Transitory *cis* Complementation: A Method for Providing Transposition Functions to Defective Transposons

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ABSTRACT

A genetic complementation system is described in which the complementing components are close together in a single linear DNA fragment; the complementation situation is temporary. This system is useful for providing transposition functions to transposition-defective transposons, since transposition functions act preferentially in *cis*. The basic procedure involves placing a transposition-defective transposon near the gene(s) for its transposition functions on a single DNA fragment. This fragment is introduced, here by general transduction, into a new host. The transposase acts in *cis* to permit the defective element to transpose from the introduced fragment into the recipient chromosome. The helper genes do not transpose and are lost by degradation and segregation. The method yields single insertion mutants that lack transposase and are not subject to further transposition or chromosome rearrangement. The general procedure is applicable to other sorts of transposable elements and could be modified for use in other genetic systems.

OVER the past decade, the use of transposable genetic elements has become a major tool in genetic research. Of particular importance for the study of gene regulation are the series of derivatives of phage Mu constructed by CASADABAN and coworkers (CASADABAN and CHOU 1984; CASADABAN and COHEN 1979; CASTILHO, OLFSON and CASADABAN 1984). One of the drawbacks associated with the use of most transposons, including the original forms of these Mu derivatives (MudI and MudII), is their ability to transpose further, forming secondary insertion mutations and rearrangements. To avoid this problem, increasing use has been made of transposition-defective derivatives of transposons that must be helped to transpose initially, but thereafter are not capable of further transposition. In the case of Mu derivatives, defective transposons (mini-Mud) have been constructed for both the operon-fusing and gene-fusing Mud-*lac* phages (CASTILHO, OLFSON and CASADABAN 1984).

In order to initially achieve insertion of defective transposons, transposase is commonly provided in *trans* from a plasmid in the recipient cell (CASTILHO, OLFSON and CASADABAN 1984; CHACONAS *et al.* 1981). For phage Mu, as for other transposons, the transposition functions act preferentially in *cis* (FOSTER *et al.* 1981; ISBERG and SYVANEN 1981; MORISATO *et al.* 1983; PATO and REICH 1984). Therefore, when transposase is to be provided in *trans* by a plasmid, it must be provided at a high level; this results in many multiple transposition events and frequent chromosome rearrangements (R. SONTI and J. R. ROTH, unpublished results). Thus, the desired insertion mutants must be moved into a genetic background free of transposase, and away from unwanted insertions and rearrangements, before they are useful in genetic studies. We present here a method to provide Mu transposase activity in a transitory manner; this allows one to generate, in one step, insertions of defective transposons in the Salmonella chromosome.

MATERIALS AND METHODS

Bacteria: All strains used in this study are derived from *Salmonella typhimurium* strain LT2 and are listed with their sources in Table 1. MudA and MudB are transposition-defective derivatives of CASADABAN's MudI and MudII phages which form operon and gene fusions, respectively; amber mutations affecting transposition cause the conditional transposition defect (HUGHES and ROTH 1984). The transposition-defective prophages used here are the MudI1734(Kan, Lac)[operon fusion] and the MudII1734(Kan, Lac)[gene fusion] elements of CASTILHO, OLFSON and CASADABAN 1984); for simplicity, we refer to these elements here as MudJ and MudK. The insertions of MudJ and MudK in the *hisD* gene used here were constructed as described below.

Media: The E medium of VOGEL and BONNER (1956), supplemented with 0.2% dextrose, was used as minimal medium. Luria-Bertani (LB) medium (Difco Tryptone, 10 g/liter; Difco Yeast Extract, 5 g/liter; NaCl, 5 g/liter) was used as rich medium. Difco agar was added to solid medium to a final concentration of 1.5%. Auxotrophic supplements were included in media at final concentrations described (DAVIS, BOTSTEIN and ROTH 1980). Antibiotics were included in media as needed (final concentrations given): kanamycin sulfate (50 μg/ml in rich media; 125 μg/ml in minimal media), sodium ampicillin (30 μg/ml in rich media; 15 μg/ml in minimal media).

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TABLE 1

List of strains

Strain	Genotype	Lac Phenotype	Source[a]
TT7216	*hisA9944*::MudI	Lac$^-$	
TT7221	*hisA9949*::MudI	Lac$^-$	
TT7692	*hisD9953*::MudA	Lac$^+$	HUGHES and ROTH (1984)
TT9734[b]	*hsiD1284*::MudB	Lac$^+$	HUGHES and ROTH (1985)
TT10270	*trp-3744*::MudK	Lac$^-$	D. ROOF
TT10286	*hisD9953*::MudJ	Lac$^+$	T. ELLIOTT
TT10288	*hisD9953*::MudJ *hisA9944*::MudI	Lac$^+$	
TT10289	*hisD9953*::MudJ *hisA9949*::MudI	Lac$^+$	
TT10380[b]	*hsiD1284*::MudK	Lac$^+$	T. ELLIOTT
TT10381[b]	*hsiD1284*::MudK *hisA9944*::MudI	Lac$^+$	T. ELLIOTT
TT10382[b]	*hsiD1284*::MudK *hisA9949*::MudI	Lac$^+$	T. ELLIOTT

[a] Unless indicated otherwise, all strains were constructed during the course of this work.
[b] The *hsi* designation is used for a second set of allele numbers assigned to histidine mutations isolated after mutation *his-9999*.

