Plasmid 65 (2011) 150–158

Contents lists available at ScienceDirect

Plasmid

journal homepage: www.elsevier.com/locate/yplas

The transfer operon of plasmid R1 extends beyond *finO* into the downstream replication genes

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ARTICLE INFO

Article history:
Received 11 June 2010
Accepted 3 December 2010
Available online 8 December 2010
Communicated by Eva Top

Keywords:
Bacterial conjugation
Gene regulation
Transcription
Copy number control
Fertility inhibition
Replication

ABSTRACT

finO is the final gene in the 35.4 kb transfer operon of IncFI plasmid F that is known to be involved in self-conjugative transfer. The genetic region distal to *finO* separates the conjugation and replication control modules of IncFII plasmid R100 and carries uncharacterized genes not found in plasmid F. However, comparison of the R100 gene organization with database entries of F-like plasmids suggests its broad conservation. We determined the DNA sequence of this region of IncFII plasmid R1 and studied its transcriptional organization. We find that transcription occurs through the entire gene region spanning from *finO* to the 5′ regulatory region of *copB*. The region lacks independent promoter activity and transcription is co-regulated with the adjacent transfer operon. We show that this extended transcriptional organization beyond *finO* is shared by plasmid R100. These findings support the hypothesis that gene products coexpressed from the *finO*-distal region in F-like plasmids are advantageous under some conditions when conjugative DNA is exchanged.

1. Introduction

The IncF plasmid F and the IncFII plasmids R1 and R100 serve as model systems for understanding several key processes in plasmid biology. These paradigm plasmids have been studied extensively to understand the mechanisms of plasmid replication, copy number control, partitioning, post-segregational killing, conjugative pilus assembly, fertility inhibition, and gene transfer via bacterial conjugation. The genetic information for this diversity of functions is carried by genomes of about 100 kb.

The process of bacterial conjugation depends on a highly sophisticated molecular machinery based on the integrated activity of proteins and requires substantial coding capacity (~35 kb). Although they provide their hosts with useful adaptive functions the potential metabolic burden of maintaining conjugative plasmids remains high (Godwin and Slater, 1979; Zund and Lebek, 1980). An effective strategy to limit this load is to repress the expression of conjugation genes. Fertility inhibition in IncF plasmids (Firth et al., 1996; Frost et al., 1994; Frost and Koraimann, 2010; Zechner et al., 2000) has been well studied with the paradigm plasmid R1 (Koraimann et al., 1991, 1996; Polzleitner et al., 1997). Organizing the majority of transfer (*tra*) genes into a long multicistronic operon, spanning from *traY* to *finO*, ensures balanced expression of the numerous proteins involved. The *tra* operon promoter (P_{traY}) of R1 is controlled by host and plasmid encoded effectors (Polzleitner et al., 1997; Strohmaier et al., 1998). The plasmid fertility inhibition (Fin) system controls expression levels of the key P_{traY} activator TraJ (Polzleitner et al., 1997; Schwab et al., 1993). The rate of translation of *traJ* mRNA is subject to FinP antisense-RNA (AS-RNA) regulation. FinO protein stabilizes the RNA hybrid complex to strengthen repression. The regulatory outcome of the Fin interactions tightly determines TraJ levels and induction of the entire *tra* operon (Koraimann et al., 1996).

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0147-619X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.plasmid.2010.12.003

Laboratory mutant derivatives with dysfunctional Fin components support highly efficient, derepressed (*drd*) conjugation frequencies by their hosts. The majority of native plasmids possess functional fertility inhibition systems.

In addition to Fin, survival of large conjugative plasmids like R1 is improved by sophisticated copy number control (CNC) systems that keep their numbers low (de la Cueva-Mendez and Pimentel, 2007; Nordstrom, 2006). The plasmid RepA protein is limiting so the key to controlling the frequency of R1 replication initiation at *oriR1* is tight regulation of the RepA concentration. The basic replicon includes additionally the CNC genes, *copB* and *copA*. Transcription of *copB* is coupled to the downstream *repA* gene yielding a bicistronic message. RepA can also be transcribed from the P_{repA} but this is subject in turn to a repressor activity of the CopB protein. CopA RNA functions as an AS-RNA that interacts with *repA*-containing transcripts blocking their translation. Together a sophisticated combination of transcriptional, post-transcriptional and coupled translational strategies limits RepA and fine-tunes R1 copy number in response to the prevailing conditions of the host and its environment.

