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Gin-mediated site-specific recombination in bacteriophage Mu DNA: overproduction of the protein and inversion *in vitro*

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Inversion of the G segment in bacteriophage Mu DNA occurs by a site-specific recombination event and determines the host specificity of Mu phage particles produced. Inversion is mediated by a Mu function (Gin). The *gin* gene has been placed under control of the inducible λ pL promoter and a synthetic Shine-Dalgarno linker upstream of the initiation codon. The Gin protein content in induced cells is boosted to ~10% of total protein. Partially purified extracts from overproducing strains promote efficient inversion of the G DNA segment *in vitro* which is visualized by agarose gel electrophoresis of the substrate DNA after cutting with appropriate restriction endonucleases. The *in vitro* reaction requires Mg^{2+}, a supercoiled DNA substrate and occurs in the absence of exogenous ATP. Inversion from the G(+) to the G(−) orientation is as efficient as the switch from G(−) to G(+).

Key words: bacteriophage Mu/site-specific recombination/*gin* gene product/overproducer/inversion *in vitro*

Introduction

In bacteriophage Mu the 3000 bp long G segment undergoes inversion (Daniell *et al.*, 1973). This process is dependent on a phage-encoded recombination function termed Gin (Kamp *et al.*, 1978) which maps to the right of the invertible G segment in the β region (Kamp *et al.*, 1979). The recombination occurs within two 34 bp sequences that flank the G segment as inverted repeats. In its site-specificity inversion of the G segment resembles other recombination systems like λ integration (Nash, 1981), and transposon-mediated resolution in Tn3 and γδ (Grindley *et al.*, 1982). G inversion regulates the host range specificity: phages with a G segment in the (+) orientation can infect *E. coli* K12 (Kamp *et al.*, 1978) whereas phages with G in the G(−) orientation are infectious for other gram-negative bacteria (Van de Putte *et al.*, 1980; Kamp, 1981). Functions similar to Gin and capable of complementing a Mu gin^- mutant have been found in other phages and bacteria: the inversion functions of phages P1, P7 and D108 (Kamp *et al.*, 1979), the Gin (Kamp and Kahmann, 1981) and Pin (Plasterk *et al.*, 1983a) functions of certain *Escherichia coli* K12 strains and the Hin function of *Salmonella typhimurium* (Zieg and Simon, 1980). A characteristic feature of these inversion systems is their low efficiency; for Mu it is $\sim 10^{-6}$ per lytic cycle (Plasterk *et al.*, 1983b). Up to now this has impeded the purification of these site-specific recombination enzymes and has made a detailed molecular analysis impossible.

The aim of our study was the isolation of an overproducing strain where the Gin protein would be produced as a substantial fraction of total cellular protein. This was achieved by manipulating the DNA region 5′ to the *gin* gene. The biological activity of a partially purified Gin protein preparation is demonstrated in an *in vitro* assay for G inversion.

Results

Construction of plasmids which overproduce Gin protein

Our rationale for the construction of a Gin overproducer was as follows. In Mu expression of the Gin function appears to be constitutive (Hsu and Davidson, 1972). From the nucleotide sequence it has been inferred that the *gin* promoter is located within the right inverted repeat sequence (IR-R) of the G segment (Plasterk *et al.*, 1983b; R.Kahmann and D.Kamp, unpublished data). By fusing the *gin* gene, including IR-R, to stronger promoters, clones had been isolated with a 100-fold increase in their inversion rate; however, even under such conditions the *gin* product was detectable in minicells but could not be visualized in normal cell extracts (Kwoh and Zipser, 1981; Plasterk *et al.*, 1983b). The low yields of Gin protein obtained under such experimental conditions could be caused by autoregulation: since the target site for Gin, the inverted repeat, coincides with the *gin* promoter, Gin protein bound to the IR-R could block access of the RNA polymerase to this promoter and thereby decrease transcription of its own gene.

As a first step we have therefore cloned the *gin* gene without its own promoter downstream from the strong, inducible pL promoter of bacteriophage λ into the high copy plasmid pLc2833. For this approach we made use of an *Xho*II site located 9 bp upstream of the GTG initiation codon of *gin* and a *Bcl*I site situated between the *gin* and the following *mom* gene (Figure 1).

The *Xho*II-*Bcl*I fragment was inserted into the *Bam*HI site of pLc2833 yielding plasmid pLMugin-X16. The plasmid was transformed into an *E. coli* strain, carrying a defective λ prophage with a thermolabile *c*I857 repressor and a heat inducible $Mu_{445\text{-}5}$G(−) gin^- prophage. The G segment of the Mu prophage, which is frozen in the G(−) orientation, can be inverted *in trans* when *gin* is made from the plasmid. *Gin* complementation can then be monitored biologically as a host range switch from G(−) to G(+), e.g., a switch from phage growth on *Enterobacter cloacae* to growth on *E. coli* K12. During the incubation at 42°C the repressor is denatured turning on transcription of *gin* from the pL promoter and simultaneously the residing Mu prophage is induced. If expression of *gin* is controlled by the pL promoter, *gin* should only be produced during the time between heat induction and lysis of the cell (which is around 1 h) and during this period invert the G segments of replicating phages. Titration of such phage lysates on CSH50 revealed that these strains do produce G(+) phage; however, the titers of G(+) phage are ~10-fold lower than titers of Mu G(−) phage (Table I). Since constitutive expression of *gin* should result in a 50:50 ratio of G(−) to G(+) phages, we conclude that expression

