A Gene Encoding a Homologue of Dolichol Phosphate-β-D-Mannose Synthase Is Required for Infection of *Streptomyces coelicolor* A3(2) by Phage φC31

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We have shown previously that a gene encoding a homologue to the eukaryotic dolichol-phosphate-β-D-mannose, protein O-β-mannosyltransferase, was required for φC31 infection of *Streptomyces coelicolor*. Here we show that a gene encoding the homologue to dolichol-phosphate-mannose synthase is also essential for phage sensitivity. These data confirm the role of glycosylation in the phage receptor for φC31 in *S. coelicolor*.

The temperate phage φC31 has been used to develop phage vectors for genetic manipulation of *Streptomyces* species, many of which are commercially useful producers of antibiotics and other bioactive secondary metabolites (4, 8). φC31 has a moderately broad host range and is able to infect around a third of the *Streptomyces* species tested (9, 18). The earliest event in phage infection is the recognition by phage tail proteins of some component on the host cell surface (7, 11, 15). While this initial step is often reversible, at some point a commitment to infection occurs in which a series of molecular changes in the phage results in the injection of DNA into the cell. Studies of the molecules on the cell surface recognized by the φC31 coat proteins will lead to a better understanding of the *Streptomyces* cell wall and to further development of phage vectors.

We have indicated previously that glycosylation of a *Streptomyces coelicolor* cell envelope protein is required for infection of the cell by φC31 (5). Mutants of *S. coelicolor* strain J1929 (ΔpglY and therefore sensitive to φC31 [1]) that are unable to support plaque formation by φC31ΔΔ, a clear-plaque mutant of φC31, were isolated. The *S. coelicolor* mutants were unable to form infective centers after contact with phage but could release phage particles after being transfected with phage DNA. These observations indicated that the mutants are blocked early in phage infection, probably at the stage of receptor binding. The *S. coelicolor* mutants fell into three classes, designated I, II, and III. A gene isolated from the *S. coelicolor* ordered cosmid library, SCE87.05, complemented the class I mutants but not those of class II or III. SCE87.05 has significant similarity to the eukaryotic dolichol-phosphate-β-D-mannose (Dol-P-Man), protein O-β-mannosyltransferases (protein mannosyltransferase), suggesting that the receptor for φC31 infection is a glycoprotein generated through an O glycosylation pathway. This suggestion was supported by observations that the plant lectin concanavalin A could inhibit phage infection and that glycoproteins could not be detected by concanavalin A in Western blots of proteins from a mutant defective in SCE87.05. A mutant phage, φC31ΔΔ, could form plaques on class I and class II mutants of *S. coelicolor* but not on class III mutants. It was proposed that φC31 normally recognizes a cell wall glycoprotein but that φC31ΔΔ recognizes the unglycosylated protein. Thus, like the class I mutants, the class II mutants are predicted to be defective in the glycosylation pathway and the class III mutants are predicted to lack the protein target of glycosylation. Here we have tested this model with experiments to investigate the role of a gene, SC6D7.16, encoding a homologue of Dol-P-Man synthase, proposed to be required for the protein glycosylation pathway.

