The possible role of ADP-ribosylation in sporulation and streptomycin production by *Streptomyces griseus*

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Mutants resistant to 3-aminobenzamide, a known inhibitor of ADP-ribosyltransferase, were obtained from *Streptomyces griseus* IFO 13189, a streptomycin-producing strain. One (strain no. 4), which had significantly reduced ADP-ribosyltransferase activity, was analysed in detail. Mutant 4 displayed a conditional phenotype with respect to cultivation temperature. At 30 °C, it exhibited severely reduced ability to produce aerial mycelium (on solid medium) and submerged spores and streptomycin (in liquid culture), but this ability was fully restored at 25 °C. The mutant produced A-factor normally, regardless of cultivation temperature, and exhibited normal ability to accumulate ppGpp intracellularly. SDS-PAGE analyses of cellular proteins labelled by [³²P]NAD revealed that an ADP-ribosylated protein with a molecular size of 44 kDa, which appeared in sporulating cultures of the parent strain, was missing from the mutant grown at the non-permissive temperature (30 °C). Genetic analysis showed that the *aba* mutation conferring resistance to 3-aminobenzamide was tightly linked to the altered phenotype. Failure to ADP-ribosylate certain cellular protein(s), presumably due to the *aba* mutation, may be responsible for impaired differentiation in this mutant.

Introduction

ADP-ribosylation is a two-step enzymic reaction. First, NAD is hydrolysed by NAD-glycohydrolase to release nicotinamide and adenosinediphosphoribose (ADPR), then ADPR is bound to specific acceptor proteins by the action of ADP-ribosyltransferase (ADPRT) (reviewed by Skorko, 1982; Ueda & Hayaishi, 1985). Mono-ADP-ribosylation can be detected in prokaryotes and eukaryotes, while poly-ADP-ribosylation occurs only in eukaryotes (Ueda & Hayaishi, 1985). ADP-ribosylation may inactivate proteins; moreover, the modified proteins may later be activated by enzymic de-ADP-ribosylation. These processes play a significant role in various biologically important events such as DNA repair, modification of RNA polymerase, inhibition of protein synthesis, regulation of cell proliferation and differentiation (Ferro *et al.*, 1983; Tanigawa *et al.*, 1983; Lucas *et*

al., 1984; Payne *et al.*, 1985; reviewed by Ueda & Hayaishi, 1985; Lai, 1986; Miwa & Sugimura, 1990). Most of the data available are from eukaryotic organisms. Our knowledge of the presence and role of ADPRT enzymes in prokaryotes is in its infancy.

T4 and N4 phage infections in *Escherichia coli* are known to result in ADP-ribosylation of the α -subunit of RNA polymerase (Skorko, 1982). Nitrogenase activity in *Rhodospirillum rubrum* and *Azospirillum lipoferum* is regulated by ADP-ribosylation of the iron subunit of the enzyme (Pope *et al.*, 1985; Hartmann *et al.*, 1986). Two cytoplasmic proteins of *Pseudomonas maltophilia* are reported to be ADP-ribosylated (Edmonds *et al.*, 1989). After ADPRT activity was reported in *Streptomyces*, first in *S. griseus* and later in other *Streptomyces* species, attempts were made to elucidate its physiological role (Barabas *et al.*, 1986, 1988). In this communication we compare the ADPRT activity in parent and mutant strains of *S. griseus*, and discuss the possible role of this enzyme in the physiological regulation of sporulation and antibiotic production. Genetic aspects of the potential significance of ADP-ribosylation in microbial differentiation are stressed.

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Abbreviations: 3-ABA, 3-aminobenzamide; ADPRT, ADP-ribosyltransferase.

Methods

Chemicals. 3-Aminobenzamide (3-ABA) was purchased from Nakarai Tesque, Kyoto, Japan. [adenine-2,8-³H]NAD and [adenylate-³²P]NAD were from Amersham and ICN Biomedicals, respectively.

Strains and preparation of mutants. *Streptomyces griseus* IFO 13189, a wild-type, prototrophic, streptomycin-producing strain, was provided by the Institute of Fermentation, Osaka, Japan. The spontaneous 3-aminobenzamide-resistant mutants were obtained as colonies that grew within 14 d after wild-type spores were spread on GYM agar (see below) containing 80 mM-3-ABA. The parent strain was able to grow, although slowly, with 50 mM-3-ABA, but its growth was completely suppressed with 80 mM. The resistant clones were purified by single colony isolation.