In addition to housekeeping modules F-like conjugative plasmids harbor genes of unknown function, which are presumably responsible for important phenotypes in natural environments, as well as antibiotic or resistance and virulence and colonization factors that are advantageous in stress situations and in pathogenic interactions. IncF plasmids are highly prevalent in natural and clinical isolates of *Escherichia coli* (Boyd et al., 1996; Johnson et al., 2007; Reisner et al., 2006a,b). Conjugative transfer of the F-like systems thus disseminates genes of the plasmid backbone and variable adaptive functions in clinically relevant and natural environments. Plasmid R1 carries genes whose functions are not understood, as well as multiple antibiotic resistance genes.

In light of the attention focused on the F family of model systems it is surprising that the regions downstream of *finO*, the last gene of the transfer operon known to be involved in conjugation (van Biesen and Frost, 1992; Yoshioka et al., 1990, 1987), remain uncharacterized. Indeed, although F, R1 and R100 are not identical, the complete sequence of plasmid R1 has not been deposited in a public database. We are interested in both the control and the consequences of bacterial conjugation mediated by F-like plasmids. This study investigates the transcriptional organization and potential functions of a block of uncharacterized genes in plasmid R1 positioned between the predicted end of the transfer operon and the distally located replication module. The data show that under laboratory conditions the region is expressed as a transcriptional unit apparently subject to control by the *tra* operon promoter P_{traY}.

2. Materials and methods

2.1. Strains, plasmids and media

E. coli strains and plasmids used in this study are listed in Table 1. Bacteria were grown in Luria–Bertani (LB)

Table 1
Strains and plasmids.

E. coli strain	Relevant genotype[a]	Reference/source
DH5α	*endA1 recA1 gyrA96 thi-l hsdR17 supE44 λ⁻ relA1 deoR Δ(lacZYA-argF)-U169 φ80dlacZΔ(M15)*	Woodcock et al. (1989)
MG1655	*Ivlg rfp50 thi*	Kjaergaard et al. (2000)
CSH26	*ara, Δ(lac-pro), thi*	Miller (1972)
MC1000	*araD139* (*ara-leu*)*7697* Δ*lac thi hsdR*	Silhavy et al. (1984)
K10636	MC1000 *galK::cat::resC-tet-resC*	Livny and Friedman (2004)
CSH26crt	CSH26 *galK::cat::resC-tet-resC*	This study
M1174	F-*sfrA*$^{+}$	Beutin and Achtman (1979) Silverman et al. (1991)
M1164	F-*sfrA5*	Beutin and Achtman (1979) Silverman et al. (1991)
Plasmid	**Description**	
R1	IncFII, *fin*+, Amp^R, Cm^R, Sm^R, Km^R	Goebel et al. (1977)
R1-16	IncFII, *fin*-, Km^R	Goebel et al. (1977)
R1*drd*19	IncFII, *fin*-, Amp^R, Cm^R, Sm^R, Km^R	Clerget et al. (1981)
R100	IncFII, *fin*+, Tet^R, Cm^R	Womble and Rownd (1988)
R100-1	IncFII, *fin*-, Tet^R, Cm^R	Womble and Rownd (1988)
pGZ119EH	P_{tac} based expression vector, ColD replicon, Cm^R	Lessl et al. (1992)
pGZarcA	*Eco*RI/*Hin*dIII fragment containing *arcA* Cloned into pGZ119EH, Cm^R	Zechner et al. (1997)
pJBA25	pUC18Not-RBSII-*gfp*mut3b∗-T_0-T_1, Amp^R	Sternberg et al. (1999)
pNUM1	Cloning vector containing *rrnB-bla-oriV* of pMS119EH and T_0 of pJBA25, Amp^R	This study
pNUM135	*tnpR* $^{RBS\ mut135}$ *lacZ* in pNUM1, Amp^R	This study
pIVET5nMut135	*oriR6K mobRP4 lacZ tnpR* $^{RBS\ mut135}$, Amp^R	Osorio et al. (2005)

[a] Tp^R, trimethoprim resistance phenotype; Sm^R, streptomycin resistance phenotype; Nal^R, nalidixic acid resistance phenotype; Amp^R, ampicillin resistance phenotype; Cm^R, chloramphenicol resistance phenotype; Km^R, kanamycin resistance phenotype; Tet^R, tetracycline resistance phenotype; Rif^R, rifampicin resistance phenotype.