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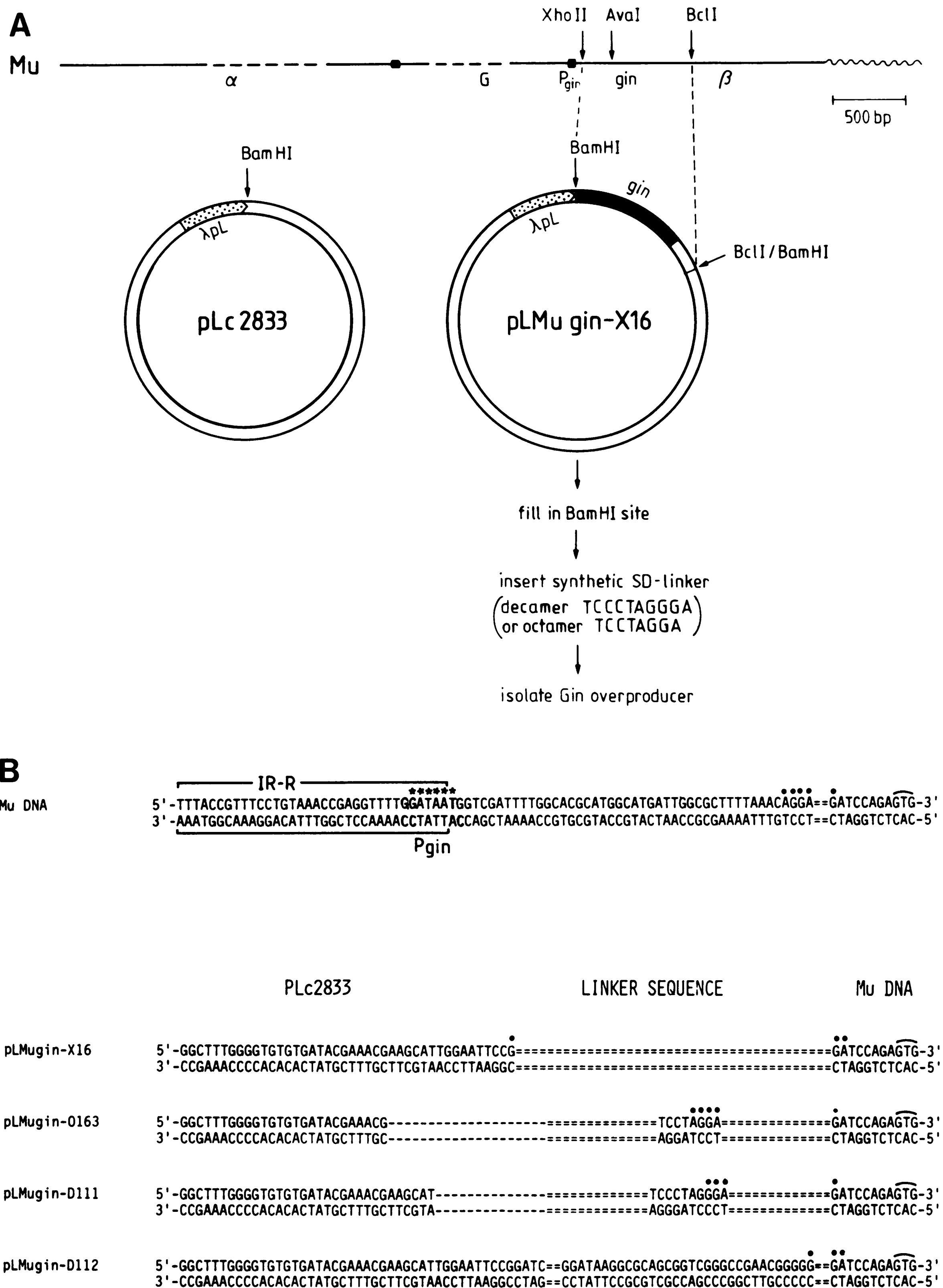

Fig. 1. **(A)** Construction of plasmids carrying the *gin* gene of bacteriophage Mu. The upper part shows a map of the Mu G region (R.Kahmann and D.Kamp, unpublished). P_{gin} marks the position of the *gin* promoter in the inverted repeat sequence IR-R. Only sites used in the construction of plasmids and for the sequence analysis are indicated. The lower part schematically shows the different steps involved in construction of plasmids overproducing Gin. Fragments containing the *gin* gene were isolated from plasmid pMu1107. Details of the construction are described in the text. All plasmids were transformed into *E. coli* K12ΔHIΔ*trp* (Mucts$62_{445\text{-}5}$G(−) *gin*$^{-}$). Plasmid structures were verified by restriction enzyme analysis. Dotted arrows show the promoter region pL and direction of transcription. Double lines represent the vector part of pLc2833. Thick lines indicate the coding sequence of *gin*. **(B)** DNA sequence of plasmids overproducing Gin protein. Double broken lines are used for connecting sequences which are directly linked. Single broken lines indicate deletions in the pLc2833 vector. Half circles mark the GUG initiation codon of *gin*. Brackets indicate the right inverted repeat of the G segment (IR-R). The Pribnow box of the putative Gin promoter is indicated by asterisks. Dots indicate SD-sequences.

Table I. Complementation of a Mu *gin*⁻ mutant by plasmids expressing *gin*

Plasmid[a]	Titer of G(−) phages on *E. cloacae*	Titer of G(+) phages on CSH50	Titer of G(+) phages / Titer of G(−) phages
pLc2833	1.2×10^7	$<10^2$	$<8.3 \times 10^{-6}$
pLMugin-X16	2.5×10^9	2.5×10^8	10^{-1}
pLMugin-D111	6.4×10^7	8.0×10^8	12.5
pLMugin-D112	8.0×10^8	5.1×10^9	6.4
pLMugin-0163[b]	2.2×10^6	9.0×10^6	4.1

[a]Host for all plasmids was *E. coli* K12ΔHΔtrp [Mucts$62_{445\text{-}5}$G(−) *gin*⁻].
[b]Titers of Mu phage propagated in the presence of pLMugin-0163 are usually very low because cell growth is arrested before the growth cycle of Mu is completed. Cell lysis is not observed.