To obtain auxotrophic mutants spores were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Ochi, 1990a). Auxotrophs were determined by replica plating on synthetic medium II (Ochi, 1990a) with or without appropriate requirements. The spontaneous reversion rate for each single auxotrophic requirement was less than 10⁻⁸.

Media. Sporulation medium contained (per litre): soluble starch, 20 g; yeast extract, 4 g; MgSO₄·7H₂O, 0.5 g; CaCl₂, 50 mg; ZnSO₄·7H₂O, 30 mg; FeSO₄·7H₂O, 25 mg; CuSO₄·5H₂O, 16 mg; and MnSO₄·4H₂O, 12 mg (adjusted to pH 7.0 with NaOH). GYM medium contained (per litre): glucose, 4 g; yeast extract, 4 g; malt extract, 10 g; peptone (NZ-amine, type A), 1 g; NaCl, 2 g (adjusted to pH 7.2 with NaOH). GYM agar contained 2% agar in addition to the above components. Synthetic medium II and starch-polypeptone-yeast extract (SPY) medium have been described previously (Ochi, 1987a). Since 3-ABA is heat-stable, media containing it were autoclaved with all components present.

Growth conditions. A spore suspension was used to inoculate SPY medium, and the culture was incubated at 30 °C as described by Ochi (1987a). After 2 d, cells were harvested by centrifugation, washed twice with 100 ml saline, and suspended in the original volume of saline. Washed cells (1 ml) were added to sporulation medium (50 ml in a 250 ml flask) and then cultured on a rotary shaker (230 r.p.m.) at the temperature indicated.

Fusion of protoplasts. Protoplast fusion was performed as described previously (Ochi, 1990a). The frequency of mycelium regeneration from protoplasts was about 10⁻³. The frequency of recombinants, given as the ratio of prototrophic colonies growing on minimal regeneration medium to the total number of viable colonies on supplemented regeneration medium, was about 3 × 10⁻³. Prototrophic recombinants selected directly on minimal regeneration medium were purified by single colony isolation and then tested for their ability to form aerial mycelium (on GYM agar) and streptomycin (in SPY medium) at 30 °C.

Assay of submerged spores. Sonication-resistant spore titres were determined by direct counting of spores, using phase contrast optics after sonication of cultured broth for 3 min. Spores, but not mycelial fragments, were visible as phase bright objects.

Assay of streptomycin and A-factor. These were determined by bioassay (Ochi, 1987a).

Enzyme assays. NAD-glycohydrolase was assayed as described previously (Ochi, 1987a), except that NAD was used as substrate instead of NADP. To assay ADP-ribosyltransferase (ADPRT), crude extract was obtained by sonic disruption (approx. 1 min at 0 °C) of cells suspended in 10 mM-MOPS buffer (pH 7.2) containing 2 mM-PMSF. Cells were centrifuged (10000 g) at 4 °C for 20 min. The supernatant thus obtained was used as a 'crude extract', which provided not only the enzyme protein but also substrate proteins to be ADP-ribosylated. It contained 1.2 to 2.4 mg protein per ml. The reaction conditions

described by Penyige *et al.* (1990) were modified as follows. A 100 µl sample of crude extract was suspended in 100 µl of a solution containing 25 mM-potassium phosphate buffer (pH 7.0), 10 mM-DTT, 0.5 mM-MgCl₂, 20 mM-KF, 0.1 mM-CaCl₂ and 30 µM-[adenine-2,8-³H]NAD (37 kBq, as a donor of ADP-ribose). Reaction mixtures were incubated at the indicated temperature for 20 min before 200 µl ice-cold 10% (w/v) TCA was mixed in. After 30–60 min at 0 °C, the suspensions were collected on membrane filters (Millipore, 0.45 µm), which were then washed with 5 ml ice-cold 5% (w/v) TCA. Radioactivity on the filters was measured with a liquid scintillation counter. The specific activity of ADPRT is expressed as the amount (d.p.m.) of radioactivity incorporated into acid-precipitable material per mg protein per min.

To obtain autoradiograms of ADP-ribosylated proteins, 30 µM-[adenylate-³²P]NAD (37 kBq) was used instead of [adenine-2,8-³H]NAD. SDS-PAGE was carried out as reported by Penyige *et al.* (1990). The gel was dried and exposed to X-ray film for 8 d at –80 °C.

