Volume 222, number 1, 199–203 FEB 05148 September 1987

Cloning and localization of the repressor gene (*c*) of the Mu-like transposable phage D108

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Received 28 July 1987

We have localized the D108 thermosensitive (cts) repressor gene to a region of DNA approx. 600 base pairs (bp) in length by sub-cloning an *Rsa*I restriction endonuclease fragment (bp 200 to bp 802 from the left-end of the D108 genome). We determined that the gene product from this fragment appears to be the same size (19 kDa) as that expressed from clones containing larger fragments of D108 DNA. Results from in vitro gel electrophoresis band-retardation and in vivo immunity assays show that the sub-cloned repressor appears to be fully functional.

Repressor; Transposable bacteriophage; Immunity; Phage D108; Phage Mu

1. INTRODUCTION

D108 and Mu are temperate *Escherichia coli* phages [1,2] that are unusual in that their linear, 37 kilobase pair (kb) genomes also behave as giant, double-stranded DNA transposable elements. Although D108 and Mu share extensive homology (>90%) at the DNA [3] and protein [4] levels, they are not homologous in their left-end regulatory regions [3]. This region of non-homology encodes the repressor (*c*), *ner* (a λ *cro*-like protein [5,6]), and the amino-terminus of the transposase (*A*) gene product [7]. It has been previously reported (deduced from the DNA sequence) that the D108 repressor is a polypeptide of 174 amino acids, similar in size and amino acid composition to the Mu repressor protein [7,8]. We report here, however, that the D108 repressor protein has an apparent molecular mass (19 kDa) lower than that of Mu repressor protein (22.5 kDa) on SDS-polyacrylamide gels. Functional assays, both in vitro and in vivo, reveal that the sub-cloned repressor gene product appears to be fully active.

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00690

2. MATERIALS AND METHODS

Bacterial strains and plasmids used in this work are listed in table 1. D108 lysates (from strain LF4028) were prepared according to DuBow and Bukhari [4]. Plasmids used in this study were constructed as follows (fig.1). Plasmid pUD78, containing 1500 bp of the left-end of D108, was constructed by ligating (with T_4 DNA ligase, Pharmacia-PL Biochemicals, Milwaukee, WI), a 3130 bp *Eco*RI (Boehringer Mannheim, Canada) fragment from pSZ5326 (a pSC101::mini-D108

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00145793/87/$3.50

Table 1

Bacterial strains and plasmids

	Characteristics	Origin
Strain		
JM103	*Δpro-lac*, *rps* L, *thi*-1, *hsd* R^- M^+, *sup* E44, *end* E, *sbc* B /F' *tra* D 36, *pro* AB, *lacI*^{q} Z Δ M15	[5]
LF120	JM103 containing pUD68	this work
LF121	JM103 containing pOP95-15	this work
LF2210	JM103 containing pDL108	this work
LF2211	JM103 containing pDL109	this work
LF4028	*Δpro-lac*, *trp* 8am, *rps* L, /F' *pro-lac*I::D108 *c*ts10	[9]
Plasmid		
pDL108	*Amp*^{R}, *Tet*^{R}, D108 *c*ts10 repressor expressed from the *lac* UV5 promotor of pOP95-15	this work
pDL109	*Amp*^{R}, *Tet*^{R}, D108 *c*ts10 repressor expressed from the *lac* UV5 promotor of pOP95-15K	this work
pUD68	*Amp*^{R}, *Tet*^{R}, Mu *c*ts62 repressor expressed from the *lac* UV5 promotor of pOP95-15	this work
pUD78	*Amp*^{R}, *Tet*^{R}, D108 *c*ts10 immunity region (*c*, *ner*, *A'*)	this work
pOP95-15	*Amp*^{R}, *Tet*^{R}, *lac* UV5 promotor/operator (p/o) containing vector	[10]
pOP95-15K	*Amp*^{R}, *Tet*^{R}, *Kan*^{R}, *lac* UV5 p/o containing vector	[5]
pMD681	*Amp*^{R}, *Tet*^{R}, Mini-Mu *c*ts62	Lalumière and DuBow (in preparation)

