disruption of *fga1* had no appreciable effect on the vegetative growth of the fungus. In contrast, two disruptants showed altered colony morphology, as shown in Fig. 4 (upper panel). The wild-type strain grew as a profusely branched circular colony. The disruptants, however, grew as elongated colonies. In microscopic observation of the colony after 24 h incubation, the wild-type strain showed radially expanding hyphae from the center of the colony, but disruptants showed a long hyphal axis (Fig. 4, lower panel). The differences in colony morphology were apparent for 2 days after inoculation of bud cells, but later these differences could not be observed clearly. The conidia-forming ability of the disruptants was found to be much reduced when compared to the wild-type strain (Fig. 3b). *fga1* disruptants produced less than half the number of conidia, as compared with the wild-type strain, but the germination frequency of conidia was not altered in the two disruptants (Fig. 3c).

The heat resistance of bud cells was compared in the disruptants and the wild-type strain. As shown in Fig. 5, a slightly higher survival was observed in the disruptants

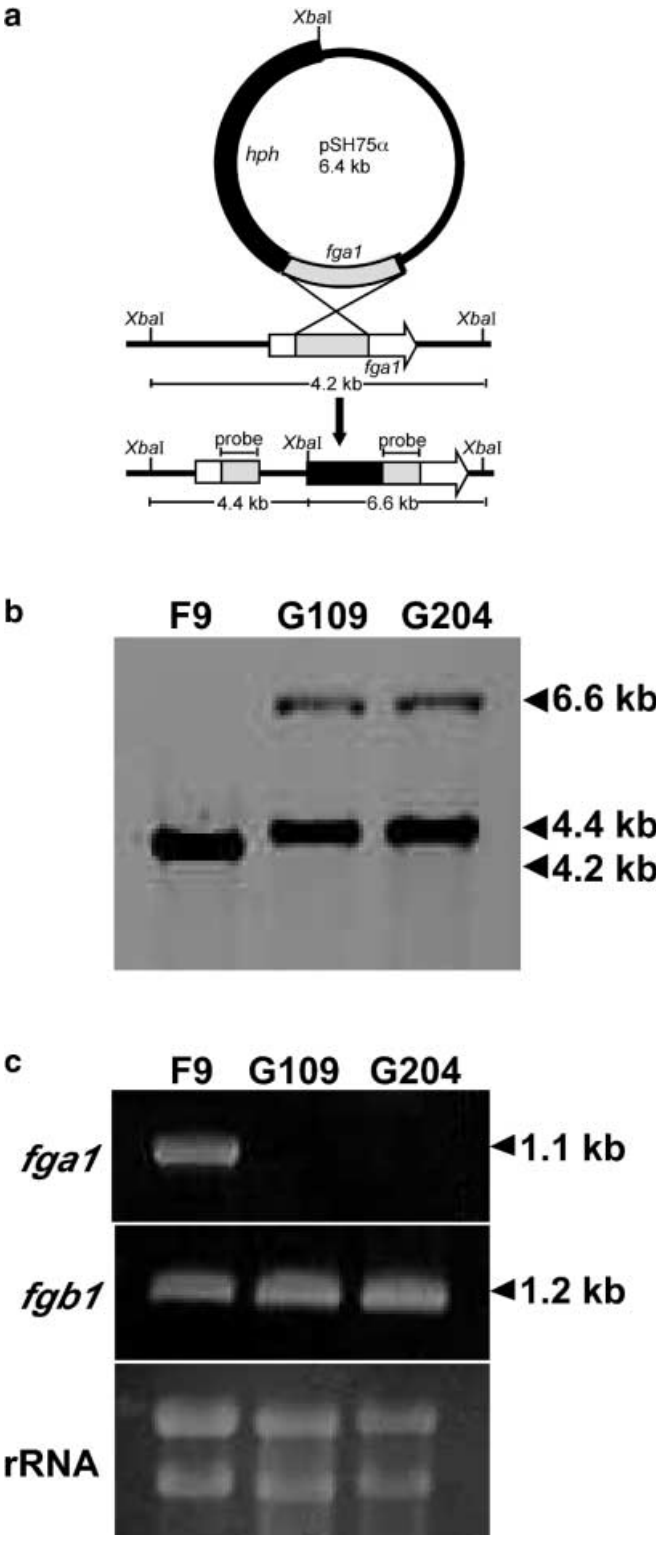

Fig. 2a–c. Disruption of *fga1* locus in *Fusarium oxysporum*. **a** Vector plasmid containing 854-bp *fga1* fragment and integration of this plasmid into the *fga1* locus by homologous recombination. **b** Southern blot analysis of DNA digested with *Xba*I from F9 (the wild-type strain) and the transformants, G109 and G204. The size of each band is *indicated on the right*. **c** RT-PCR analysis of *fga1* and *fgb1* transcripts in F9 and the transformants. *fgb1* is the gene for the G protein β subunit in *F. oxysporum*