Alkaline hydrolysis of ³²P-labelled proteins. A crude extract was prepared from parent-strain mycelium harvested in late (48 h) growth phase as described above. One ml reaction mixture containing 30 µM-[adenylate-³²P]NAD (9.25 MBq) was incubated at 35 °C for 20 min. Then proteins were precipitated by adding 1 ml of 20% TCA. After 30 min at 0 °C, then 10 min at 80 °C (to redissolve lipids and nucleic acids), the precipitated proteins were collected by centrifugation at 10000 g for 20 min at 4 °C. The pellet was washed once with ice-cold 10% (w/v) TCA, and then twice with 70% (v/v) ethanol. It was resuspended in a small amount (approx. 3 ml) of 10 mM-Tris/HCl buffer (pH 7.0) containing 6 M-guanidine.HCl to solubilize proteins. For alkaline hydrolysis of ³²P-labelled proteins (Jouanneau *et al.*, 1989), portions (1 ml) of the protein suspension were incubated in 0.1 M-KOH at 27 °C or 37 °C for 3 h. At the end of the incubation, proteins were precipitated with excess perchloric acid (which also removes K⁺) at 0 °C. After 30 min, the samples were centrifuged at 12000 g for 10 min at 4 °C, and the supernatants were concentrated under high vacuum to 200 µl. After further centrifugation (12000 g), 20 µl portions of the supernatant solutions were applied to silica gel TLC plates (60 F₂₅₄, Merck). Reference samples (10 nmol each) of NAD, ADP-ribose, ADP and AMP were also applied in one spot. The chromatograms were developed with *n*-butanol:acetone:acetic acid:5% (w/v) ammonia:water (9:7:5:5:2) for 3 h. The standards were detected by their UV absorption, and the chromatogram was exposed to X-ray film with an intensifying screen for 8 h at –80 °C.

Assay of nucleotide pools and intracellular 3-ABA. Intracellular concentrations of GTP and guanosine 5'-diphosphate 3'-diphosphate (ppGpp) were assayed by HPLC as described earlier (Ochi, 1986b, 1987a). Intracellular concentrations of 3-ABA were determined as follows. Cells (10 ml portions) grown in 50 ml GYM medium containing various amounts of 3-ABA were rapidly collected on a filter paper (diameter 9 cm, no. 2, Toyo Roshi Co.) and washed with 200 ml 0.1 M-LiCl. The filter was then immediately placed upside down in a Petri dish containing 10 ml 1 M-formic acid; the entire collection procedure was completed within 30 s. A separate 40 ml sample was used to determine the dry cell weight. After 1 h incubation in a cold room, a sample for assay by HPLC was prepared as described previously (Ochi, 1986b). The 3-ABA in the extract was determined by HPLC as follows. A 50 µl sample (equivalent to about 0.2 mg dry cell weight) was applied to a column (Hibar LiChlospher 100 RP-18, 5 µm, 4 × 250 mm, Merck) developed with a solution containing 5% (v/v) methanol, 5% (v/v) acetonitrile, 1% (v/v) tetrahydrofuran, and 0.025% phosphoric acid at a flow rate of 1 ml per min. Absorbance was measured at 235 nm; 3-ABA was eluted at a retention time of 4.2 min. The intracellular concentration (mM) of 3-ABA was calculated assuming that 1 mg dry cell weight represented 3.3 µl intracellular water (Ochi, 1987b).

Results and Discussion

Isolation of 3-ABA-resistant mutants

The possible significance of ADP-ribosylation of cellular protein(s) in sporulation of certain *S. griseus* strains has been suggested by Barabas *et al.* (1988). In preliminary experiments, only slight growth inhibition of *S. griseus* was observed on GYM agar containing 50 mM-3-aminobenzamide (3-ABA), whereas aerial mycelium formation (and thus sporulation) was severely depressed. 3-ABA, an analogue of nicotinamide, is a potent inhibitor of poly(ADP-ribosyl)transferase (Purnell & Whish, 1980; Banasik *et al.*, 1992), which plays a central role in ADP-ribosylation reactions in eukaryotes. Although Banasik *et al.* (1992) showed benzamide and its derivatives to be weak inhibitors of mono(ADP-ribosyl)-transferase from hen heterophils, Penyige *et al.* (1990) have demonstrated that 3-ABA strongly inhibits ADP-ribosylation in *S. griseus*. Therefore, some 3-ABA-resistant mutants might have an altered ADPRT affecting sporulation. To assess this possibility, we isolated and analysed mutants. Four colonies (1, 4, 5 and 7) of *S. griseus* 13189 that developed spontaneously on

