Behavior of IncQ Plasmids in Agrobacterium tumefaciens

Hille, Jacques; Schilperoort, Rob

Published in:
Plasmid

DOI:
10.1016/0147-619X(81)90045-7

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1981

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
https://doi.org/10.1016/0147-619X(81)90045-7

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 09-03-2021
Behavior of Inc-Q Plasmids in Agrobacterium tumefaciens

JACQUES HILLE AND ROB SCHILPEROORT

Department of Biochemistry, State University of Leiden, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

Received July 1, 1981

Inc-Q plasmids were introduced into Agrobacterium tumefaciens, by mobilization from Escherichia coli with an Inc-P plasmid, or by transformation with purified plasmid DNA. It was found that they were stably maintained. The presence of an Inc-Q plasmid did not influence tumorigenicity. These results suggest that these plasmids may be used in genetic complementation studies of Ti plasmid mutants in A. tumefaciens.

The Ti plasmid of Agrobacterium tumefaciens is essential for crown gall tumor formation (1–3). Part of the Ti plasmid, the T-DNA region, is transferred to the plant cell during tumor induction (4,5). Another segment of the Ti plasmid, distinct from the T-DNA region, was also found to be essential for tumorigenicity (6,7). This segment, the so-called Vir-region, was shown to be complementable in trans by R prime plasmids (8; J. Hille, I. Klasen, and R. Schilperoort, submitted for publication).

Detailed complementation studies of such Ti plasmid mutants might give information on the mechanism of tumor induction by the bacterium. To this end, we sought possible cloning vehicles that: (i) can replicate in A. tumefaciens, (ii) do not themselves influence tumorigenicity, (iii) have a suitable size and can be used for cloning with insertional inactivation, and (iv) most preferably can also replicate in Escherichia coli. A vector that fits these four conditions might give information on the mechanism of tumor induction by the bacterium.

Purified pKT214 DNA, isolated from E. coli, was used to transform A. tumefaciens strain C58 by a freeze/thaw method (10). About 2000 Tc'Sm'Cm' transformants were obtained with 1 pg plasmid DNA and 10^9 bacteria. The transformation frequency was not influenced when the same plasmid, isolated from Agrobacterium, was used. This value is comparable to the frequency of transformation of small Ti plasmid derivatives (11), which indicates that pKT214 is not sensitive to any restriction/modification system of A. tumefaciens strain C58.

In order to obtain an extra selectable marker on pKT214, this plasmid was made linear with BamH1. A small Ti plasmid derivative, pAL2832 (11), was also digested with BamH1, which gives fragments of 0.9 and 1.6 Mdalton. The 0.9-Mdalton fragment carries the Ch resistance marker of transposon Tn1. These fragments were mixed,
Fig. 1. Map of the Inc-Q plasmid pRL220. In the circle PstI sites are shown; the dark part represents the largest PstI fragment of RSF1010, from which this plasmid is derived. Abbreviations: Cb, resistance to carbenicillin; Cm, resistance to chloramphenicol; Sm, resistance to streptomycin; Tc, resistance to tetracycline.

ligated, and used to transform A. tumefaciens to Cb resistance. One out of forty transformants turned out to carry pKT214-Cb, the plasmid of which was named pRL220 (see Fig. 1).

To test for possible influence of an Inc-Q plasmid on the tumorigenicity of Agrobacterium, several different strains were transformed with pRL220 and thereafter tested for tumor-inducing capacity on two different plants (see Table 1). No influence on oncogenicity was observed. This is in contrast to Inc-W plasmids. It was reported that, in their presence, Agrobacterium strains failed to induce tumors (12).

We have shown that Inc-Q plasmids do conform to the conditions stated. They replicate in E. coli as well as in A. tumefaciens, they can be transferred reciprocally between these species by mobilization and transformation, and they do not influence the tumor-inducing capacity of A. tumefaciens. Furthermore, cloning with the use of insertional inactivation is possible in the unique sites for BglII and XbaI in the Tc resistance locus of pKT214 and pRL220.

These plasmids can be utilized for site-specific mutagenesis of Ti plasmids in the manner described by Ruvkun and Ausubel (13). Ti plasmid fragments, cloned on pKT214, can, e.g., be transposon mutagenized in E. coli. Mutagenized recombinant plasmids can be introduced into an Agrobacterium strain, carrying a Ti plasmid. Upon introduction of pRL220, the described recombinant plasmid is lost because of incompatibility. With a certain frequency the transposon is rescued by homologous recombination with the corresponding region in the Ti plasmid.

The unique BglII site of pRL220 can be applied for cloning the cos site of phage λ, which is conveniently located between BglII sites on the plasmid pHC79 (14). This per-

**TABLE 1**

TUMOR-INDUCING CAPACITY OF STRAINS CARRYING pRL220

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid(s)</th>
<th>Tested on tomato</th>
<th>Tested on sunflower</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without pRL220</td>
<td>In the presence of pRL220</td>
</tr>
<tr>
<td>LBA288</td>
<td>Cryptic</td>
<td>-c</td>
<td>-</td>
</tr>
<tr>
<td>LBA677</td>
<td>Cryptic, pTi</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LBA937</td>
<td>Cryptic, pTi, R772</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LBA973</td>
<td>Cryptic, pAL969a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LBA958</td>
<td>Cryptic, pTi-C58</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

|              |                    |                   |                     |

|              | Plasmid pRL220 was introduced into these strains by transformation. |
|              | The cryptic plasmid in A. tumefaciens is described by Casse et al. (1979). |
| (−)          | No tumor induction; (+) tumor induction. |
| a Plasmid pAL969 is an R772::Ti cointegrate plasmid. |
mits the use of this plasmid for cosmid cloning in E. coli. Fragments of about 15 Mdalton can be cloned on such a plasmid. The possibility to do so will contribute to complementation studies with Ti plasmid mutants.

ACKNOWLEDGMENTS

We thank our colleagues of the MOLBAS research group for their help and especially Paul Hooykaas for critically reading this manuscript.

REFERENCES