Stabilization of Rhizobium symbiosis plasmids

The ability of Rhizobium sp. NGR234 to participate in symbiosis is determined by a 536 kb symbiotic plasmid, pNGR234a (5). This plasmid contains genes essential for symbiosis, but NGR234 can be cured of pNGR234a, yielding strain ANU265 (10). These can be divided into mechanisms that actively distribute the plasmid to daughter cells at cell division, those which resolve plasmid oligomers (when they occur) into monomers and those which cause the death of plasmid-free daughter cells by post-segregational killing. In all cases of post-segregational killing, a plasmid-encoded gene system produces a stable poison and an unstable antidote. In a plasmid-deficient offspring a protein antidote is more readily digested by cellular proteases than the poison and the cell is killed by the toxic effects of the latter. The chromosomally encoded two-gene hip operon of Escherichia coli described by us (4) and others (2, 3, 11, 12, 14) exhibits many of the characteristics of the two-protein plasmid stabilization system, other examples of which include ccd of the F plasmid, parD/pem of plasmids R1 and R100, parDE of plasmids RP4 and RK2, and phd/doc of the PI plasmid (for review, see ref. 8). The hip operon, at 33.8 min on the E. coli chromosome, consists of two genes, hipA and hipB, encoding proteins of 50 and 10 kDa, respectively. Under normal conditions, HipA and HipB cannot be isolated unbound to one another (3). If hipA, encoding the poison, is expressed in excess of hipB, encoding the antidote, then E. coli cell division is rapidly inhibited (4). As in other stabilization systems, hipA and hipB are adjacent genes in an operon in which the antidote is encoded by the upstream gene of the operon and the bound complex of antidote and poison acts to autoregulate the system by binding an upstream regulatory region (3). In addition to functioning as an antidote, HipB, a Cro-like protein, also acts as a repressor reducing the activity of the hip promoter to barely detectable levels (3). This, coupled to the fact that the stop codon of hipB overlaps the start codon of hipA, indicates the presence of strict transcriptional and translational regulation to keep levels of unbound HipA, the poison, to an absolute minimum. It could therefore be concluded that, if expressed on a plasmid, the hip operon would act as a stabilization system in which plasmid loss would result in HipB being more rapidly degraded than HipA causing death of the plasmid-free cell.

There are examples of chromosomal operons that have homology to plasmid-encoded genes responsible for plasmid maintenance, such as chpA and chpB, which have homology with pem of the R100 plasmid (9). As with hip, the function of such chromosomal systems is unknown, but ChpA and ChpB have similar effects upon cell growth as the corresponding Pem protein. In the case of chpA and chpB, homologues do exist on E. coli plasmids. However, in the case of hip there appear to be only two intact homologues of the poison gene hipA, and both are on the Rhizobium symbiosis plasmid pNGR234a (5). These are genes y4mE and y4dM, previously of unassigned function, but encoding proteins with 28 and 27 % identity, respectively, to HipA. As in the case of hipA and hipB, both y4mE and y4dM are downstream of repressors y4mF and y4dL, the stop codons of which overlap the start codons of y4mE and y4dM, respectively. This strongly indicates that, as seen in E. coli, unregulated expression of the hipA analogues would be detrimental to the cell (4). It is therefore tempting to speculate that y4mE/mF and y4dM/dL act as plasmid stabilization systems for the symbiosis plasmid pNGR234a, a plasmid that can be cured from Rhizobium without affecting survival of the organism (10).

The known targets for protein killer genes include DNA gyrase (ccd) (1) and DnaB (pem) (13). In the case of hip the target is unlikely to be DNA gyrase as its inhibition leads to filamentation of E. coli, a phenomenon not observed with induction of hipA (4). The parD and pem systems of plasmids R1 and R100 are located close to their origins of replication and this is also the case for one of the hip homologues (y4dM) in Rhizobium in which the locus is situated equidistant between the origin of replication (oriV) and origin of transfer (oriT). The pem system consists of PemK (poison) and PemI (antidote) and the target of the system is believed to be DnaB, as DnaB-dependent initiation of DNA replication is inhibited by the addition of PemK in vitro (13). Due to the similar location of the hip analogue to y4dM, it is tempting to speculate that both y4dM and hipB are regulated by the same mechanism (10).

