Gene, 139 (1994) 133–134

GENE 07613

Brief Notes

A protein involved in stabilization of a large non-symbiotic plasmid of *Rhizobium meliloti* shows homology to eukaryotic cytoskeletal proteins and DNA-binding proteins*

(α-Tubulin; partition mechanisms; homeobox domain)

Jesús Mercado-Blanco and José Olivares

Departamento de Microbiología, Estación Experimental del Zaidín, CSIC, 18008 Granada, Spain

Received by F. Bolivar: 10 May 1993; Revised/Accepted: 10 August/20 August 1993; Received at publishers: 20 September 1993

SUMMARY

An open reading frame, denoted ORF2, present in the replication and stabilization region of plasmid pRmeGR4a of *Rhizobium meliloti* GR4, was identified by sequence analysis. This 1068-bp ORF2 potentially codes for a 356-amino-acid protein that seems to play a role in pRmcGR4a stabilization. Similaritics of the ORF2-encoded protein with eukaryotic cytoskeletal proteins and DNA-binding proteins were found.

The presence of large plasmids in rhizobia species is a common trait. *Rhizobium meliloti* strain GR4 carries two highly stable non-symbiotic plasmids in addition to the pSyms. Until now there is no information on the replication and stabilization mechanisms of rhizobial plasmids although isolation of minimal replicons has been achieved (Neilan et al., 1986; Mozo et al., 1990). These large plasmids, that usually are considered as cryptic, are stably maintained both in free-living rhizobia and in symbiosis. Several stabilization mechanisms have been identified in different replicons. Some of them, the so-called partition mechanisms, are being extensively studied and involvement of proteins with similarities to cytoskeletal proteins has been proposed (for a review, see Williams and Thomas, 1992).

Correspondence to: Dr. J. Olivares, Departamento de Microbiología, Estación Experimental del Zaidín, CSIC, Prof. Albareda 1, 18008 Granada, Spain. Tel. (34-58) 121011; Fax (34-58) 129600.

*On request, the authors will supply detailed experimental evidence for the conclusions reached in this Brief Note.

Abbreviations: aa, amino acid(s); bp, base pair(s); HTH, helix-turn-helix; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); *ori*, origin of replication; ORF, open reading frame.

In the course of DNA sequencing and characterization of the replication region of the highly stable plasmid pRmeGR4a (segregation rate of $<0.1\%$ loss per generation according to Durland and Helinski, 1987), we identified an ORF (ORF2) with a coding capability for a 356-aa (38.5-kDa) protein (Fig. 1A). Deletions affecting this ORF did not abolish replication but did affect stabilization. Thus, the segregation rate of recombinant plasmids pJMB40 and pJMB45 harbouring the minimal pRmeGR4a replicon was 0.2% loss per generation (Mercado-Blanco and Olivares, 1993). In contrast, the segregation rate of a 1980-bp deletion derivative affecting the whole ORF2-coding region was tenfold higher. These experiments were conducted in continuous log-growth cultures during 80 generations in strain GRM8SR (a pRme-cured derivative of GR4) harbouring the recombinant plasmids. Comparison of this putative protein with sequences in the Swissprot and PIR protein data bases showed homology with α-tubulin of different species (Fig. 1B). In addition, homologies were found with other cytoskeletal proteins as is represented in Fig. 1A. Also, ORF2 has partially conserved the type-I ATP-binding motifs (Walker et al., 1982) (Fig. 1A) that have been de-

