Improved Generalized Transducing Bacteriophage for *Caulobacter crescentus*

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*Caulobacter* phage φCr30T, a temperature-sensitive derivative of the lytic, generalized transducing phage φCr30, was isolated as a double temperature-sensitive recombinant in a cross between φCr30ts1 and φCr30ts2. φCr30T mediated generalized transduction of *Caulobacter crescentus* at frequencies comparable to those of φCr30 and eliminated the requirement for irradiation of transducing lysates to prevent killing of transductants on the plate.

*Caulobacter crescentus* with its unusual life cycle has been proposed by several authors as an ideal procaryotic system in which to study the basic principles of cellular differentiation. Studies using synchronized populations, single-cell microcultures, cell cycle mutants, and inhibitors of macromolecular synthesis have yielded a rather detailed picture of the events occurring during the *C. crescentus* developmental program, as reviewed recently (1, 7, 9). The genetic analysis of *C. crescentus* was greatly aided by the discovery of a generalized transducing phage, φCr30, by Ely and Johnson (4). This phage has already been used extensively in the characterization of the mutants affecting flagellar morphogenesis (6), nitrogen metabolism (3), and phospholipid metabolism (2). In addition a combination of φCr30-mediated generalized transduction and plasmid RP4-mediated chromosome mobilization has yielded a linkage map for *C. crescentus* containing an ever-increasing number of genetic markers, greatly assisting future genetic studies (J. T. Barrett, C. S. Rhodes, D. M. Ferber, B. Jenkins, S. A. Kuhl, and B. Ely, J. Bacteriol., in press).

Unfortunately phage φCr30 is a lytic phage and the yield of transductants is low, since most of the transductants are killed by phage released from nearby productive infections. Those transductants that survive are usually resistant to the phage and thus removed from further genetic analysis (6). To avoid this problem, Ely and Johnson developed a technique of irradiating the transducing lysate with UV light to inactivate the phage particles (6). Since the phage is large relative to a single transduced gene, the titer of viable phage is thus reduced by many orders of magnitude without seriously reducing the transduction frequency for the marker of choice. This procedure, however, introduces the complication that the transducing DNA has been irradiated heavily and thus is likely to carry mutations not originally present in the donor. It is even possible that the introduction of so large a piece (greater than 100 × 10^6 daltons) of irradiated DNA may cause an indirect induction of something comparable to the error-prone SOS repair system of *Escherichia coli* (5). In short, the irradiation step compromises attempts to maintain isogenic strains for careful genetic comparisons.

To avoid the irradiation step and the problem of the lytic nature of the phage, temperature-sensitive derivatives of phage φCr30 were isolated and tested for their ability to carry out generalized transduction without prior irradiation with UV light. A lysate of φCr30 grown on *C. crescentus* CM5000 (an isolate of strain CB15 carried in this laboratory for several years) was treated with the mutagen hydroxylamine (8) until survival was about 0.1%. The lysate was plated on strain CM5000 at room temperature (22 to 24°C), and plaques were picked with sterile toothpicks to lawns of strain CM5000 either at room temperature or prewarmed to 33°C. Plaques that gave lysis at room temperature but not at 33°C were purified by two single-plaque isolations and retested. From 600 plaques tested, two temperature-sensitive mutants were obtained, φCr30ts1 and φCr30ts2. The reversion frequency of each of the mutants was about 10^-6. Plating about 10^9 of either mutant on a plate allowed the growth of a normal lawn from an inoculum of about 4 × 10^3 cells, suggesting that infection at the nonpermissive temperature may not be lethal for the cells.

To test transduction ability, a proline auxotroph, strain CM5068 (*pro-501*), was transduced at 33°C with φCr30 and φCr30ts1 grown on the wild-type strain CM5000, with and without UV
irradiation of the lysates. In the absence of irradiation, the φCr30 lysate gave transductants with highly irregular colony morphology ("nibbled colonies"). The φCr30ts1 lysate gave transductants with normal morphology whether the lysate was irradiated or not. The transduction frequency in this experiment was about 32 × 10⁻³ Pro⁺ cells per plaque-forming unit (measured at 23°C). This frequency is similar to that observed with φCr30 after irradiation.

To assure that the temperature sensitivity would not be lost by reversion during subsequent cultivation of the phage, a cross was performed between φCr30ts1 and φCr30ts2 to generate a double mutant. About 3 × 10⁶ cells (log-phase culture of strain CM5000 grown in PYE broth [2]) were mixed with 2 × 10⁶ of each mutant phage; adsorption was allowed for 45 min at 24°C. The infected cells were washed once with PYE broth to remove unadsorbed phage and were resuspended in four times the original volume. After incubation for 135 min at 24°C with vigorous aeration, chloroform was added to the mixture, debris was removed by centrifugation, and the resulting lysate was titrated at permissive and nonpermissive temperatures. The ratio of the titer at 33°C to that at 24°C was 0.15 (3.5 × 10⁶/1.9 × 10⁷), suggesting a recombination frequency as high as 30%. Twenty plaques were picked from the permissive plate and were tested for growth at 24 and 33°C. Two of the 20 gave good lysis at both temperatures. Seven of the remaining 18 picked plaques were grown into lysates by the plate stock method and were titrated for reversion frequency at 33°C. One of the seven gave fewer than 10⁻⁹ revertants and was assumed to be the double mutant φCr30ts1,ts2, which was named φCr30T for convenience. We have not yet obtained a revertant of this strain.

The mutant φCr30T was grown on strain CM5000 and used to transduce strains SC146 (argG105), SC309 (metD106), and SC451 (proC104) to prototrophy without prior irradiation of the transducing lysate. Transductants were obtained at frequencies of 10⁻⁶ to 10⁻⁷ for each of the markers. Thus φCr30T is a nonreverting derivative of φCr30 that can carry out generalized transduction without irradiation of the transducing lysate.

The cross that generated the double mutant (the first bacteriophage cross reported in the genus Caulobacter) cannot be used reliably to determine the recombination frequency. The adsorption of φCr30 to strain CM5000 is slow under any conditions and very slow at 24°C. It is difficult to know, therefore, what the actual multiplicity of infection was, especially since about a third of the cells survived the infection. Nevertheless, the value reported here is a minimum estimate of possible recombination frequencies and suggests that these frequencies are high enough to make a genetic study of Caulobacter phages, or at least φCr30, feasible. The original phage φCr30 can be propagated easily either as a liquid lysate or as a plate lysate; φCr30T gives a low yield when propagated on a liquid culture, but gives yields of 10¹⁰ to 10¹¹ per ml when propagated as a plate lysate.

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LITERATURE CITED