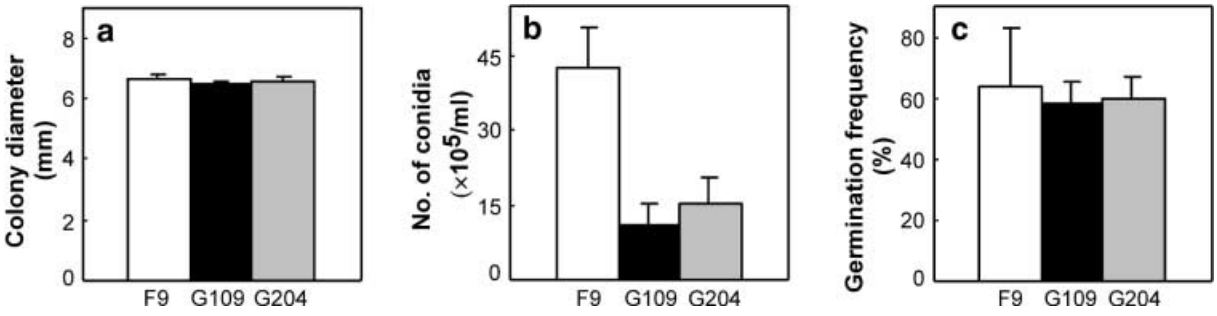

Fig. 3a–c. Effect of *fga1* disruption on growth and development of *F. oxysporum*. **a** Vegetative growth of the wild-type strain (F9) and the disruptants G109 and G204 on potato/sucrose (PS) agar plates, represented as colony diameter after an incubation of 6 days. **b** Conidiation analysis in the wild-type strain and the disruptants. Conidia were collected from 6-day-old fungal colonies and their number was counted. **c** Germination frequency, expressed as a percentage of germinated cells in 200 conidia analyzed. In all three figure-parts, values represent the mean ± SD for ten replicates from two independent experiments. In **a** and **c**, no statistically significant differences were observed between the wild-type and the disruptants, as determined by Student's *t* test ($P > 0.05$). In **b**, significant differences were observed between the wild-type and the disruptants ($P < 0.001$)

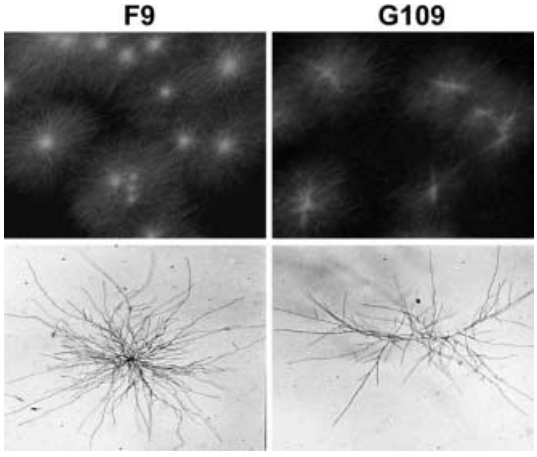

Fig. 4. Colony morphology of a *fga1* disruptant. The wild-type strain (F9) and the disruptant G109 were cultivated on a PS agar plate at 26 °C and photographed at ×4 magnification after 48 h (*upper panel*) or at ×20 magnification after 24 h (*lower panel*)