of the *gin* gene in pLMugin-X16 is under the control of the pL promoter but the amounts made are not sufficiently high to achieve 50% inversion (e.g., comparable titers of G(+) and G(−) phages) within one generation. Furthermore, the amount of Gin protein synthesized by plasmid pLMugin-X16 is not high enough to visualize the protein in cell extracts (Figure 3). From the DNA sequence, the mol. wt. of Gin is predicted to be 21 700 (R.Kahmann and D.Kamp, unpublished). A possible explanation for our failure to achieve substantial levels of Gin production with plasmid pLMugin-X16 might be that the *gin* transcript is not efficiently translated. The *Xho*II site we made use of during construction of pLMugin-X16 is located within the proposed SD-sequence of *gin* (R.Kahmann and D.Kamp, unpublished results; Figure 1B). By cloning into the *Bam*HI site of pLc2833 the SD-sequence of *gin* has been altered from AGGAG to GGA. We reasoned that restoring or improving the SD-sequence in pLMugin-X16 by inserting a synthetic oligonucleotide linker containing a SD-sequence might raise the translation efficiency. In pLMugin-X16 the vector-*gin* fusion *via* *Bam*HI-*Xho*II has regenerated the *Bam*HI site. This site was subsequently used for insertion of a SD-linker (Figure 1B). Out of 700 transformants tested, three (pLMugin-D111, pL-Mugin-D112, pLMugin-0163) were found to yield comparable numbers of G(+) and G(−) phages in the complementation assay for *gin* (Table I). This indicated that these clones produce enough Gin protein to reach a balance between Mu G(+) and Mu G(−) phages in one generation.

Characterization of plasmids which overproduce Gin protein

In contrast to cells containing pLMugin-X16 which continue growth after induction of the pL promoter, the growth rate of cells harbouring these new Gin-producing plasmids is reduced; the most severe reduction is observed with pLMugin-0163 (Figure 2). When cell lysates of these putative Gin overproducers were analyzed on an SDS-polyacrylamide gel (Figure 3), after induction of the pL promoter a new peptide with the size expected for Gin is seen. In pLMugin-0163 the amount of Gin approaches 10% of the total cellular protein (Figure 3). In time course experiments we have shown that pLMugin-0163 extracts contain maximum levels of Gin already 30 min after pL induction, while it took ~1 h to detect visible amounts of Gin protein in extracts of strains containing pLMugin-D111 or pLMugin-D112 (results not shown). The amounts of Gin protein synthesized can be directly correlated with cell growth (Figure 2): cells harbour-

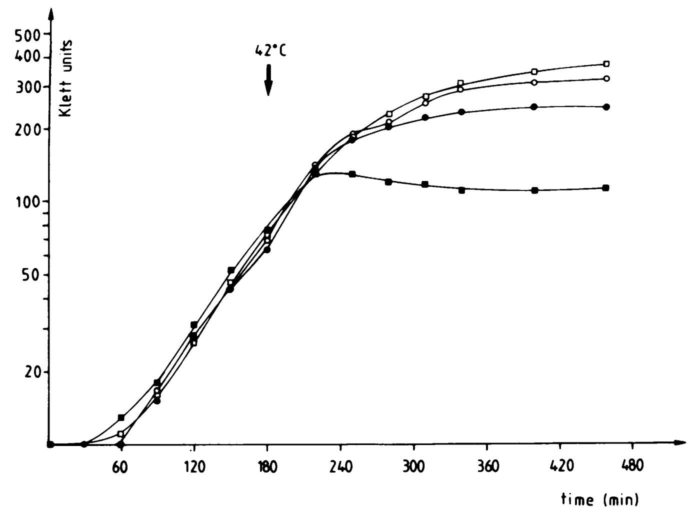

Fig. 2. Growth curves of cells harbouring plasmids that overproduce Gin. K12ΔHIΔ*trp*/pLMugin-X16 (-□-), pLMugin-0163 (-■-), pLMugin-D111 (-●-) and pLMugin-D112 (-○-) were grown in dYT + ampicillin. Cell density was determined using a Klett photometer. At 70 Klett units the cultures were shifted to 42°C and grown for another 280 min.

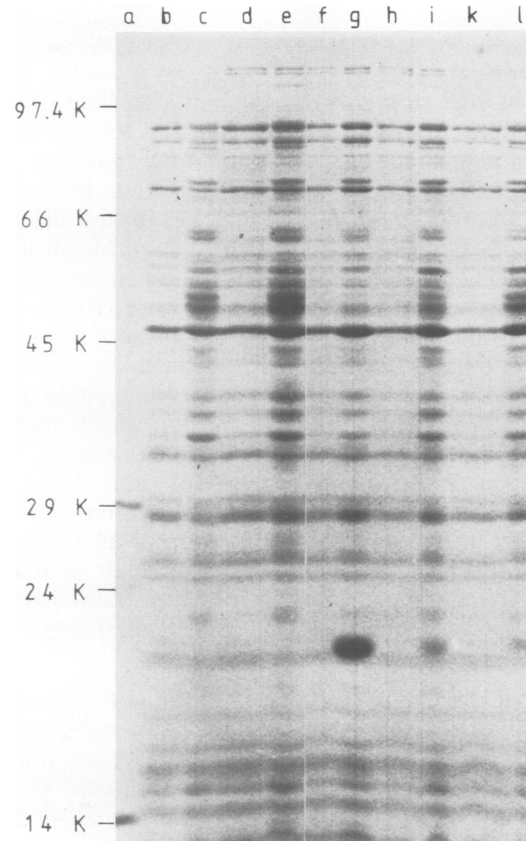

Fig. 3. SDS-polyacrylamide gel of total cell lysates from strains expressing Gin protein. Cells were grown and heat-induced under conditions described in Figure 2. Cell lysates from cultures at the time of heat induction and 2 h after heat induction are loaded side by side. Separation is on a 15% SDS polyacrylamide gel. **(a)** Mol. wt. standards: lysozyme (14 000), trypsinogen (24 000), carbonic anhydrase (29 000), ovalbumin (45 000), bovine serum albumin (66 000), phosphorylase b (97 400); **(b,c)** pLc2833; **(d,e)** pLMugin-X16; **(f,g)** pLMugin-0163; **(h,i)** pLMugin-D111; **(k,l)** pLMugin-D112. The arrow marks the Gin protein.

