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Production and modification of Mu (G−) phage particles in *E. coli K12* and *Erwinia*

BY ARIANE TOUSSAINT AND ERIC SCHOONEJANS

Laboratoire de Génétique, Université libre de Bruxelles, rue des Chevaux, 67, B 1640 Rhode St Genèse, Belgium

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SUMMARY

We studied the amount of Mu(G+) and Mu(G−) phages in different Mu lysates prepared either upon induction or upon infection of *E. coli* and *Erwinia* strains. We also looked at the level of expression of the modification function (*mom*) by Mu(G−) phages, both after induction and after infection of *E. coli* and *Erwinia*. The expression of *mom* seems to be regulated in the same manner in *E. coli* and in the strain of *Erwinia carotovora* tested. The proportion of both types of Mu(G+) and Mu(G−) phages in induced lysates is very variable and we found growth conditions favouring the production of Mu(G−) particles. This should extend the use of Mu as a genetic tool and as a generalized transducing phage to many enterobacteria.

INTRODUCTION

The temperate mutator phage Mu which was first isolated in *E. coli K*12 (Taylor, 1963) has since been found to grow in and to infect many different species of enterobacteria (van de Putte, Cramer & Ghiphart-Gassler, 1980; Kamp, 1981; Faelen *et al.* 1981*a*). This is also the case for the closely related but hetero-immune phage D108 (Hull, Gill & Curtis, 1978 and Gill, Hull & Curtis, 1981). The two phages carry on their genome a segment called the G region, which inverts in the prophage. The G segment of Mu is now fairly well characterized. It has been entirely sequenced (Kahmann & Kamp, to be published). Two genes, *S* and *S′*, which are both involved in specifying the phage host range (Howe, Schumn & Taylor, 1979; Toussaint *et al.* 1978; van de Putte *et al.* 1980; Kamp, 1981) seem to share a common part, *Sc*, encoded by the extreme right of the α region of the Mu genome and a variable part, *Sv* and *S′v* respectively, which map at the extreme left and right ends of the G segment (see Fig. 1). Inversion of G is a slow process (Symonds & Coelho, 1978). Consequently, in a culture of a bacterium lysogenic for Mu, one expects an equilibrium to be reached, where about half of the bacteria carry a Mu prophage with the G region in one orientation and the other half have a prophage with the G region in the opposite orientation. After induction, since the Mu genome is only transcribed from one strand (Bade, 1972; Wijffelman *et al.* 1974), one type

of prophage will give progeny which will express the *S* gene and have the *S* host range, while the other prophages will give progeny which will express the *S'*gene and consequently will have the *S'* host range. This process provides a Mu population with two distinct but related host ranges (Sandulache *et al.* to be published). Some bacteria, either wild type or mutant, including *E. coli K*12, *Salmonella typhimurium* LT2 (Faelen *et al.* 1981*a*) and *E. coli* B (van de Putte, personal communication) are sensitive to Mu with the *S* host range (Mu(G+) phages) while other strains as *Citrobacter freundii*, *Shigella sonneii*, *E. coli C* (van de Putte

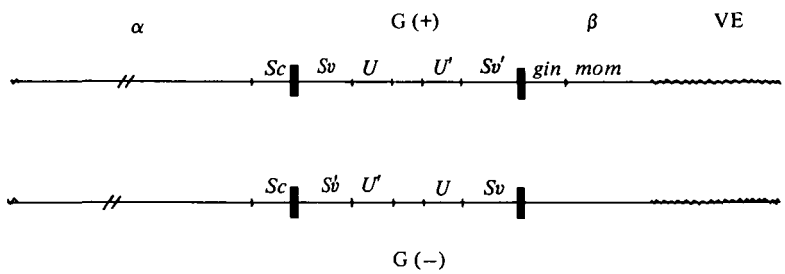

Fig. 1. The G and β regions of the Mu genome. The G and β regions are represented approximately to scale. The zig-zag lines represent the variable ends of the phage genome. For details about genes *S*, *S'*, *U*, *U'*, *gin* and *mom* see text.

et al. 1980), *Enterobacter cloacae* (Kamp, 1981), *Klebsiella pneumoniae* (Lefèbvre, unpublished results) and different varieties of *Erwinia* (*carotovora*, *uredovora*, *amylovora* and *herbicola*) (Faelen *et al.* 1981*b*; Lefèbvre, unpublished results) are sensitive to Mu phages with the *S'* host range (Mu(G−) phages).