Mud cassette replacement: The *hisD*::MudJ and *hisD*::MudK insertions used were constructed by transductional conversion of previously characterized insertions *hisD9953*::MudA(operon fusion) and *hsiD1284*::MudB (gene fusion). Strains carrying these insertions were transduced to kanamycin resistance (Kan^r) using P22 grown on strain TT10270 (*trp-3744*::MudK). The Kan^r transductants that remained Trp$^+$ and lost the Amp^r phenotype of the recipient's Mud element were recombinants whose MudA or B prophage had inherited the transposition defect and Kan^r determinant of the donor by recombination events between the *lac* and Mu *c* regions at the ends of the Mud prophages. These recombinants were about 0.2% of the total Kan^r transductants. Note that in these recombinational interconversions, the nature of the recipient fusion (operon or gene fusion) is not altered, since the recombinational events are internal to the prophages. This interconversion method was described for *E. coli* by CASTILHO, OLFSON and CASADABAN (1984).

RESULTS

In the method described here, transposition of a defective Mud phage is achieved by providing transposase functions in *cis* on a short-lived transduced fragment. The strategy for *cis* complementation is to place a transposition-proficient Mud prophage [the original Mud1(Amp, *lac*), here designated MudI] near a transposition-defective MudJ or MudK element such that the Mu transposase genes (*A* and *B*) of MudI are close to the defective MudJ or MudK element. Phage P22 can package the entire MudJ (or K) prophage. Fragments carrying the MudJ or MudK insertion very often include the proximal portion of the nearby MudI prophage and the transposase genes which are located there. When this fragment is transduced into a recipient cell, the transposase genes are induced by zygotic induction and transposase acts on the nearby defective Mud element, causing its transposition into the recipient chromosome. The rest of the fragment (including the transposase genes) is eventually degraded or lost by segregation, leaving an insertion mutant carrying a single stable insertion mutation free of transposase. The result is a one-step method applicable to any recipient strain.

The helper prophage (MudI): The helper MudI prophages were isolated as MudI-induced His$^-$ auxotrophs that are phenotypically HisD$^+$; they use histidinol as a source of histidine and therefore must express the *hisD* gene. These insertions, which must map downstream of the *hisD* gene, were screened for their Lac phenotype. Insertions with a Lac$^-$ phenotype were chosen, since *his*::Mud insertions that are not fused to the *his* promoter are oriented with their transposase genes near to the promoter-proximal *hisD* gene. Two MudI insertions mapping in the *hisA* gene were chosen to provide helper transposase functions in this study.

Double-mutant donors for *cis* complementation: The transposition-defective transposons (MudJ or MudK) are placed near a functional copy of Mu transposase genes by constructing double mutants that have the *hisD*::MudJ (or K) element near the helper *hisA*::MudI element. The double mutants were made by transduction crosses using P22 phage grown on strains carrying a *hisD*::MudJ (or a *his*::MudK) insertion to transduce recipient strains carrying the *hisA*::MudI insertion. Transductants that inherit the donor Kan^r and retain the recipient Amp^r are double mutants which carry both insertions in the *his* operon. The structure of these double mutants is diagrammed in Figure 1; the rationale for this structure is described below.

The Mud sequences in the above double mutants are such that when the transducing phage P22 is grown on the double mutant, a single virion can package the entire MudJ (or MudK) element present in the *hisD* gene and the transposition functions of the nearby MudI helper prophage. P22 packages 44 kb of chromosome (SUSSKIND and BOTSTEIN 1978). MudI is 37 kb in length while the MudJ and MudK prophages are 11.3 and 9.7 kb in length, respectively (CASTILHO, OLFSON and CASADABAN 1984; O'CONNOR

TABLE 2

***Cis*-complemented transposition of MudJ and MudK[a]**

Donor strain	Relevant genotype	No. of Kan^r transductants screened	No. of His$^-$ recombinants	No. of transposition mutants		Frequency of transposition mutants among Kan^r transductants (%)	Frequency of new auxotrophs among transposition mutants (%)
				Auxotrophic (His$^+$)	Prototrophic		
TT10288	MudJ	394	54	17	323	86	5
TT10289	MudJ	286	101	9	176	65	5
TT10381	MudK	537	32	19	486	94	4
TT10382	MudK	595	42	25	528	93	4

[a] Growth conditions and genetic methods used in this study are described elsewhere (HUGHES and ROTH 1984). The recipient in all crosses was the wild-type strain LT2.