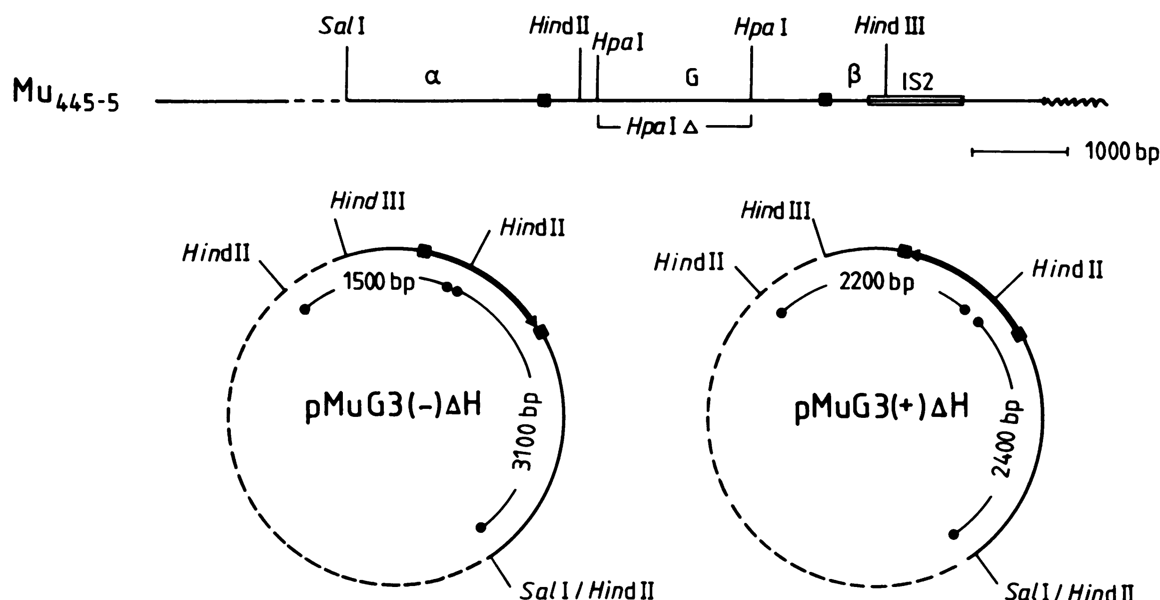

Fig. 4. Restriction map of the substrate DNA used to monitor G inversion. The upper part shows a map of Mu_{445-5} and indicates the region which was cloned in pBR322 to obtain plasmid pMuG3(+)ΔH. pMuG3(−)ΔH differs from pMuG3(+)ΔH only in the orientation (indicated by the arrow) of the G segment. Inversion can be monitored by *Hind*II cleavage because of the asymmetric location of one *Hind*II site within the G segment (Allet and Bukhari, 1975). This yields fragments characteristic for the two G orientations, numbers give their sizes in bp. The G segment is represented by thick lines, flanking Mu_{445-5} β and α sequences by thin lines and vector DNA by broken lines. Boxes indicate the inverted repeat sequences.

ing pLMugin-0163 stop growth ~45 min after induction of the pL promoter, a visible growth reduction for pLMugin-D111 becomes apparent only ~120 min after induction and for pLMugin-D112 growth inhibition manifests itself only after 3.5 h. In order to understand the basis for the different levels of Gin synthesis (pLMugin-0163 >>pLMugin-D111 >pLMuginD112), we sequenced the region between the pL promoter and the start of the *gin* gene (Figure 1B). Two of the three plasmids analyzed carry an SD-linker as expected, the third one contains DNA derived from an unknown source (pLMugin-D112). pLMugin-D111 and pLMugin-0163 have acquired small deletions during construction which, starting at the *Bam*HI site, extend towards the pL promoter. The deletions span 20 bp in pLMugin-0163 and 14 bp in pLMugin-D111, respectively. For unknown reasons we have not succeeded in finding a simple insertion of an SD-linker without a concomitant deletion. Several plasmids exhibiting the same level of *gin* expression as pLMugin-X16 were sequenced and were found to have filled in *Bam*HI sites.

G inversion in vitro

To show that the Gin overproducers facilitate the isolation and purification of active Gin protein, we have established an *in vitro* assay for a crude extract. As substrate we constructed a plasmid, pMuG3ΔH, which contains a shortened G segment in either G orientation but lacks a functional *gin* gene (Figure 4). *In vivo* the G segment of pMuG3(−)ΔH can be inverted to G(+) by providing the Gin function *in trans* on a prophage (data not shown).

The assay to detect inversion is based on changes in the *Hind*II restriction enzyme cleavage pattern of plasmid pMuG3ΔH when it is converted from G(−) to G(+) or *vice versa* (Figure 4). Extracts were made from strains containing plasmid pLMugin-0163 following a fractionation scheme similar to the one described for the resolvase of Tn3 (Krasnow and Cozzarelli, 1983). In such extracts obtained by differential salt precipitation, Gin is >85% homogeneous (Figure 5). Initially G(−) substrate DNA was incubated with a Gin extract or the pLc2833 control extract for 60 min and after deproteinization transformed into CSH50. Plasmid DNA from 100 transformants each was isolated and cut with *Hind*II. Thirty of the 100 transformants, where the substrate DNA had been treated with the Gin extract, had switched the G segment from the G(−) to the G(+) orientation. In the case of the pLc2833 extract all 100 transformants tested had retained their G segment in the G(−) orientation. This result demonstrates that the protein overproduced by pLMugin-0163 is able to catalyze the switch and furthermore that the control extract lacks such an activity.