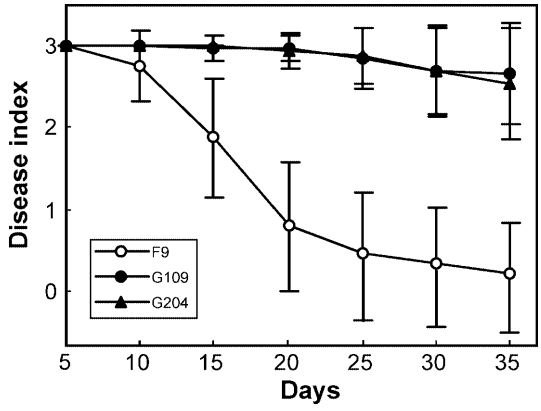

Fig. 6. Incidence of *Fusarium* wilt caused by *F. oxysporum* wild-type strain and *fga1* disruptants on cucumber plants (cv. Suyo). The severity of disease symptoms was recorded at different times after inoculation, using an index ranging from 0 (dead plant) to 3 (healthy plant). Values represent the mean ± SD of 40 plants tested for each fungal strain in two independent experiments

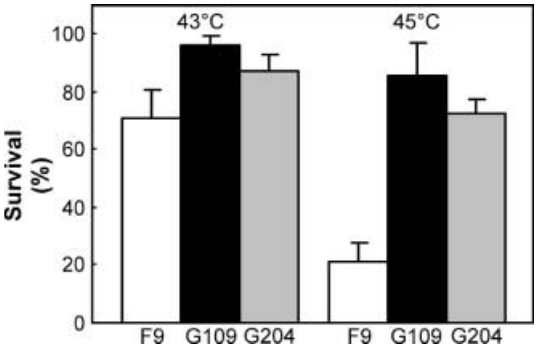

Fig. 5. Effect of *fga1* disruption on the survival of *F. oxysporum* after heat treatment. Bud cells of the wild-type strain (F9) and the disruptants G109 and G204 were heat-treated at the temperatures indicated. Then, approximately 100 cells were spread on PS plates and incubated at 26 °C. Survival is expressed as a percentage of the 26 °C control. Values represent the mean ± SD for ten replicates from two independent experiments. Statistically significant differences were observed between the wild-type and the disruptants, according to Student's *t* test ($P < 0.001$)

at a heat shock of 43 °C, although this difference was more pronounced after heat shock at 45 °C. Approximately 21% of wild-type bud cells survived a heat shock of 45 °C, while more than 70% survival was observed in the two disruptants.

Pathogenicity of the wild-type strain and the two disruptants was analyzed by the root-dip method. As shown in Fig. 6, the wild-type strain induced yellowing of the plants after 15 days and caused wilting or death of the plant within 25 days, but the two disruptants did not induce yellowing till 35 days. The results indicate that pathogenicity is markedly reduced in the *fga1*-disrupted fungus.

In mammalian systems, the $G\alpha_i$ subunit negatively regulates adenylate cyclase, leading to a decrease in cAMP levels (Neer 1995). In order to determine whether the *fga1* product has a function similar to that of the mammalian $G\alpha_i$ subunit in *F. oxysporum*, intracellular cAMP levels were measured in the wild-type strain and the *fga1* disruptants. As shown in Table 1, both the *fga1*

Table 1. Intracellular cAMP accumulation in the wild-type strain F9 and the two *fga1* disruptants, G109 and G204. Values represent the mean ± SD of six replicates from two independent experiments. Statistically significant differences were observed between the wild-type strain and the disruptants according to Student's *t* test ($P < 0.001$)

Strains	cAMP (pmol/mg of protein)	Percentage of the wild-type strain
F9	4.00 ± 0.22	100.00
G109	2.53 ± 0.15	63.25
G204	2.58 ± 0.19	64.50

disruptants show reduced levels of cAMP (approximately 65% of the wild-type strain). This result suggests that the *fga1*-encoded α subunit is involved in the cAMP pathway in *F. oxysporum* and it regulates cAMP levels positively, unlike mammalian $G\alpha_i$, since the gene disruptants show decreased cAMP levels.

Discussion

Fungal α subunits are grouped into three distinct subfamilies (Regenfelder et al. 1997). The deduced amino acid sequence of *fga1* from *F. oxysporum* shows high identity with the members of one group, which corresponds to the mammalian $G\alpha_i$ family. In addition, *fga1*-encoded protein contains possible sites for *N*-myristoylation and ADP-ribosylation, which are characteristic of G protein α_i members (Simon et al. 1991). These results indicate that *fga1* from *F. oxysporum* is a member of the $G\alpha_i$ family.