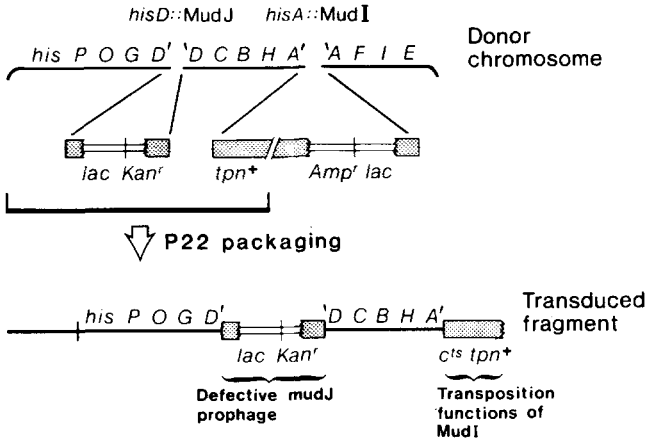

FIGURE 1.—The MudJ donor strain for transposition by *cis*-complementation. This strain carries two closely linked Mud insertions within the *his* operon. The MudI(Amp) prophage inserted in the *hisA* gene is fully functional (for transposition); the MudJ(Kan) prophage inserted in the *hisD* gene is transposition-defective. The P22 virion is too small to include all of both Mud prophages. However, many of the P22 transducing fragments that include MudJ will also include the proximal end of MudI and the transposition genes that are located there. Fragments of this type, when introduced into a recipient cell, produce transposition proteins which can act in *cis* to permit transposition of the defective MudJ prophage into the recipient chromosome.

and MALAMY 1983). Roughly 4 kb of *his* sequence (including the *hisH*, *B* and *C* genes) lies between the two insertion sites. Thus, P22 cannot transduce both inserted elements simultaneously, but transduced fragments that carry MudJ or K frequently include the transposition functions at the end of the nearby MudI element.

Transposition of defective elements by *cis* complementation: General transducing lysates (phage P22) were grown on the double mutants described above. These lysates were used to transduce a wild-type recipient strain selecting for inheritance of kanamycin resistance (encoded by the defective prophage). This selection demands inheritance of MudJ or K, either by transposition or by recombination into the chromosome. The latter recombinants can be avoided as described below. Results are presented in Table 2. Transposition transductants arise only if the helper MudI prophage is present in the donor; no such transposition types arise if the donor carries only the defective *hisD*::MudJ or K mutation. Donors with both elements give Kan^r transductants including up to 94% transposition types which have acquired MudJ or K insertions at a variety of sites, distributed apparently randomly throughout the chromosome. These transposition types include 4–5% auxotrophs, typical of the frequency seen when the chromosome is randomly mutagenized by phage Mu. The new auxotrophs are of many distinct types and appear to be randomly distributed. The Kan^r transductants that arise by homologous recombination are all phenotypically HisD$^-$ and can be avoided if the transposition crosses are performed on medium lacking histidine. Both the recombinants (HisD$^-$) and the transposition types are listed separately in the summary presented in Table 2.

As expected, neither the recombinants nor the transposition transductants inherit the ampicillin resistance of the helper prophage. Both types have been tested for the ability of the Mud prophage to transpose when transduced into a wild-type recipient; none of the tested transductants carry Mud elements that are capable of independent transposition. All of the tested auxotrophs appear to be due to single transposition events as judged by the fact that, when transduced to prototrophy, they simultaneously lose drug resistance and auxotrophy. If secondary insertions had been present, these transductants should continue to show drug resistance.

DISCUSSION

From the results presented above we infer the following sequence of events. Phage P22 transducing particles that carry the defective mini-Mud frequently include transposase genes of the nearby MudI prophage. When this transduced fragment is introduced into a recipient strain, the Mu transposase functions

are zygotically induced. Transposase acts on the ends of the adjacent defective Mud element, causing its transposition into the recipient chromosome. This results in acquisition of kanamycin resistance, which is selected in the cross. After transposition of the defective prophage, the donor DNA flanking the defective Mud, including the Mu transposase genes, is degraded or lost by segregation; thus, transposase is no longer produced by the transductant clone. This is inferred since the Amp^r phenotype of the helper prophage is not inherited and the transductants carry single Mud prophages that are not capable of further transposition.

The complementation scheme described here was developed to permit simple isolation of single mutants due to insertion of defective elements. The method as described can be applied to any strain sensitive to infection by phage P22 and does not require removal of the new insertion from the strain of origin.

While this method is applied here to the Salmonella/P22 system, the basic idea can be applied to any genetic subject to transduction or transformation. Modifications of the procedure for use in other systems might include different spacing of insertion elements, or use of donor DNA sequences that are not homologous with the recipient chromosome so that homologous recombination could be prevented. Simultaneous introduction of entire MudI and MudJ(K) elements might not interfere with the effectiveness of the method since selection is made only for inheritance of the defective element. Recombinants that coinherited the MudI helper could easily be identified (by Amp^r) and discarded.

This method, using P22 transduction, has been successfully applied to defective derivatives of Tn*10* (T. ELLIOTT, unpublished results). A method generally similar to this one has been used in *Escherichia coli* by WAY *et al.* (1984) to achieve transposition of defective Tn*10* transposons. In their method, the Tn*10* transposase gene, expressed at high level by a TAC promoter, is present on a derivative of phage lambda that also includes the defective Tn*10* element.

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