Requirements for G inversion in vitro

Experiments described here were performed with Gin extracts which have been partially freed of endogenous DNA from the extract (see Materials and methods). As shown above the *in vitro* inversion frequency is sufficiently high to employ a direct physical assay for inversion. In a time course we have compared inversion from G(−) to G(+) and G(+) to G(−) in a pMuG3ΔH substrate DNA (Figure 6). Fragments characteristic for inversion appear already after a 2 min incubation period with the extract and these bands have reached their maximal intensity after 10 min. The inversion frequency is not affected by the initial orientation of the G segment in the substrate DNA. Employing the same physical assay for G inversion we have shown that the substrate DNA has to be supercoiled; linear, nicked or relaxed DNA are not inverted efficiently (Table II). The addition of 10 mM EDTA to the reaction buffer abolishes inversion while the addition of ATP has no measurable effect (Table II).

Discussion

Here we have described the construction of plasmids which overproduce Gin protein and have shown in an *in vitro* assay that cell-free extracts from overproducers promote efficient inversion of the G DNA segment.

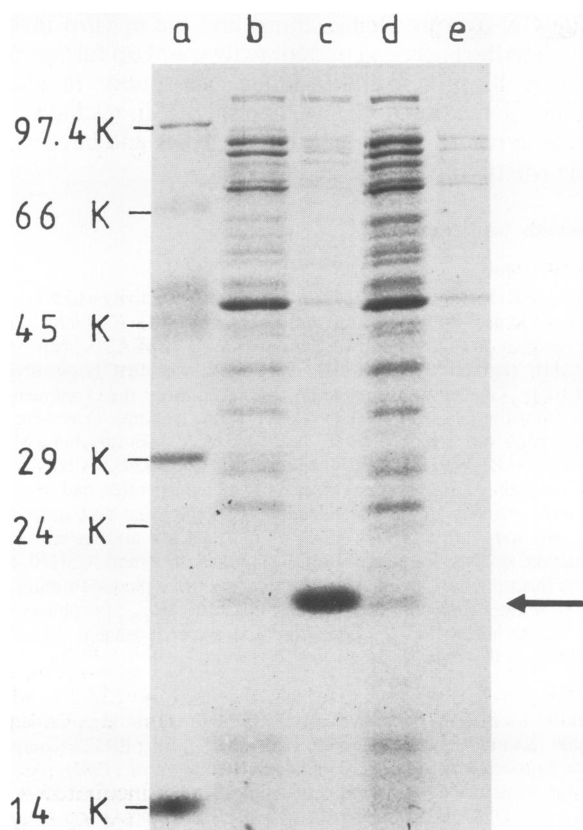

Fig. 5. SDS-PAGE of extracts used for G inversion. The equivalent of 5 µl extract (extract fraction) and 70 µl supernatant (supernatant fraction), respectively, were applied to a 15% SDS-polyacrylamide gel after dialysis against 25 mM Tris-HCl, pH 6.8. **(a)** Mol. wt. standards as in Figure 3; **(b)** pLMugin-0163 supernatant; **(c)** pLMugin-0163 extract; **(d)** pLc2833 supernatant; **(e)** pLc2833 extract. The Gin protein is marked by an arrow.

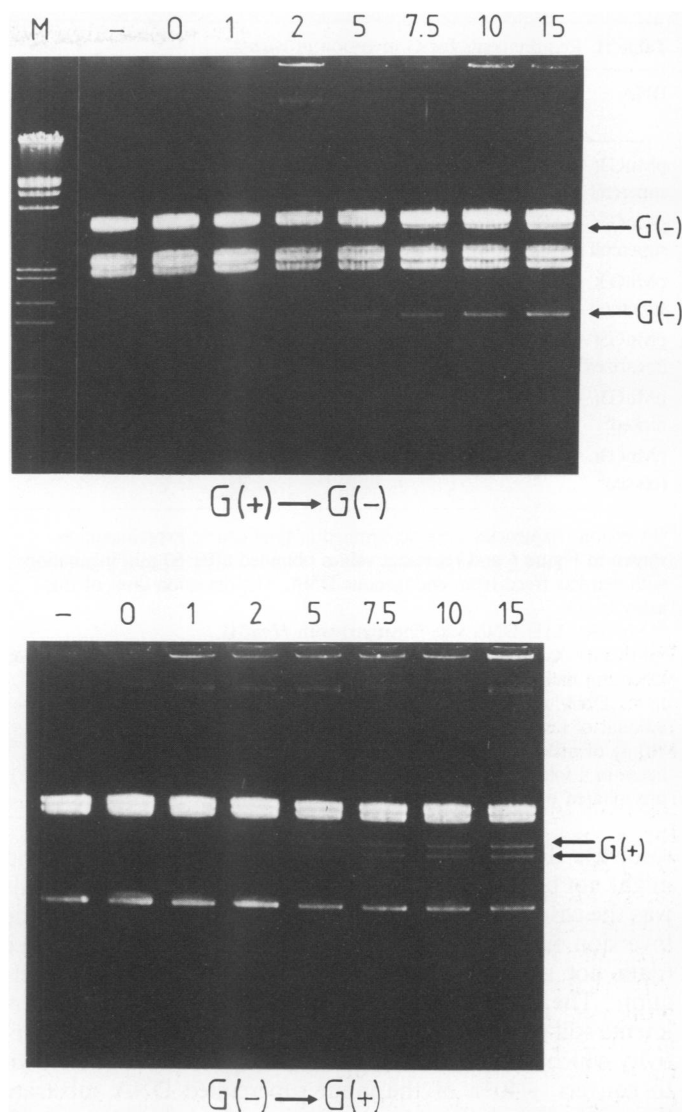

Fig. 6. Time course of G inversion *in vitro*. DNAs analyzed are: pMuG3(+)ΔH **(upper part)** incubated with a pLMugin-0163 extract for 0–15 min as indicated above each slot, – indicates that no extract has been added. In the **lower part** the substrate DNA was pMuG3(–) ΔH DNA treated under exactly the same conditions as above. M is λ DNA cleaved with a mixture of *Eco*RI and *Hind*III as size marker. Fragments generated by inversion are indicated with G(–) and G(+), respectively.