The function of the Gα subunit has been extensively investigated in mammalian systems. Release of α_i subunit from heterotrimeric complex lowers intracellular cAMP levels by inhibition of adenylate cyclase activity (Chen et. al 1996; Childers and Deadwyler 1996). In fungi, *Cryphonectria parasitica* strains containing a *cpg1* disruption show an increase in cAMP level, suggesting that Gα encoded by *cpg1* inhibits adenylate cyclase activity, like mammalian $G\alpha_i$ (Gao and Nuss 1996). In *N. crassa*, however, deletion of *gna1* leads to lower adenylate cyclase activity and intracellular cAMP level in the cells grown on solid medium (Ivey et al. 1999). The result suggests that the Gα subunit encoded by *gna1* positively controls adenylate cyclase activity. In *M. grisea*, *magB* disruptants fail to develop an appressorium and this defect is overcome by the addition of exogenous cAMP (Liu and Dean 1997), suggesting that the *magB*-encoded α subunit may actually stimulate adenylate cyclase, as observed with *N. crassa*. The present results indicate that disruption of *fga1* in *F. oxysporum* leads to a lower cAMP level in the cell similar to *gna1* disruptants in *N. crassa*. Based on sequence alignment, the G protein α_i subunit from simple eukaryotes forms a unique class, distinct from mammalian $G\alpha_i$ subunits (Wilkie and Yokoyama 1994). Therefore, the function of the $G\alpha_i$ subunit in fungi may differ from that in mammalian counterparts. The role of *C. parasitica cpg1* in the negative regulation of cAMP levels suggests that not all fungal $G\alpha_i$ proteins differ from their mammalian counterpart. It seems likely that there are subclasses of $G\alpha_i$ proteins in the filamentous fungi.

The functional role of *fga1* was inferred from physiological analysis of the *fga1*-disrupted strains. Disruption of *fga1* results in altered colony morphology, reduced conidiation, increased heat resistance and reduced pathogenicity. Similar phenotypes were also observed in $G\alpha_i$ disruptants of other filamentous fungi (see Introduction). The results reported before have shown that disruption of the $G\alpha_i$ gene in phytopathogenic fungi is usually accompanied by a loss or reduction of pathogenicity, except in *Cochliobolus heterostrophus* (Horwitz et al. 1999) and *U. maydis* (Regenfelder et al. 1997).

Increased heat resistance was observed in *N. crassa* strains with a low level of cAMP-dependent protein kinase activity (PKA). Deficiency of adenylate cyclase results in a decreased cAMP level and subsequent lower PKA activity, leading to increased heat resistance (Cruz et al. 1988). Disruption of *gna1* in *N. crassa* also shows a reduction of the intracellular cAMP level (Ivey et al. 1999); and increased sensitivity to heat shock was observed in constitutively activated *gna1* strains (Yang and Borkovich 1999). Similarly, the increase of heat resistance observed in *F. oxysporum fga1* disruptants may be caused by the decrease of intracellular cAMP concentration because of Gα deletion. These results suggest that heat-shock response in fungi is under the control of the cAMP-PKA pathway.

Although no effect of *fga1* disruption was observed on the vegetative growth in the nutrient medium, disruptants showed altered colony morphology, as compared with the wild-type strain. In *B. cinerea*, disruptants of *bcg1* also show altered morphology. *bcg1* disruptants build smaller, more compact, colonies than the wild-type; and the colonies have an atypically even margin (Gronover et al. 2001). In addition to morphological change, *fga1* disruptants showed reduced conidiation. All these observations suggest an important role played by G protein signaling during growth and development in *F. oxysporum*.

F. oxysporum is a soil-borne fungus that infects plants via roots. The ability of this fungus to cause disease depends on the attachment of conidia to the roots, germination of conidia, formation of infection structures, penetration of the host and finally colonization of host tissue. *fga1* disruptants may have a defect in one of these processes. In *M. grisea*, disruptants of *magB* (*fga1* homologue) cannot form an appressorium (an infection structure in this fungus), suggesting that reduction of appressorium formation leads to reduced pathogenicity (Liu and Dean 1997). In *F. oxysporum*, *fga1* may play some role in the infection process, although this fungus has no distinct infection structure, such as an appressorium. Microscopic analysis of the infection and disease development of *fga1* disruptants is now in progress. Further analysis of the downstream effector

molecules of this signaling pathway may provide a better understanding of molecular mechanisms related to fungal pathogenicity.

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