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the origin of replication of the symbiosis plasmid, DnaB is a possible ydM target in \textit{Rhizobium}.

This is the first evidence that a plasmid stabilization system may exist in \textit{Rhizobium} and suggests a mechanism by which such a system might operate to retain certain symbiosis plasmids.

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**A complex group I intron in \textit{Nectria galligena} rDNA**

An article by Crockard and coworkers in a recent issue of \textit{Microbiology} reported a polymorphic insertion element of 363, 1185 or 1423 bp within the nuclear small subunit (SSU) rDNA of the ascomycete fungus \textit{Nectria galligena} (3). The insertion site was mapped to position 1199 (corresponding to \textit{Escherichia coli} SSU rRNA numbering), a site known to harbour nuclear group I introns in fungi. However, the authors failed to assign this insertion to any known class of insertion elements.

Several classes of insertions (introns and retroelements) are known to interrupt genes encoding rRNA, with the group I introns being the most frequent (10). Despite the fact that all group I introns perform the same catalytic reaction at the RNA level, resulting in intron excision and exon ligation (1), almost none of the sequence positions are universally conserved. However, the group I intron RNAs can be folded into a common well-defined secondary and three-dimensional structure (12), and 12 subgroups, based on distinct primary sequence motifs and characteristic structural features in peripheral regions, have been proposed (5, 13).

We have re-analysed the \textit{Nectria} sequences reported by Crockard et al. (3) and discovered that the smallest insertion (the 363 intron) contains a typical group I intron structure (Fig. 1a). The intron, which belongs to subgroup IC4, is distantly related to the more common subgroup IC1 introns in nuclear rDNA (5). A homologous structure is also present at the 5′ end of the \textit{Nectria} intermediate-sized insertion (the 1185 intron). We have previously reported functional analyses of a group IC4 intron in the SSU rDNA of the myxomycete \textit{Didymium} (4, 11). The group IC4 intron follows the same splicing pathway as the prototype group IC1 intron from \textit{Tetrahymena} (1).

A FASTA search (GCG Computer Package) using the 363 intron sequence as a ‘probe’ identifies three related fungal group IC4 introns inserted at the SSU rDNA position 1199 (the same as the \textit{Nectria} intron). These include two \textit{Cryptophyta} introns (2) and an intron from \textit{Tilletiopsis flava} (15). The fact that similar group IC4 introns are found in distantly related eukaryotic micro-organisms suggests that the \textit{Nectria} 363 intron, in contrast to what was suggested by Crockard et al. (3), has been gained by horizontal transfer during evolution.

Some nuclear group I introns are known to contain ORFs encoding functional endonucleases involved in intron mobility by homing at the DNA level (6, 8, 14). All these nuclear homing endonucleases contain the characteristic His-Cys box motif of a 30-aa histidine- and cysteine-rich sequence (9). An identical His-Cys box is shared by the 1185 intron and in the large \textit{Nectria} insertion (the 1423 intron), and the motif in \textit{Nectria} (Fig. 1b) is very similar to those previously reported in homing endonucleases from the myxomycete \textit{Physarum}, the amoeboidlale \textit{Naegleria} and the red alga \textit{Porphyra} (8). The \textit{Physarum} homing endonuclease is well studied and known to be directly involved in group I intron mobility (14). The recently deduced crystal structure of this endonuclease suggests that the His-Cys box resides are directly involved in novel zinc-binding motifs and in the active site of the enzyme (7). In \textit{Nectria}, the His-Cys box-