Three plasmids which were analysed in detail differ in the amount of Gin protein synthesized. This correlates with growth characteristics of cells containing these plasmids: the growth rate of cells decreases with increasing amounts of Gin being made. Since initiation of transcription in pL plasmids starts within the region common to all plasmids, the different levels of Gin protein produced in the three clones must be attributed to sequence differences upstream of the SD-sequence or the SD-sequence itself. The SD-sequence in front of the genuine Mu *gin* gene is 5 bp long. The same sequence is found in pLMugin-0163. pLMugin-D111 has a 4-bp match with the original Mu SD-sequence and in pLMugin-D112 the SD-sequence is shortened as in the starting plasmid pLMugin-X16 (Figure 1B). Secondary structure of the RNA transcript has been implicated in determining the efficiency of translation. It has been postulated that an initiation codon freely accessible in a hairpin-loop raises the translation efficiency for a given RNA. As a factor of somewhat less importance, the SD-sequence should also be accessible (Iserentant and Fiers, 1980; Gheysen *et al.*, 1982). From computer calculated possible secondary structures of the initiation region of our clones (not shown), we have learned that the *gin* initiation codon GUG exists in a loop or a non-base-paired state in overproducing plasmids pLMugin-0163 and pLMugin-D111 while it is likely to exist in a base-paired structure in the original Mu sequence, in pLMugin-X16, and in the overproducer pLMugin-D112. The SD-sequences of all our plasmids seem to be in a non-base-paired or partially base-paired configuration. These calculations fit into the concept that maximal expression of a gene is correlated with an accessible initiation codon and SD-sequence in the RNA transcript. Although we have succeeded in isolating *gin* overproducing cells, we cannot convincingly ascertain what the major cause for overproduction is (removal of IR-R or structural alterations in regions 5′ to the SD-sequence) because of the small deletions our constructs have acquired. Therefore we cannot yet comment on the possible autoregulatory scheme for *gin* expression. We are currently designing experiments to test this.

Extracts from Gin-overproducing strains promote efficient inversion of the G segment from the G(–) to the G(+) orientation and *vice versa*. If inversion goes to completion, we expect 50% of both G orientations. In all experiments we have been slightly short of reaching this value. An explanation could be that under our assay conditions, Gin, like $\gamma\delta$

Table II. Requirements for G inversion *in vitro*

DNA	Buffer	Inversion frequency[a]
pMuG3(−)ΔH supercoil	TMD	40%
pMuG3(−)ΔH supercoil	TMD 10 mM EDTA	<5%
pMuG3(−)ΔH supercoil	TMD 1 mM ATP	40%
pMuG3(−)ΔH linearized[b]	TMD	<5%
pMuG3(−)ΔH nicked[c]	TMD	<5%
pMuG3(−)ΔH relaxed[d]	TMD	<5%

[a]Inversion frequencies were determined in time course experiments as shown in Figure 6 and represent values obtained after 60 min incubation with extracts freed from endogenous DNA. The detection limit of this assay is 5%.
[b]pMuG3(−)ΔH DNA was linearized with *Hind*III.
[c]pMuG3(−)ΔH DNA was nicked by incubating 20 μg DNA with 0.005 μg DNase in nick-translation buffer (Maniatis *et al.*, 1982) for 15 min at 15°C. DNA was extracted with phenol and precipitated with ethanol. The amount of linear molecules was estimated to be <5%.
[d]10 μg of nicked pMuG3(−)ΔH DNA were religated with 2.5 units T4 ligase in a total volume of 100 μl for 16 h, extracted with phenol and precipitated with ethanol.

resolvase (Reed, 1981), might precipitate from solution and might not be able to catalyze multiple inversion events. If this was the case, repeated addition of extract should increase the inversion frequency, for which we have found no evidence (data not shown). Therefore we favour another interpretation. The partially purified extracts used in these experiments still contain small amonts of an unspecific nicking activity which in experiments not described here has been shown to convert ~40% of the input supercoiled DNA substrate into nicked circles in 15 min. Since nicked DNA is not efficiently inverted by Gin we consider this to be the major reason for our failure to reach maximum inversion. We are not certain that the Gin protein alone is sufficient to catalyze the inversion reaction and at present we cannot exclude the possibility that additional host factors are required for its activity. The substrate and reaction requirements for Gin closely resemble those determined for resolvases of Tn3 and γδ (Reed, 1981; Krasnow and Cozzarelli, 1983), especially in their need for a supercoiled substrate and lack for an external energy source. We therefore expect that the energy for inversion comes from breakage of the phosphodiester bonds and is stored in a covalent complex between Gin and DNA as has been demonstrated for resolvase (Reed and Grindley, 1981). In contrast with resolvases which recombine only directly repeated sites efficiently (Reed, 1981), Gin shows a strong preference for inversions between inverted repeated sequences (Plasterk *et al.*, 1983b). This implies that the relative orientation of sites is sensed and that directionality of the system is an inbuilt feature. Since Gin and resolvases of Tn3 and γδ share ~35% homology on the amino acid level (R.Kahmann and D.Kamp, unpublished) and are therefore likely to be derived from a common ancestor, it will be particularly interesting to compare these proteins on a structural and physio-chemical level with respect to what determines directionality.

The Gin overproducing strains and the *in vitro* inversion assay described here will undoubtedly speed up further purification of this protein and facilitate such studies. In addition, the characterization of the mechanism of Gin-mediated inversion, with regard to binding sites, cleavage and intermediates in the reaction, has become tangible.