SSDI 0378-1119(93)E0609-H

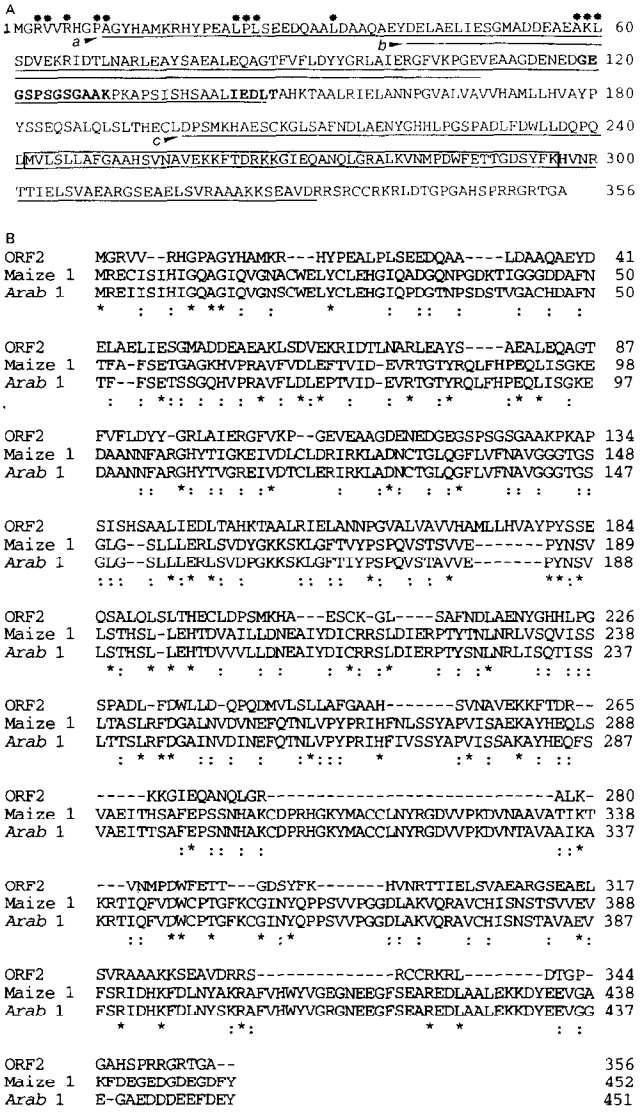

Fig. 1. Alignment of the aa sequence deduced from ORF2 present in the replication region of plasmid pRmeGR4a and proteins of different origins. (**A**) The aa sequence deduced from ORF2. Data on nt sequence were obtained from plasmid templates using Sequenase T7 DNA polymerase for sequencing reactions according to the manufacturer's indications (US Biochemical, Cleveland, OH, USA). For these experiments we used derivatives of pBluescriptIIKS carrying a 4.8-kb *Pst*I fragment containing the *ori* region of pRmeGR4a. Three segments of the protein that are underlined showed the following alignments: (***a***) homology to a microtubule-associated protein (human), 16.4% identity in 146 aa overlapped; (***b***) 25.4% identity with neurofilament protein (human); (***c***) homology to *hox-2,4* homeobox gene product (*Mus musculus*) (Kongsuwan et al., 1989). Boxed region corresponds to the homeobox domain that is involved in DNA binding (26.8% identity in this segment). In this region identities were also found with the N-terminal domain of NodD proteins, also involved in DNA binding. Furthermore, in this domain is located the HTH motif detected for NodD proteins. Asterisks above aa in the N-terminal domain of the sequence indicate identities with NodD domains that showed homology to vertebrate nuclear receptors (Györpal and Kondorosi, 1991). Positions 119–130 and 143–147 that have partially conserved the type-I ATP-binding motifs proposed by Walker et al. (1982) are indicated in boldface. The nt and aa sequences reported in this paper are deposited in the EMBL, GenBank data bases under accession No. X69105. (**B**) Alignment of the aa sequences for ORF2 and two α-tubulins from maize (Maize 1) and *Arabidopsis* (*Arab* 1). The overall identity and similarity were 14.3% and 30.6% in 356 aa overlapped, respectively. It must be noted that two important regions (a Gly-rich domain involved in GTP binding and the highly variable acidic C-terminal domain) are absent in the *Rhizobium meliloti* GR4 protein. (*) identity; (:) similarity.

tected in the A proteins of the partition systems (Williams and Thomas, 1992). On the other hand, we have found that this protein also showed identity with proteins involved in DNA binding such as an eukaryotic gene with a homeobox domain (*Hox2,4*) and the DNA-binding domain of the NodD proteins where HTH is located (Fig. 1A). These two findings (domains with homology to DNA-binding proteins and similarities to cytoskeletal proteins) along with the fact of destabilization displayed by ORF2-deleted clones suggest the presence of a yet uncharacterized stabilization mechanism in which ORF2 protein may be involved.

AKNOWLEDGEMENTS

This research was supported by Comisión Interministerial de Ciencia y Tecnología, grant BIO90-0747, and the EC BRIDGE Program, grant BIOT159-C. The authors are indebted to P. Puigdomenech and J. Rigau for the alingment of α-tubulin sequences.

REFERENCES

Durland, R.H. and Helinski, D.R.: The sequence encoding the 43-kilodalton TrfA protein is required for efficient replication or maintenance of minimal RK2 replicons in *Pseudomonas aeruginosa*. Plasmid 18 (1987) 164–169.

Györpal, Z. and Kondorosi, A.: Homology of the ligand-binding regions of *Rhizobium* symbiotic regulatory protein NodD and vertebrate nuclear receptors. Mol. Gen. Genet. 226 (1991) 337–340.

Kongsuwan, K., Allen, J. and Adams, J.M.: Expression of *hox-2,4* homeobox gene directed by proviral insertion in a myeloid leukemia. Nucleic Acids Res. 17 (1989) 1881–1892.

Mercado-Blanco, J. and Olivares, J.: Stability and transmissibility of the cryptic plasmids of *Rhizobium meliloti* GR4. Their possible use in the construction of cloning vectors for rhizobia. Arch. Microbiol. 160 (1993) in press.

Mozo, T., Cabrera, E. and Ruiz Argüeso, T.: Isolation of the replication region from a *Rhizobium* plasmid and examination of its potential as a replicon for rhizobiaceae cloning vectors. Plasmid 23 (1990) 201–205.

Neilan, J., Heery, D. and Dunican, L.K.: Isolation of replication loci of plasmid origin from *Rhizobium trifolii*. Biochem. Soc. Trans. 14 (1986) 478.

Walker, J.E., Saraste, M., Runswick, M.J. and Gray. N.J.: Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinase, and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. 1 (1983) 945–951.

Williams, D.R. and Thomas, C.M.: Active partitioning of bacterial plasmids. J. Gen. Microbiol. 138 (1992) 1–16.