plasmid [9]) into the *Eco*RI site of the *lac* UV5 expression vector pOP95-15 [10]. A 900 bp *Dra*I-*Pvu*II fragment from pUD78 was purified by the 'crush and soak' mehod [11]. After *Eco*RI linker addition [12], the fragment was ligated to *Eco*RI-linearized pOP95-15, resulting in plasmid pDL108. A 600 bp *Rsa*I (BMC) fragment (bp 200 to bp 802 from the left-end of the D108 phage genome [7]) was isolated from pDL108 and ligated into the *Sma*I site of pOP95-15K [5], resulting in plasmid pDL109. To clone the Mu *c*ts62 repressor gene, the left-most 1 kb of Mu DNA was isolated from plasmid pMD681 (Lalumière and DuBow, in preparation) as an *Eco*RI fragment and ligated into *Eco*RI-linearized pOP95-15, resulting in plasmid pUD68 (see table 1). *E. coli* strain JM103 (table 1) was transformed [13] and colonies containing the recombinant plasmids were isolated on LB plates [14] containing 40 μg/ml ampicillin. Plasmid-encoded gene products were labelled in vivo with [^{35}S]methionine (1120 Ci/mmol, Amersham) by the chloramphenicol release procedure of Neidhardt et al. [15] and visualized by fluorography [16] after electrophoresis on 16% SDS-polyacrylamide gels [17]. Crude protein extracts and band-retardation assays were prepared and conducted as described by Tolias and DuBow [5]. Relative immunity to D108 superinfection was tested by applying serial dilutions [10^2–10^7 D108 plaque-forming units (PFU)/ml] prepared in Mu buffer [4] to bacterial lawns in 0.75% soft agar, on LB agar plates containing 2.5 mM $CaCl_2$ and 2.5 mM $MgSO_4$ [14], and 1 mM isopropyl-β-D-pyranoside (IPTG) to induce plasmid-encoded gene expression. The plates were incubated overnight at either 32 or 43°C, and the presence or absence of bacterial lysis (plaque formation) was noted.

3. RESULTS

Fig.2 displays the proteins expressed from plasmids pDL108 and pDL109 (fig.1) labelled in

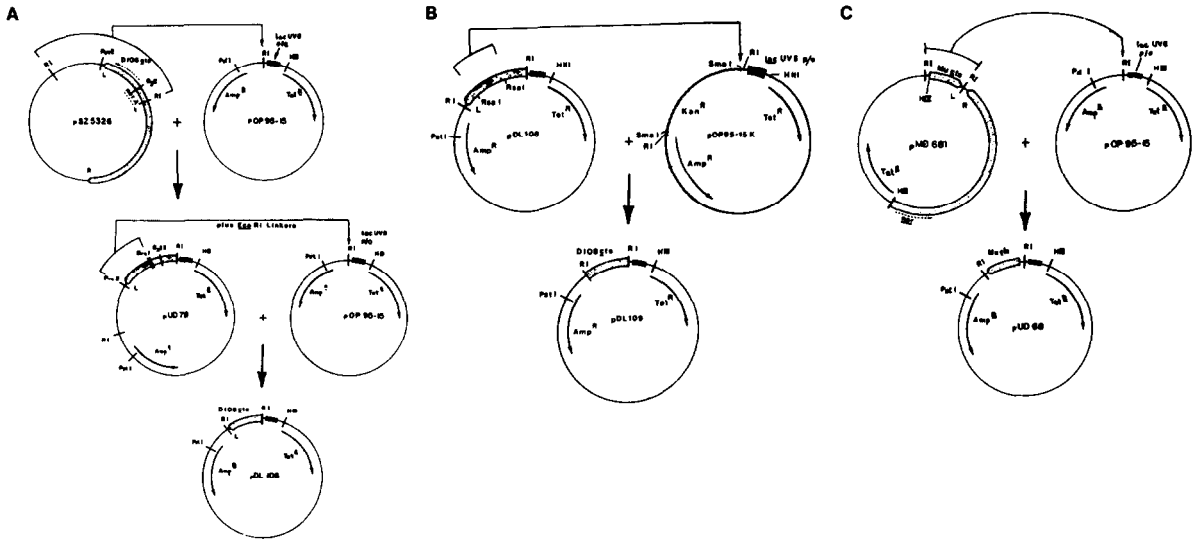

Fig.1. Construction of recombinant plasmids: (A) the D108 *c*ts10 repressor gene, cloned with a *Dra*I restriction fragment containing the left-most 865 bp of the D108 genome, (B) the D108 *c*ts10 repressor gene, cloned as a 600 bp *Rsa*I restriction fragment (bp 200–802 from the left-end of the D108 genome), and (C) the Mu *c*ts62 repressor gene, cloned with an *Eco*RI restriction fragment containing the left-most 1000 bp of the Mu genome, all under the control of the *lac* UV5 promotor in pOP95-15 [10].