The *mom* gene of Mu, which is responsible for the modification of Mu DNA, maps in the middle of the β region of the Mu genome (Toussaint, Desmet & Faelen, 1980) (Fig. 1). It is the only Mu gene which has so far been found to be expressed at a higher level after induction than after infection. Transcription of the *mom* gene requires expression of the host DNA-adenine methylase function *dam* (Hattman, submitted for publication). Mu *mom*$^+$ DNA extracted from lysates grown upon induction of a *dam*$^+$ lysogen contains ~ 15% modified adenine residues. The precise nature of the modification remains to be defined (Hattman, 1979), but it allows Mu DNA to escape restriction by a whole series of type I and type II restriction enzymes (Allet & Bukhari, 1975; Toussaint, 1976; Kahman & Kamp, unpublished results). Among the *Erwinia* strains sensitive to Mu, we found one which is naturally restrictionless. This allowed us to easily measure the amount of Mu(G−) phages in Mu lysates grown lytically on *E. coli* or *Erwinia* and to measure the number of bacteria carrying Mu(G+) and Mu(G−) prophages in different cultures. We have also been able to measure the level of *mom* expression by induced and lytically grown Mu(G−) phages, both in *Erwinia* and in *E. coli*.

Table 1. *Bacteria, bacteriophages and plasmids*

Strain	Characters	Origin or reference
Bacteria		
B374	*Erwinia carotovora* prototroph	Hamon and Peron, 1961 (via Ledoux)
20D3	*Erwinia uredovora* prototroph	Kado (via Ledoux)
RH6001	*Erwinia herbicola* prototroph	Hopital Brugmann, Brussels
Citrobacter freundii	prototroph	van de Putte *et al.* 1980
*E. coli K*12 *strains*		
JM260	Δ(*ara-leu*), *his*, *argE*, *lac*, *gal*, *mtl*, *xyl*, *recB*, *sbcB*, *supE*, *hsr*, *Str*^{r}, *araD*, *leu*::Mu*cts*61	Sanchez, unpublished
MH125	*araD*, *leu*::Mu*cts*61	Howe, 1973
C600	*leu*, lac_Y, *supE*	Campbell, 1961
JA112	H(λ::Mu*cts*61, c^+), *lacZam*, *argH*, Mu inserted in late genes, left of gene *F*	Toussaint, unpublished
JA114	H(λ::Mu*cts*61, c^+) (Mu*cts*61), *lacZam*, *argH*; Mu inserted in λ late genes, left of gene *F*	Toussaint, unpublished
N100	*gal2*, *recA*, Str^r	Gottesman & Yarmolinsky, 1968
DM1	W3110 (Mu*cts*62)	Taylor
2379	*thr*, *leu*, *met*, *lac*, *supE*, *hsA*	Arber & Wauters-Willems, 1970
Bacteriophages		
Muc$^+$		Taylor, 1963 (via Starlinger)
Mu Vir3051 *mom*3452		van Vliet *et al.* 1978
Mu*cts*61	Thermoinducible	Howe, 1973
Mu*cts*62	Thermoinducible	Howe, 1973
D108*cts*10	Thermoinducible	Hull *et al.* 1978
MP11	Mu*cts*62 with P1 host range	Toussaint *et al.* 1978
MPh1	Mu*cts*62 with P1 CM host range	Toussaint *et al.* 1978
Mu*cts*62Δ*S*	Plaque forming phage derived from Mu*cts*62 *S*::Tn*9*. Has lost Cml and part of the *S* gene	Résibois *et al.* 1981
Plasmids		
pULB15	RP4::Mu*cts*62 *mom*3452	Toussaint & Bade, unpublished

MATERIAL AND METHODS

Bacteria and bacteriophages are listed in table 1.

