SHORT COMMUNICATIONS

A Study of Spontaneous and *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine-induced Phage Resistant Mutants in *Caulobacter crescentus*

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INTRODUCTION

Studies of the developmental steps in the cell cycle of *Caulobacter crescentus* have involved the isolation of non-motile mutants (Kurn *et al.*, 1974) and mutants resistant to phage ϕ CbK (Agabian-Keshishian & Shapiro, 1971), to the flagellotropic phage ϕ Cp34 and to the RNA phage ϕ Cp2 which is thought to adsorb to pili (Fukuda *et al.*, 1976). Kurn *et al.* (1974) reported the isolation of a non-motile mutant which had lost sensitivity to phage ϕ Cb5, a pilus-specific phage, and ϕ CbK. A motile revertant of this mutant was shown to have regained phage sensitivity. Fukuda *et al.* (1976) isolated mutants resistant to either phage ϕ CbK or ϕ Cp34 or ϕ Cp2. Many of these mutants showed pleiotropic loss of sensitivity to all three phages and/or loss of motility. Some motile revertants of a number of these non-motile phage resistant mutants were shown to have simultaneously regained wild-type phage sensitivities. On the basis of the pleiotropic phenotypes of these mutants and of the phenotypes of the revertants isolated, Fukuda *et al.* (1976) suggested that the synthesis of flagella, pili and phage ϕ CbK receptor sites can be inhibited by a single mutation and that the expression of these surface structures is under a common control mechanism.

This communication reports studies on phage ϕ CbK resistant strains of *C. crescentus* CB15 isolated either after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) mutagenesis or as spontaneous mutants.

METHODS

Strains. All strains were derived from *Caulobacter crescentus* CB15 which was a gift from Dr L. Shapiro, Albert Einstein College, New York. The peptone yeast extract (PYE) medium of Poindexter (1964) was used throughout. All incubation was carried out at 30 °C and broth cultures were agitated on a rotary shaker. Phage ϕ CbK was a gift from Dr Shapiro and phages ϕ Cb12r (ATCC 19089-B1) and ϕ Cb13 (ATCC 19089-B2) were obtained from the American Type Culture Collection.

Mutagenesis and the isolation of phage resistant mutants. Strain CB15 was mutagenized using NTG and phage ϕ CbK resistant mutants were isolated by the method of Fukuda *et al.* (1976). The plaque forming ability of phages ϕ Cb12r and ϕ Cb13 was tested on each phage ϕ CbK resistant mutant, both spontaneous and induced.

Motility. All phage resistant variants isolated were examined by phase-contrast microscopy. A strain was designated non-motile if no motile cells were observed on examination of three successive subcultures. Phage resistant variants isolated after NTG mutagenesis were streaked on 0.35 % PYE agar plates as a further test for motility. The presence of extensive spreading from a control streak after 3 d incubation was taken as evidence of motility.

Isolation of revertants. Revertants to motility of non-motile phage resistant variants were isolated by the method of Fukuda *et al.* (1976).

Table 1. *Phenotypes of motile revertants of four non-motile mutants of C. crescentus*

Parent	Sensitive to all 3 phages			Resistant to all 3 phages		
	Normal	Abnormal	Extremely abnormal	Normal	Abnormal	Extremely abnormal
Mutant 3	0	0	0	6	5	3
Mutant 24	2	7	3	0	0	3
Mutant 33	1	1	6	1	1	3
Mutant 36	0	0	0	2	5	2

RESULTS

Spontaneous phage ϕ CbK resistant mutants

One hundred and fifty independent phage ϕ CbK resistant variants of strain CB15 were isolated without the aid of mutagenesis. Swarmer cells were observed by microscopy in broth cultures of all of these variants. However, approximately one-third of the 150 strains showed impaired motility, either having very small numbers of swarmer cells or exhibiting sluggish movement. The morphology of these strains was normal with the exception of one variant, strain GU1, which produced filaments of 10 to 70 μ m in length in approximately 20 % of cells at all stages of growth in culture. One hundred and forty-nine of the phage ϕ CbK resistant variants were pleiotropic with respect to resistance to phages ϕ Cb12r, a pilus-specific RNA phage, and phage ϕ Cb13, a DNA phage. One strain was resistant to phage ϕ CbK only.

NTG-induced phage ϕ CbK resistant mutants

Fifty phage ϕ CbK resistant variants were isolated from 50 independent subcultures of NTG-treated strain CB15. None of the variants showed altered morphology on microscopic examination. Four of the mutants were non-motile. Impaired motility, similar to that described for spontaneous phage resistant mutants, was noted as a property of 18 of the 50 variants. All 50 strains were also resistant to the phages ϕ Cb12r and ϕ Cb13.

Motile revertants of the four non-motile phage resistant variants were isolated from 0.35 % agar plates. These were purified on PYE agar and their motility was confirmed on 0.35 % agar and by phase-contrast microscopy. The sensitivities of the motile revertants to the three phages ϕ CbK, ϕ Cb12r and ϕ Cb13 were tested. The morphology of these revertant strains was examined by phase-contrast microscopy of 18 h PYE broth cultures. The strains were classified into three morphological groups: normal, less than 1 % of all cells examined showed abnormalities; abnormal, between 1 and 10 % of all cells were over five times the length of the parent CB15 cells; and extremely abnormal, over 10 % of all cells were over five times the normal cell length. Strains in the 'extremely abnormal' group frequently manifested cells with tightly coiled spirals, swollen cells or Y-shaped branches. The cells of the parent CB15 never showed these last two abnormalities and only produced their characteristic loose spiral filaments in old (4 d) PYE broth cultures. The filaments produced by the 'abnormal' or 'extremely abnormal' strains never showed the loose spiral form of the wild-type. The results of these tests are summarized in Table 1. In all, only three of the 51 revertants (two from mutant 24 and one from mutant 33) regained phage sensitivity and had a normal morphology.

DISCUSSION

The results suggest that the use of NTG mutagenesis in studying the possible connections between the formation of DNA phage receptors, pili and flagella generates strains with pleiotropic phenotypes that are not detected in spontaneous variants. While impaired motility was observed in many spontaneous mutants, no non-motile strains were isolated

in 150 phage ϕ CbK resistant variants; in contrast, four of 50 NTG-induced phage ϕ CbK resistant mutants showed complete loss of motility. This suggests that the association of phage resistance with loss of motility only occurs in NTG-induced mutants or at a low frequency in spontaneous mutants. It is possible that the phenotypes of the NTG-induced mutants are the results of more than one mutation. NTG has been shown to cause multiple linked mutations both in *C. crescentus* (Johnson & Ely, 1977) and in *E. coli* (Guerola *et al.*, 1971).

Reversion analysis has been used by Kurn *et al.* (1974) and by Fukuda *et al.* (1976) to demonstrate that motility and phage sensitivity could be regained simultaneously in some non-motile phage resistant strains. In this work we have also shown that two out of four non-motile phage resistant mutants spontaneously produced phage sensitive motile revertants at a high frequency (8 of 13 motile revertants, and 12 of 15 motile revertants). The abnormal morphology of the majority of these revertants (17 out of 20), however, suggests that they are the result of suppression rather than a true back mutation (Gorini & Beckwith, 1966).

Reversion data on such NTG-induced mutants in *Caulobacter* therefore warrants careful analysis.

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