GYM agar containing 80 mM-3-ABA were selected randomly. These all exhibited severely reduced ability to form aerial mycelium when cultured at 30 °C. Their ability to produce A-factor was normal. The mutants grew as well as the parent strain in sporulation medium or SPY medium, but somewhat slowly in synthetic medium. Mutant no. 4 was used for most further analyses since it exhibited the highest resistance to 3-ABA (up to 100 mM).

Concentrations of 3-ABA within cells grown in the presence of the drug (Table 1) were roughly proportional to, but far lower than, those outside the cells; presumably 3-ABA was taken up through passive, rather than active, transport. The similar intracellular 3-ABA concentrations of the parent and mutant 4 imply that the mutation had not altered the transport system. The intracellular 3-ABA concentration in the presence of 80 mM-3-ABA was about 0.5 mM (Table 1), at which concentration the ADPRT activity of *S. griseus* is known from *in vitro* experiments to be inhibited by 50–60% (Penyige *et al.*, 1990). Thus, the observed growth inhibitory effect of 3-ABA in the parent strain could be due, at least in part, to its action on ADPRT.

Characterization of the mutants

The parent strain began to produce sonication-resistant spores after incubation for 24 h at 30 °C in sporulation medium, and almost all cells (>80%) were present as spores at 48 h. However, the mutant produced 30-fold less spores (Table 2), and was impaired in producing streptomycin. Strikingly, the ability of mutant 4 to produce submerged spores or streptomycin was temperature-dependent; when the incubation temperature was lowered to 25 °C both abilities were completely restored (Table 2). A-factor was produced normally (0.4–1 µg ml⁻¹) regardless of cultivation temperature. The ability to produce aerial mycelium on surface culture was also completely restored at 25 °C. Mutant strains 1, 5 and 7

Table 1. Intracellular 3-ABA concentration in cells cultured in the presence of 3-ABA

Strains were grown at 30 °C for 12 h (mid-exponential phase) in GYM medium, filtered and transferred to the same volume of fresh GYM medium containing various amounts of 3-ABA; incubation was then continued for 5 h. The 3-ABA concentration was assayed as described in Methods.

3-ABA added (mM)	Intracellular concentration (mM)	
	Parent	Mutant 4
20	0.09	0.10
50	0.37	0.32
80	0.52	0.56

Table 2. Sporulation and streptomycin production by parent and mutant strains in liquid culture

Data represent mean values from three independent experiments. There was no marked difference in biomass between parent and mutant strains (2.5–3.1 mg dry weight ml⁻¹ in sporulation medium, 6.8–7.7 mg dry weight ml⁻¹ in SPY medium).

Strain	Sonication-resistant spores ml ⁻¹ *		Streptomycin produced (µg ml ⁻¹)†			
			30 °C		25 °C	
	30 °C	25 °C	2 d	3 d	2 d	3 d
Parent (13189)	3 × 10 ⁸	5 × 10 ⁸	83	95	41	132
Mutant 4	1 × 10 ⁷	4 × 10 ⁸	0	0	50	122
Mutant 7	1 × 10 ⁸	2 × 10 ⁸	8	40	42	97

* Cells were grown in sporulation medium for 3 d at the indicated temperature.

† Cells were grown in SPY medium for 2 d or 3 d at the indicated temperature.

also showed this conditional phenotype for aerial mycelium formation, submerged spore formation, and streptomycin production, but the restoration at 25 °C was incomplete. The impaired ability of mutant 4 to produce aerial mycelium at 30 °C was not restored by the addition of decoyinine, adenine, guanine, nicotinamide, citrulline, ornithine or calcium chloride at any concentration tested (0.1–10 mM).

Aerial mycelium formation by mutant 4 at 25 °C showed an increased sensitivity to nutrients; adding 1% Casamino acids completely suppressed the development of aerial mycelium in mutant 4 but not in the parent strain.