Media. Bacteria and phages were grown in LB (Miller, 1972). Phage titrations were made on L agar (LB supplemented with 1·1 % Difco agar).

Preparation of Mu lysates.

E. coli and *Erwinia* lysogens were grown with aeration at 32° in LB up to a concentration of ~ 2×10^8 bacteria/ml. *E. coli* strains were induced by shifting the

cultures to 42° for 1 h by which time they had lysed. *Erwinia* lysogens were shifted to 42° for 30–40 min then returned to 36° for 60 min by which time they had usually lysed. This treatment was applied because the *Erwinia* strains used, even when not lysogenic, do not grow at 42°. Lysed cultures were sterilized by the addition of a few drops of $CHCl_3$ and centrifuged to remove cell debris.

Muc$^+$ and Muc*ts*62 lysogenize B374 very poorly. Stable lysogens can be obtained only by isolating B374 auxotrophs which most probably result from Mu insertion, or by repeated selections of Ap^r derivatives of B374 infected with a Muc*ts*62 carrying a non-transposable Ap^r marker.

Plate lysates on *E. coli* were prepared on L agar as described by Adams (1959). Plate lysates on *Erwinia* were grown at 35–36°. 0·1 ml of a saturated culture were mixed with 10^6 phages and poured with 3 ml of 0·5% soft agar on 1 L agar plate. Phages were harvested after 6 h incubation.

Single burst analysis. JA112 was grown at 32° in LB up to a concentration of $\sim 2 \times 10^8$ bacteria/ml. Cultures were diluted so as to have ~ 25 bacteria in 60 ml LB and then aliquots of 1 ml were incubated at 42° for 1 h without aeration. Any bacteria present were killed by addition of a few drops of $CHCl_3$ which was then allowed to evaporate. Half of each aliquot was mixed with JM260 and plated on LB agar. The other half was plated with B374.

RESULTS

B374 is naturally restrictionless

Plate lysates of Mu *Vir*3051 *mom*3452 were grown on B374 (*Erwinia carotovora*), RH6001 (*Erwinia herbicola*), 20D3 (*Erwinia uredovora*), JM260 (*E. coli K*12 r^-m^+) and *Citrobacter freundii* and were titred on lawns of the same strains at 35°. The results are summarized in table 2. The lysate grown on B374 is restricted by both RH6001 and 20D3. On the other hand lysates grown on RH6001, 20D3 and *Citrobacter* are not restricted by B374 showing that it is r^-. Since both B374 and JM260 are r^- they can be used to measure directly the number of Mu(G−) and Mu(G+) phages in any lysate. Table 2 shows that, as expected, the lysate grown on JM260, which is sensitive to Mu(G+) phages, contains mostly Mu(G+) phages while lysates grown on *Erwinia* strains or on *Citrobacter*, which are sensitive to Mu(G−) phages, contain mostly Mu(G−) particles. The proportion of Mu(G−) phages in the lysate grown on JM260 ($\sim 2 \times 10^{-4}$) is about the same as the proportion of Mu(G+) phages in the lysates grown on *Erwinia* and on *Citrobacter* ($\sim 5 \times 10^{-4}$).

Analysis of proportions of G(+) and G(−) phage produced

We measured the number of each type of Mu particles in lysates grown on different strains, either by induction or by infection. The results are given in Table 3. The proportion of Mu(G+) and Mu(G−) phages in the lytically grown lysates is fairly constant. The minority population represents between 10^{-3} Mu(G+) in lysates grown on *Erwinia* and 10^{-4}–10^{-5} Mu(G−) in lysates grown on *E. coli*.