Materials and methods

Bacterial strains

The *E. coli* K12 strain CSH50 (Miller, 1972) lacks an activity which can complement a Mu *gin*⁻ mutant (Kamp and Kahmann, 1981). *E. coli* K12ΔHIΔ*trp* is the designation for M72 Sm[R] *lac*Zam Δ*bio-uvr*B Δ*trp*EA2 (λ *N*am 7-*N*am53 cI857ΔHI) (Remaut *et al.*, 1981). This strain was first lysogenized with Mucts62$_{445\text{-}5}$ G(+) *gin*⁻ (Chow *et al.*, 1977). To invert the G segment in this strain from the G(+) to the G(−) orientation, lysogenic cells were mated (Miller, 1972) with strain MXR (F⁻ Δ*lac pro rec*A *gal*E) carrying a Mu *gin*⁺ prophage on RP4 (PULB11:RP4; Mucts62 *gin*⁺ *mom*3452) kindly provided by A.Toussaint. K12ΔHI Δ*trp* (Mucts62$_{445\text{-}5}$) recipients that had received the RP4 were selected on YT plates containing kanamycin and streptomycin. Such cells were cured (Miller, 1972) of the RP4:Mu and tested for phage production on MuG(+) and MuG(−) indicator strains CSH50 and *E. cloacae* (Kamp, 1981), respectively. Strains producing plaque forming Mu on *E. cloacae* only were considered to carrying Mucts62$_{445\text{-}5}$ prophages with G now frozen in the G(−) orientation and were designated K12ΔHIΔ*trp* [Mucts62$_{445\text{-}5}$ G(−)*gin*⁻].

Plasmids

pMu1107 is a pBR322 derivative carrying the entire G and β region of the Mu genome (Kahman, 1983). pLc2833, derivatized from pBR322, contains the leftward promoter (pL) of bacteriophage λ (Remaut *et al.*, 1981). pMuG3(−) ΔH was constructed by insertion of a *Hind*III-*Sal*I fragment from Mucts 62$_{445\text{-}5}$ G(−)*gin*⁻ DNA (Chow *et al.*, 1977) (containing the G segment, ~2000 bp of α, 400 bp of β and 200 bp of IS2 DNA) into the *Sal*I-*Hind*III sites of pBR322. In a second step a 1600-bp *Hpa*I fragment within G was deleted. pMuG3ΔH is *gin*⁻. Other plasmids are described in Results. Plasmid DNA was isolated by the Triton X-100 lysis procedure and purified by equilibrium centrifugation in cesium chloride-ethidium bromide gradients (Davis *et al.*, 1980). For analytical purposes plasmid DNA was isolated by the alkaline lysis method (Birnboim and Doly, 1979).

Media and buffers

dYT medium and YT plates have been described (Maniatis *et al.*, 1982). Ampicillin was added to final concentrations of 60 μg/ml when necessary. TMD-buffer: 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 0.1 mM dithiothreitol. SE: 0.1 mM Na_2 EDTA, 0.15 M NaCl, 0.5% SDS, pH 7.5.

Nucleic acid procedures

Agarose gel electrophoresis, cleavage of DNA with restriction endonucleases, filling in recessed 3′ ends, ligation and transformation of *E. coli* were done according to standard methods (Maniatis *et al.*, 1982). The self-complementary olignucleotides d(TCCTAGGA) and d(TCCCTAGGGA) were chemically synthesized following established phosphotriester procedures and were characterized by 2-dimensional fingerprint and FAB-MS (fast atom bombardment mass spectometry) techniques (Grotjahn *et al.*, 1982; Frank *et al.*, 1983).

For the construction of plasmids containing the synthetic SD-linkers, 100 pmol SD-linkers were phosphorylated in a volume of 20 μl containing 500 pmol ATP, 7 units T4 polynucleotide kinase (Boehringer) in 70 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM dithiothreitol. Phosphorylated SD-linkers were ligated into the filled-in *Bam*HI site of pLMugin-X16. DNA sequences were determined using the chemical degradation method of Maxam and Gilbert (1980). Sequences were read from an *Ava*I site located 195 bp downstream of the GTG initiation codon in *gin* (R.Kahmann and D.Kamp, unpublished) towards a *Hind*II site in the promoter region of pLc2833 (Remaut *et al.*, 1981).

Biological assay for gin complementation

Plasmids to be tested for their *gin* complementing activity were transformed into K12ΔHI Δ*trp* [Mucts62$_{445\text{-}5}$ G(−)*gin*⁻]. Transformants were grown in dYT medium containing ampicillin to a cell density of 5 x 10^8 cells/ml at 28°C, shifted to 42°C and incubated with vigorous aeration until lysis. The resulting phage lysates were treated with chloroform and titers were determined on CSH50 and *E. cloacae*. Plaques on CSH50 indicate that the G segment in progeny phages has been switched from the G(−) to the G(+) orientation *via gin* mediated recombination.