ADPRT activity

To relate the altered phenotype to ADPRT function we measured the activity of the enzyme, together with that of NAD-glycohydrolase, which is presumably indirectly involved in ADP-ribosylation reactions. Mutants 4 and 7 grown at the non-permissive temperature (30 °C) both had substantially reduced ADPRT activity, with the larger decrease in mutant 4 (Table 3). The NAD-glycohydrolase activity in the mutants was also lower than in the parent, but the reduction was not so severe. ADPRT activity was compared in the parent and mutant 4 cells grown at permissive and non-permissive temperatures. Much less activity was detected in cells grown at 25 °C than in cells grown at 30 °C (Table 4). Unexpectedly, mutant 4 grown at 25 °C had less ADPRT activity than the parent strain grown under similar conditions.

ADP-ribosylation of cellular proteins

Previous investigations (Barabas *et al.*, 1988; Penyige *et al.*, 1990) with another *S. griseus* strain (52-1) demonstrated that several membrane proteins are labelled enzymically during incubation with [³²P]NAD. We used the same method to detect ADP-ribosylation of proteins in the parent and mutant strains, except that 'crude extract' was employed instead of a membrane preparation as the source of enzyme and of substrate proteins. The results of SDS-PAGE analyses of labelled cellular proteins are shown in Fig. 1. A 44 kDa protein (band A) appears in sporulating cultures (48 h age) of the parent strain grown at 30 °C but is missing from similar cultures of mutants 4 and 7. Two other labelled proteins with molecular sizes of 39 and 37 kDa (bands B and C) were present in 48 h cultures, but appeared also in mutants 4 and 7; however the extent of labelling was different from the parent strain. A similar experiment with crude extracts from cells grown at the permissive temperature (25 °C) failed to give an informative autoradiogram,

Table 3. Intracellular NAD-glycohydrolase and ADPRT activity in parent and mutant strains grown in sporulation medium at 30 °C

Data are mean values from two independent experiments.

Strain	Specific activity at various times (h)				
	NAD-glycohydrolase [U (mg protein) ⁻¹]*			ADPRT† [d.p.m. (mg protein) ⁻¹ min ⁻¹]	
	12	24	48	24	96
Parent (13189)	8.2	19.4	9.6	11 270	18 000
Mutant 4	4.4	9.2	7.5	3150	3800
Mutant 7	6.9	13.5	7.3	5400	8570

* One unit is the amount of enzyme that catalyses the decomposition of 1 µmol of NAD per min.

† Reaction at 34 °C.

Table 4. Effect of culture temperature on ADPRT activity in *S. griseus* mycelium

Strains were grown in SPY medium at 30 °C for 36 h or at 25 °C for 42 h. ADPRT was assayed as in Table 3, except that the reaction was carried out at 25 °C as well as at 34 °C.

Strain	Temperature used for		Specific activity* [d.p.m. (mg protein) ⁻¹ (min) ⁻¹]
	Growth	Enzyme assay	
Parent (13189)	30	34	6500 ± 310†
		25	5770 ± 200
	25	34	1290 ± 310
		25	1560 ± 270
Mutant 4	30	34	2170 ± 120
		25	2750 ± 210
	25	34	740 ± 120
		25	870 ± 200

* Values are mean of two determinations ± SD.

† ADP-ribose incorporation was calculated to be 0.61 pmol (mg protein)⁻¹ min⁻¹.

apparently because of lower enzyme activity. Although the altered ADP-ribosylation patterns might have resulted simply from the absence or decreased amount of proteins available for ADP-ribosylation, no difference was observed between protein bands of parent and mutant strains in SDS-PAGE gels stained with Coomassie Blue (data not shown).

Identification of labelled modifying group

We attempted to identify the labelled modifying group by subjecting a preparation of ³²P-labelled proteins to alkaline treatment. When ³²P-labelled proteins prepared as described above from a sporulating culture of the parent strain were incubated under alkaline conditions

Fig. 1. Autoradiogram of an SDS-PAGE gel on which ADP-ribosylated proteins of *S. griseus* strains were separated. Crude extracts from cells grown in sporulation medium at 30 °C to early (24 h) or late (48 h) growth phase were incubated at 34 °C for 20 min with [γ - ^{32} P]NAD. Equivalent amounts of protein from the assay mixtures were analysed by SDS-PAGE. Positions of molecular mass standards are shown at the right hand side. Lanes: 1, parent strain; 2, mutant 4; 3, mutant 7. A, B and C in the figure indicate the labelled proteins that appeared only in the late growth phase.