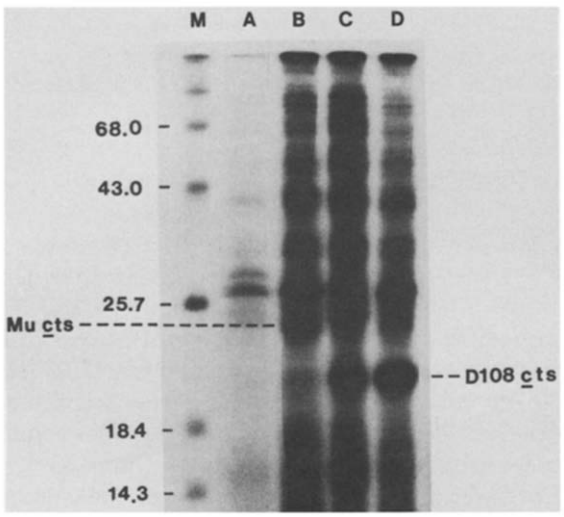

Fig.2. Autoradiogram of [^{35}S]methionine-labelled proteins expressed from (A) LF121 (containing the vector, pOP95-15), (B) LF120 (containing the Mu *c*ts62 repressor gene), (C) LF2210 (containing the D108 *c*ts10 repressor gene cloned from the *Dra*I site), and (D) LF2211 (containing the D108 *c*ts10 repressor gene cloned on the 600 bp *Rsa*I fragment). (M) ^{14}C-labelled marker proteins (BRL): 68.0 kDa, bovine serum albumin; 43.0 kDa, ovalbumin; 18.4 kDa, α-chymotrypsinogen; 14.3 kDa, β-lactoglobin.

vivo with [^{35}S]methionine [14]. Lane A of fig.2 shows the proteins expressed in the vector-containing (pOP95-15) strain LF121, while lane B shows the proteins expressed from the Mu repressor plasmid pUD68. A polypeptide with an apparent molecular mass of 22.5 kDa found in lane B, but not in lane A, most likely represents the 174 amino acid Mu *c*ts repressor gene product [18,19]. Both lanes C (pDL108) and D (pDL109) contain strongly expressed polypeptides with an apparent molecular mass of 19 kDa that are not represented in lanes A and B, which suggests that the 19 kDa proteins are the cloned D108 *c*ts repressor gene products.

Fig.3A and C displays the retarded migration of a ^{32}P-labelled restriction fragment (473 bp, *Sau*96-*Bgl*II) containing the D108 immunity/operator region [7] by increasing amounts of crude protein extract containing the D108 *c*ts repressor derived from either LF2210 (pDL108) or LF2211 (pDL109). Fig.3B and D demonstrates that these extracts do not affect the migration of a 385 bp *Hin*fI-*Eco*RI control fragment derived from D108 sequences adjacent to and downstream of the D108 immunity/operator region [7].

