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# Thermosensitive step associated with transfer of the Ti plasmid during conjugation: Possible relation to transformation in crown gall

(plant tumors/octopine)

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**ABSTRACT** **It is reported here that transfer by means of a conjugative process of an oncogenic plasmid from a virulent strain of *Agrobacterium tumefaciens* to a strain of that organism that had been cured of the plasmid is thermosensitive. Since the thermosensitive step found in the conjugative process appears similar in every respect to a thermosensitive step that is involved in the transformation of a normal cell to a tumor cell in the crown gall disease of plants, it is suggested that the observed results may reflect the existence of a thermosensitive step that is common to these two processes.**

It has long been known that crown gall tumors initiated in plant species such as the tomato (*Lycopersicon esculentum*) (1), *Vinca rosea* (2), and *Kalanchoe daigremontiana* (3) develop optimally between 20° and 27° but not above 30°. This was found to be true despite the fact that both oncogenic strains of the crown gall bacteria and the host plants develop well at or somewhat above 30°. Subsequent studies showed that a thermosensitive step is involved in the inception phase of the disease and that once the cellular transformation has occurred at or below 27° the transformed cells develop into neoplastic growths equally well above and below the critical temperature of 30° (2, 4). When reaction rates were determined between 27° and 30° and applied to the Arrhenius equation, activation energies of the order of 80,000 cal (334 MJ)/mol were obtained (3). Although reactions of this order of magnitude are suggestive of protein denaturation, the meaning of these findings as they relate to the inception phase of the crown gall disease of plants has remained obscure.

Studies have now shown that a large genetic element, which has been identified as the Ti plasmid, may be transmitted by means of a conjugative process from oncogenic strains of *Agrobacterium tumefaciens* to strains of that bacterium that have been cured of the plasmid as well as to strains of *A. radiobacter* (5, 6). The possibility existed, therefore, that the transfer of the Ti plasmid from virulent to avirulent strains of the bacterium by a conjugative mechanism and the transfer of the plasmid from oncogenic strains of the bacterium to conditioned host cells have a common thermosensitive step. It is the purpose of the present paper to show that a thermosensitive step does, in fact, exist in the conjugative process which appears similar in every respect to that found during the transformation of a normal cell to a tumor cell in the crown gall disease of plants, suggesting that a common thermosensitive step may exist in the two processes.

## MATERIALS AND METHODS

The Ti plasmid, which is present in all virulent strains of the crown gall bacterium, has been found not only to carry genetic information required for oncogenicity (7–9), but to code for specific amino acids—the opines—of which octopine [$N^2$-(D-1-carboxyethyl)-L-arginine] and nopaline [$N^2$-(1,3-dicarboxypropyl)-L-arginine] are examples (10–12). These same amino acids are specifically utilized as both carbon and nitrogen sources by bacterial strains that carry plasmid-derived genetic information required for their synthesis. This fact was utilized in preparing selective culture media used in these studies.

**Bacterial Strains.** *A. tumefaciens* B6S3, a derivative from strain B6, was described by Vervliet *et al.* (13). *A. tumefaciens* C58C9 was obtained by curing strain C58 (14, 15) and made resistant to rifampicin (50 mg/liter) and streptomycin (500 mg/liter).

**Crosses.** A single colony isolate of donor strain, B6S3, was grown to exponential growth phase in liquid minimal medium‡ containing glucose (2 g/liter) and octopine (10 mM). Similarly, a single colony of the recipient strain C58C9 *rif*$^r$*str*$^r$ was grown to the exponential growth phase in a liquid minimal medium containing glucose (2 g/liter) only. Upon suitable dilution, with sterile water, these cultures were mixed in a ratio of 10 donors to 1 recipient, to a final $OD_{680\,nm}$ of 0.2. Drops of the mating mixture were deposited onto agar octopine medium (glucose 2 g/liter, octopine 10 mM), and the dishes were incubated at various temperatures for 48 hr. Controls were performed by plating separately donor and recipient cells on the same medium. The bacterial mixture, harvested with a platinum loop, was suspended in sterile water, diluted, and plated on minimal agar with octopine (10 mM) as the sole source of carbon and nitrogen, plus rifampicin (50 mg/liter) and streptomycin (500 mg/liter). After incubation for 1 week at 25°, the dishes were examined and the colonies were scored. Donors were completely eliminated by the antibiotics; recipients showed only thin growth, whereas transconjugants gave characteristic colonies. Phage typing was used to demonstrate homology between transconjugants and recipients (13).

## EXPERIMENTAL RESULTS

We have studied the influence of temperature on the transfer of the Ti plasmid from a virulent strain of *A. tumefaciens* to a strain of that organism that had been cured of the plasmid. The results of this study, which are shown in Table 1, demonstrate that the conjugative process involved in the transfer of the plasmid, like the transformation process in the crown gall disease, is thermosensitive with the critical temperature at or

‡ $KH_2PO_4$, 10.7 g/liter; $MgSO_4 \cdot 7H_2O$, 160 mg/liter; $CaCl_2 \cdot 2H_2O$, 10 mg/liter; $FeSO_4 \cdot 7H_2O$, 5 mg/liter; $MnSO_4 \cdot 7H_2O$, 2 mg/liter; $(NH_4)_2SO_4$, 2 g/liter. The last component was omitted in the selection medium, where octopine was the source of both carbon and nitrogen.

Table 1. Crosses between *Agrobacterium tumefaciens* C58C9 *rif*$^r$*str*$^r$ and B6S3

| Mating temperature | Frequency of transfer* |
|---|---|
| 23° | $4.0 \times 10^{-2}$ |
| 27° | $3.5 \times 10^{-2}$ |
| 30° | $0.9 \times 10^{-2}$ |
| 33° | $<10^{-6}$ |
| 37° | $<10^{-6}$ |

* No. of transconjugants per recipient cell.

near 30° in both instances. The results of these studies suggest, therefore, that both processes may share a common step which is thermosensitive.

## DISCUSSION AND CONCLUSIONS

Braun has established that several steps are involved in the formation of tumors in the crown gall disease of plants (16). Of these, only one, that involved in the inception phase, is thermosensitive. The results of studies reported here demonstrate that a conjugative process involving the transfer of the oncogenic Ti plasmid from a virulent strain of *A. tumefaciens* to a strain of that bacterium that has been cured of the plasmid is also thermosensitive in precisely the same temperature range as is the transformation process. The critical temperature in both instances is at or near 30°. The results of these studies suggest, therefore, that both transformation and conjugation share a common thermosensitive step. If, moreover, the transformation of a normal plant cell to a crown gall tumor cell and the conjugative transfer of the Ti plasmid from virulent to avirulent strains of that bacterium result from similar mechanisms, it would provide an explanation of why transformation does not occur above 30° in the crown gall disease.

That plasmid DNA is importantly involved in the development of the tumor phenotype in crown gall now appears established (9, 10, 17). Whether these newly introduced plasmid genes are themselves coding for one or more tumor-promoting factors or are acting indirectly to persistently activate host cell genes that are involved in the establishment and maintenance of the tumorous state remains to be determined.

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