Fig. 2. TLC analysis and autoradiography of alkaline hydrolysate of proteins of *S. griseus* 13189 previously incubated with [γ - ^{32} P]NAD. ^{32} P-labelled proteins of a crude cell extract were precipitated with TCA, washed and incubated in 0.1 M-KOH at 27 °C or 37 °C. Lane 1, hydrolysis at 37 °C; lane 2, at 27 °C.

(0.1 M-KOH at 27 °C or 37 °C), radioactivity was released. Analysis of the released products by TLC (Fig. 2) showed ADP-ribose and AMP at the lower temperature, but only AMP at the higher temperature. This hydrolysis pattern is typical for ADP-ribosylated proteins (Jouanneau *et al.*, 1989). Thus, our results indicate that the labelled modifying group represents ADP-ribose. Accordingly, we suggest that the appearance of ADP-ribosylated proteins is age-dependent and, for some of them (especially the 44 kDa protein), correlated with sporulation and antibiotic production.

Correlation between the aba mutation and the altered phenotype

The *aba* mutation leading to resistance to 3-ABA was genetically analysed by the protoplast fusion technique. Auxotrophic *aba* mutants (*arg aba-1*, *pro aba-1*, and *his aba-1*) were derived from mutant 4 by chemical mutagenesis. Each auxotrophic *aba* mutant was crossed with the auxotrophic *aba*⁺ strain (*ura trp aba*⁺), and then prototrophic recombinants that grew on minimum regeneration medium were analysed. In a cross between *arg aba-1* and *ura trp aba*⁺, all 38 prototrophic and 3-ABA-resistant recombinants were defective at 30 °C in streptomycin production, aerial mycelium formation, and submerged spore formation, while all 23 *aba*⁺ recombinants tested exhibited the normal parental phenotype for these characteristics. Similar results were obtained in two other crosses (*pro aba-1* $\times$ *ura trp aba*⁺, *his aba-1* $\times$ *ura trp aba*⁺). Furthermore, in four recombinants examined, those carrying the *aba* mutation exhibited 4-fold less ADPRT activity than did the *aba*⁺ strains. Thus, the *aba* mutation is tightly linked with all of the altered phenotypes.

We also attempted a reversion analysis by searching for revertants that could sporulate in nonselective media (lacking 3-ABA) at 30 °C. However, no strain ($< 5 \times 10^{-6}$) with the parental phenotype was detected.

Changes in nucleotide pool size

From evidence obtained with several *Streptomyces* species, including *S. griseus* 13189, Ochi (1986a, b, 1987a, b, 1990a, b) proposed that morphological differentiation of *Streptomyces* results from a decrease in the GTP pool size, whereas physiological differentiation (e.g. antibiotic production) results more directly from a function of a *rel* gene affecting ppGpp accumulation. A recent study with *Streptomyces antibioticus* demonstrated that mRNA levels for phenoxazinone synthase, an enzyme involved in actinomycin synthesis, were much lower in a *relC* mutant than in the parent strain (Kelly *et al.*, 1991). Therefore, we measured changes in nucleotide

pools during growth at the non-permissive temperature. Mutant 4 displayed virtually the same pattern of nucleotide pool changes as reported for the parent strain (Ochi, 1987a). Furthermore, mutant 4 exhibited the normal parental ability to accumulate ppGpp [430 pmol (mg dry wt)⁻¹] during Casamino acid deprivation. Apparently, the effects of the *aba* mutation on *S. griseus* differentiation are not exerted through changes in the pool size of ppGpp or GTP.

To our knowledge, this is the first report of a possibly altered ADPRT; the system may be useful for studying the role of ADP-ribosylation in development and secondary metabolite production in *Streptomyces*. In particular, the conditional phenotype of mutant 4 may be useful. The reduced ADPRT activity in mutant 4, despite its normal parental phenotype at the permissive temperature, was unexpected. No explanation can be offered at present, but it may be that differentiation in *S. griseus* is initiated somewhat differently at 25 °C and 30 °C. Alternatively, it might indicate that ADPRT activity has nothing to do with sporulation or streptomycin production. Some important questions to be answered are (i) whether the altered activity in ADPRT is caused directly by a lesion in the gene encoding the enzyme, and (ii) what role (if any) the 44 kDa protein plays in promoting physiological and morphological differentiation. Whether *S. griseus* ADPRT exists in single or multiple forms should also be clarified.

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