Cells containing pDL108 (LF2210) and pDL109

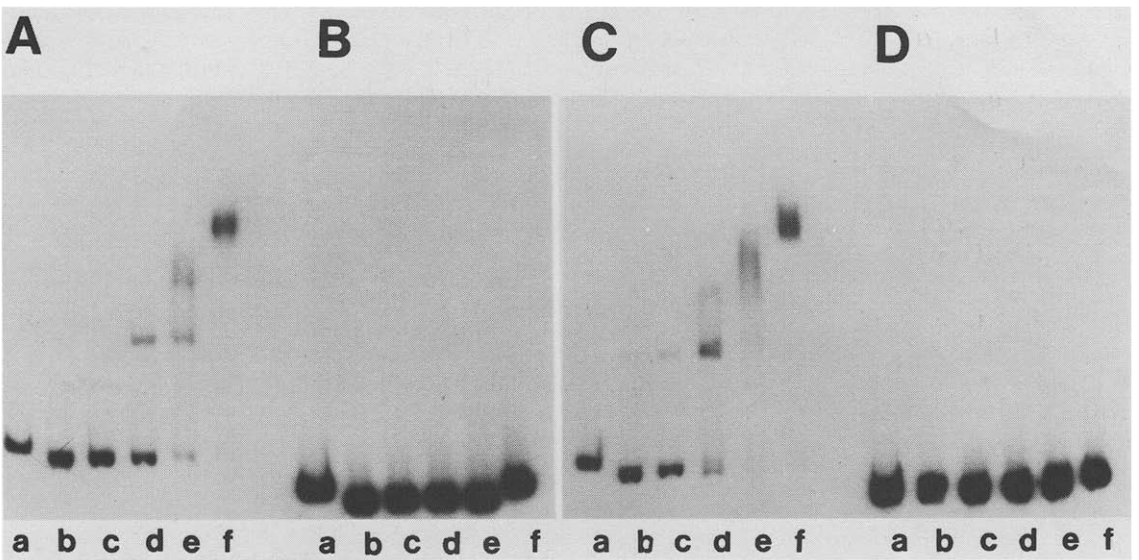

Fig.3. Band-retardation assays with D108 *cts* repressor-containing crude protein extracts. 2–10 ng ^{32}P-labelled DNA restriction fragment with (A,C) or without (B,D) the D108 repressor binding site were mixed with 1–5 μg unlabelled, sonicated calf thymus (non-specific) competitor DNA and increasing amounts of crude protein extract made from either LF2210 or LF2211. (A) Crude protein extract of LF2210 with the 473 bp *Sau*96-*Bgl*II restriction fragment containing the D108 immunity/operator region (specific substrate); (B) crude protein extracts of LF2210 with the 385 bp *Hin*fI-*Eco*RI restriction fragment (non-specific substrate); (C) crude protein extract of LF2211 with the 473 bp (specific) restriction fragment; (D) crude protein extract of LF2211 with the 385 bp (non-specific) restriction fragment. The amount of crude protein extract added in each reaction mixture was: (b) 0.05 μg (1.4 μg/ml), (c) 0.10 μg (2.8 μg/ml), (d) 0.50 μg (14.0 μg/ml), (e) 1.0 μg (28.0 μg/ml), and (f) 5.0 μg (140.0 μg/ml). (a) ^{32}P-labelled DNA fragment incubated without crude protein extract.

(LF2211) were immune to superinfection by D108 phage at 32°C but not at 43°C, while the vector-containing control strain (LF121) was not immune at either temperature (table 2).

Table 2

Comparative immunity test

Strain	Temperature	Phage	PFU/ml					
			10^2	10^3	10^4	10^5	10^6	10^7
LF2210	32°C	D108	–	–	–	–	–	+
	43°C	D108	*	*	*	*	+	+
LF2211	32°C	D108	–	–	–	–	–	+
	43°C	D108	*	*	*	*	+	+
LF121	32°C	D108	+	+	+	+	+	+
	43°C	D108	+	+	+	+	+	+

+, clearly discernible plaque; *, a poorly defined but detectable turbid zone of lysis; –, no detectable zone of lysis

4. DISCUSSION

We have localized a functional D108 repressor gene to a region of DNA approx. 600 bp in length. Mizuuchi et al. [7] proposed (from the DNA sequence) that the open reading frame of the D108 repressor gene begins at bp 864 from the D108 left-end to encode a Mu-sized repressor protein. However, functional assays both in vitro (band-retardation assays) and in vivo (immunity to superinfection) show that sequences located between bp 200 and 802 from the left-end of the D108 genome most probably encode the D108 repressor. Examination of the nucleotide sequence of this region [7] reveals a GTG codon at position 768 bp (from the D108 left-end), in the same reading frame as the putative 174 amino acid D108 repressor, which could serve as the initiation codon of the smaller repressor protein. This protein would consist of 142 amino acids and appears to migrate on SDS-polyacrylamide gels more slowly

(19 kDa) than its amino acid sequence would predict (15.8 kDa), as does the Mu repressor protein (22–26 kDa vs 19 kDa) [8]. The initiation codon of the *lac* repressor is also GTG [20] and this protein also migrates on SDS-polyacrylamide gels more slowly (40–50 kDa) than would be predicted from its amino acid sequence (38 kDa) [20,21]. The 142 amino acid D108 repressor retains its homology with the amino-terminus of the D108 A protein [7], and contains a bi-α-helical structure (amino acid residues 7–27 of the putative 142 amino acid D108 repressor) observed in many DNA-binding proteins [22] within which is found the putative D108 thermosensitive mutation [7]. It is possible that the 174 amino acid polypeptide predicted by Mizuuchi et al. [7] can be expressed from a D108 prophage, but may perform a different role from that of the smaller (142 amino acid) D108 repressor protein, as is the case for Tn*5* protein 1 and its internally initiated, in-frame protein 2 [23,24]. The difference in the size of the D108 and Mu repressor proteins suggests that they may be biochemically different and perform different roles in the regulation of D108 and Mu transposition. The nature of these differences is currently under investigation.

ACKNOWLEDGEMENTS

We extend our thanks to Dr P. van de Putte for his useful discussions. M.S.D. is a Chercheur-Boursier of the Fonds de la Recherche en Santé du Québec (FRSQ). D.B.L. was supported by a Medical Research Council of Canada Postgraduate Studentship. This work was supported by Medical Research Council of Canada grant MA6751.

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