Dol-P-Man catalyzes the transfer of mannose from GDP-mannose to Dol-P in fungi and in mammals (10, 17). Dol-P-Man is then the substrate for the transfer of the mannose to a serine or threonine by the protein glycosyltransferase in the O-glycosylation pathway. The protein sequences of the fungal and mammalian Dol-P-Man synthases were used to search the *S. coelicolor* genome sequence (http://www.sanger.ac.uk/Projects/S_coelicolor/). The closest homologue was the predicted product of SC6D7.16 (Fig. 1). The cosmid SC6D7 was therefore introduced by protoplast transformation into two of the class II phage-resistant mutants, DT1029 and DT2021, and into the *S. coelicolor* class III mutant, DT2017, isolated previously (5). Seven individual transformants of each strain were tested for sensitivity to phage by using a plate assay. This was performed by preinoculating one-half of an R1M plate with φC31ΔΔ and then streaking spores from the transformants across the plate from the phage-free half to the phage-containing half. SC6D7 conferred sensitivity to phage φC31ΔΔ in approximately 50% of the transformants of DT1029 and DT2021 (the class II mutants). This incomplete complementation appears to be typical of complementation by cosmids, and it is believed that the mutant and wild-type alleles undergo homogenization (5, 13). To confirm the complementation, we constructed a plasmid, pDT16, that carried only SC6D7.16. PCR was used to amplify the open reading frame and flanking DNA from cosmid SC6D7, and the PCR product was inserted into the integrating vector pSET152 (3). This plasmid was introduced by conjugation into a class I strain, DT1017; the class II strains DT1028, DT1029, DT1035, DT2008, and DT2021; and the class III mutant DT2017. Seven individual transconjugants from each mating were tested for sensitivity to phage by using the plate assay. Phage sensitivity was restored to all the...
transconjugants tested from the class II mutants DT1029, DT1035, and DT2021, and this was confirmed in a plaque assay (Fig. 2). The class II mutants DT1028 and DT2008 remained phage resistant (data not shown). As expected, pDT16 did not restore phage sensitivity to \( \phi H9278 \) C31 in either the class I or class III mutant.

To confirm the requirement for SC6D7.16 in phage infection, we constructed an \( S.\ coelicolor \) strain, J1929::pDT15, containing a targeted insertion in SC6D7.16. A DNA fragment internal to SC6D7.16 was amplified by using primers DT23 and DT24, inserted into the suicide vector pSET151 (3), and introduced into the phage-sensitive strain J1929 (1) by conjugation. Integration of this construct by insert-directed homologous recombination into \( S.\ coelicolor \) J1929 should give rise to a disrupted version of SC6D7.16. All seven transconjugants tested were resistant to \( \phi C31r\Delta25 \) by the plate assay. As expected, pDT16 did not restore phage sensitivity to \( \phi C31r\Delta25 \) in either the class I or class III mutant.

In addition, J1929::pDT15 spores could support plaque formation by \( \phi C31h \) (data not shown). This phenotype is consistent with a class II phage resistance phenotype (5).

These observations indicate that the protein product of SC6D7.16 is essential for \( \phi C31r\Delta25 \) infection of \( S.\ coelicolor \). SC6D7.16 is a homologue of the eukaryotic Dol-P-Man synthases (Fig. 1). In eukaryotes, Dol-P-Man provides the mannosyl residues in glycosylphosphatidylinositols and in N, O, and C glycosylation. A knockout of the single-copy gene DPM1, which codes for Dol-P-Man synthase, resulted in complete loss of protein mannosylation in \( Saccharomyces\ cerevisiae \) (12).

Prokaryotes do not have dolichol. They contain instead other polyprenols, in particular undecaprenol phosphate (14). There is little information on the polyprenols in the \( Streptomyces \) cell envelope; however, the closely related bacteria \( Mycobacterium\ tuberculosis \) and \( Mycobacterium\ smegmatis \) contain a variety of polyprenol phosphates, which are covalently attached to mannoside (Pol-P-Man) (2, 6). Pol-P-Mans in mycobacteria are thought to be synthesized in addition, J1929::pDT15 spores could support plaque formation by \( \phi C31h \) (data not shown). This phenotype is consistent with a class II phage resistance phenotype (5).
by Ppm1, a functional analogue to the eukaryotic Dol-P-Man synthase and the closest homologue to SC6D7.16 (Fig. 1).

The data presented here, together with previous findings (5), indicate that SCE87.05 and SC6D7.16, which encode a putative protein glycosylation pathway in S. coelicolor, are required for the synthesis of the protein glycosylation receptor in S. coelicolor. Similar to the roles of equivalent proteins in eukaryotes, these enzymes probably catalyze two steps in a protein glycosylation pathway in S. coelicolor. The observation that not all of the class II mutants are complemented by SC6D7.16 suggests that DT2008 and DT1028 are defective in other genes, possibly those encoding other enzymes in the glycosylation pathway. It is clear from these studies that these proteins are not essential for the growth of S. coelicolor, implying that they are not exclusively required components of cell wall biosynthesis. The role of protein glycosylation is not known either in the mycobacteria or in Streptomyces, but it will hopefully become clearer once the targets for glycosylation have been characterized.

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