Preparation of cell-free extracts and conditions for G inversion in vitro

100 ml dYT medium with ampicillin were inoculated with K12ΔHIΔ*trp*/

pLc2833 and K12ΔHI Δ*trp*/pLMugin-0163, respectively, grown to 80 Klett units (8 x 10^8 cells/ml) and then shifted to 42°C for 30 min. Cells were harvested, washed with 10 ml ice-cold TMD buffer, resuspended in 2 ml of the same buffer containing 10% sucrose, 0.1% Triton X-100 and broken in a French pressure cell press at 1.300 p.s.i. NaCl was added to a final concentration of 1 M, the extract was kept on ice for 15 min and centrifuged for 40 min at 40 000 *g*. The supernatant was dialyzed for 2 h against 500 ml of 50 mM NaCl, 0.1% Triton X-100 in TMD. The precipitate was collected by centrifugation for 20 min at 17 000 *g* and resuspended in 0.2 ml TMD, 0.1% Triton X-100, 1 M NaCl (extract fraction). The supernatant from this centrifugation is designated supernatant fraction. To remove DNA from the extract fraction the suspension was diluted 1:4 with the same buffer and after the addition of two volumes 4 M NaCl in TMD layerered on a CsCl shelf (1.5 ml, 0.9 g CsCl/ml in TMD, 1 M NaCl) and centrifuged in an SW50 Rotor for 3 h at 40 000 r.p.m., 4°C. The fraction above CsCl was carefully removed with a Pasteur pipette and dialyzed against 200 mM NaCl, 0.1% Triton in TMD for 2 h at 4°C. Standard reactions included in a total reaction volume of 25 μl: 1 μg pMuG3ΔH DNA, 1 μl pLMugin-0163 extract (or 5 μl extract after removal of DNA) or 1 μl pLc2833 extract, 1 x TMD buffer. Reactions were carried out at 37°C and were stopped by the addition of 75 μl SE. The DNA was extracted with phenol (three times), precipitated with ethanol, digested with *Hind*II and analyzed on a 1.2% agarose gel. Gels were stained with ethidium bromide and photographed under short wave u.v. light.

SDS-PAGE of total cell extracts

Cells were grown in dYT medium supplemented with ampicillin at 28°C to a cell density of ~7 x 10^8 cells/ml. Cultures were shifted to 42°C for 2 h. At the time of heat induction (0 min) and 120 min later, 1 ml of cells was withdrawn, washed and resuspended in 100 μl of 25 mM Tris-HCl, pH 6.8. The suspension was sonicated for 10 s (Branson Sonifier B-30), 100 μl of Laemmli sample buffer (Laemmli, 1970) was added, followed by 3 min incubation at 100°C and a 15 min spin in an Eppendorf centrifuge. 30 μl of the 0 min and 10 μl of the 120 min samples, respectively, were loaded side by side on a 15% polyacrylamide gel using the Laemmli system. Gels were stained with Coomassie Brilliant Blue G-250 and destained as described by Weber and Osborn (1969).

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References

Allet,B. and Bukhari,A.I. (1975) *J. Mol. Biol.*, **92**, 529-540.
Birnboim,H.C. and Doly,J. (1979) *Nucleic Acids Res.*, **7**, 1513-1523.
Chow,L.T., Kahmann,R. and Kamp,D. (1977) *J. Mol. Biol.*, **113**, 591-609.
Daniell,E., Abelson,J., Kim,J.S. and Davidson,N. (1973) *Virology*, **51**, 237-239.
Davis,R.W., Botstein,D. and Roth,J.R. (1980) *Advanced Bacterial Genetics*, published by Cold Spring Harbor Laboratory Press, NY.
Frank,R., Heikens,W., Heisterberg-Moutsis,G. and Bloecker,H. (1983) *Nucleic Acids Res.*, **11**, 4365-4377.
Gheysen,D., Iserentant,D., Derom,C. and Fiers,W. (1982) *Gene*, **17**, 55-63.
Grindley,N.D.F., Lauth,M.R., Wells,R.G., Wityk,R.J., Salvo,J.J. and Reed,R.R. (1982) *Cell*, **30**, 19-27.
Grotjahn,L., Frank,R. and Bloecker,H. (1982) *Nucleic Acids Res.*, **10**, 4671-4678.
Hsu,T. and Davidson,N. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 2823-2827.
Iserentant,D. and Fiers,W. (1980) *Gene*, **9**, 1-12.
Kahmann,R. (1982) *Cold Spring Harbor Symp. Quant. Biol.*, **47**, 639-646.
Kamp,D. (1981) in Schlessinger,D. (ed.), *Microbiology - 1981*, American Society for Microbiology, Washington, pp. 73-76.
Kamp,D. and Kahmann,R. (1981) *Mol. Gen. Genet.*, **184**, 564-566.
Kamp,D., Kahmann,R., Zipser,D., Broker,T.R. and Chow,L.T. (1978) *Nature*, **271**, 577-580.
Kamp,D., Chow,L.T., Broker,T.R., Kwoh,D., Zipser,D. and Kahmann,R. (1979) *Cold Spring Harbor Symp. Quant. Biol.*, **43**, 1159-1167.
Krasnow,M.A. and Cozzarelli,N.R. (1983) *Cell*, **32**, 1313-1324.
Kwoh,D.Y. and Zipser,D. (1981) *Virology*, **114**, 291-296.
Laemmli,U.K. (1970) *Nature*, **227**, 680-685.
Maniatis,T., Fritsch,E.F. and Sambrock,J. (1982) *Molecular Cloning. A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499-559.
Miller,J.H. (1972) *Experiments in Molecular Genetics*, published by Cold Spring Harbor Laboratory Press, NY.
Nash,H.A. (1981) *Annu. Rev. Genet.*, **15**, 143-167.
Plasterk,R.H.A., Brinkman,A. and van de Putte,P. (1983a) *Proc. Natl. Acad. Sci. USA*, **80**, 5355-5358.
Plasterk,R.H.A., Ilmer,T.A.M. and van de Putte,P. (1983b) *Virology*, **127**, 24-36.
Reed,R.R. (1981) *Cell*, **25**, 713-719.
Reed,R.R. and Grindley,N.D.F. (1981) *Cell*, **25**, 721-728.
Remaut,E., Staussens,P. and Fiers,W. (1981) *Gene*, **15**, 81-93.
Shine,J. and Dalgarno,L. (1974) *Proc. Natl. Acad. Sci. USA*, **71**, 1342-1346.
Van de Putte,P., Cramer,S. and Giphart-Gassler,M. (1980) *Nature*, **286**, 218-222.
Weber,K. and Osborn,M. (1969) *J. Biol. Chem.*, **244**, 4406-4412.
Zieg,J. and Simon,J. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 4196-4201.

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