Table 2. *Titration of different lysates of Mu* Vir3051 *mom*3452

Phage lysate*	Titration on B374	RH6001	20D3	*Cit. fr.*	JM260
Mu(G−).B374	2×10^9	4×10^6	10^6	$\geqslant10^7$	10^6
Mu(G−).RH6001	10^{10}	10^{10}	6×10^7	nd	6×10^6
Mu(G−).*Cit. fr.*	10^9	nd	nd	$\geqslant10^8$	6×10^3
Mu(G−).20D3	10^9	nd	2×10^9	nd	10^6
Mu(G+).JM260	2×10^6	2×10^5	3×10^4	nd	10^{10}

* Mu*vir*3051 *mom*3452 plate lysates were grown on various hosts and assayed on different indicator strains. Mu(G−) and Mu(G+) indicate the predominant orientation of the G region found in the progeny phage.

nd: not done. *Cit. fr.*: *Citrobacter freundii*.

Table 3. *Proportion of G(+) and G(−) phages in Mu lysates grown either by infection or by induction*

Host	Phage	Mode of growth	Titre of Mu(G+)	Mu(G−)	Mu(G+)/Mu(G−)
MH125*recA*	Muc*ts*61	Induction	3×10^8	2×10^9	0·15
C600	Muc*ts*62	Induction	5×10^9	10^9	5
C600	Muc*ts*62	Infection	3×10^9	3×10^5	10^4
C600	D108*cts*10	Induction	10^9	10^8	10
C600	D108*cts*10	Infection	10^{11}	8×10^5	$1{\cdot}2\ 10^5$
C600	MP11	Induction	8×10^8	5×10^9	0·16
C600	MP11	Infection	5×10^9	5×10^5	10^4
C600	MPh1	Induction	2×10^9	4×10^9	0·5
C600	MPh1	Infection	10^{11}	10^6	10^5
B374	Muc*ts*62	Induction	3×10^9	6×10^8	55
B374	Muc*ts*62	Infection	5×10^5	5×10^8	10^{-3}
B374	MP11	Induction	3×10^7	10^8	0·3
B374	Muc$^+$	Infection	5×10^5	10^8	$5\ 10^{-3}$
B374	Muc*ts*61	Infection	7×10^5	5×10^8	$1{\cdot}4\ 10^{-3}$
594	Muc*ts*61	Induction	9×10^8	10^9	0·9
JA112	Muc*ts*61	Induction	5×10^8	10^{10}	$5\ 10^{-2}$
JA114	Muc*ts*61	Induction	2×10^9	4×10^9	0·5
N100	Muc*ts*61	Induction	7×10^9	4×10^6	$1{\cdot}75\ 10^3$
N100	Muc*ts*62 *mom*3452	Induction	2×10^9	10^8	20
B374/pULB15	Muc*ts*62 *mom*3452	Induction	7×10^8	4×10^6	1·75

Lysates were prepared as described in material and methods, and were titrated at 35° on JM260 for Mu(G+) and B374 for Mu(G−).

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Muc*ts*62 and D108*cts*10 lysates grown by induction contain either 50% of each phage type or a majority (up to 90%) Mu(G+) particles, while Muc*ts*61, MP11 and MPh1 induced lysates contain either ~ 50% of each phage type or a majority of Mu(G−) particles.

*Single burst analysis of phages produced by JA*112

Most of the lysogens which we have used carry an unknown number of Mu prophages. However, JA112 is a monolysogen and produces about 95% Mu(G−) phages. Symonds & Coelho (1978) found that individual bacteria of a Muc*ts*62 monolysogen produce only one type of phage. Since JA112 produces mostly Mu(G−), a culture of JA112 should contain a majority of bacteria producing only Mu(G−) phages.

Table 4. *Single burst analysis of JA*112 *phage productions*

	JA112 (*a*)	
Number of fractions producing plaques on:		
B374 only	40	i.e. 69% of the producers
JM260 only	15	i.e. 26% of the producers
B374 and JM260	3	i.e. 5% of the producers
Total number of fractions analysed	335	
Proportion of producers	0·17	
Predicted fraction of tubes containing more than one producer (*b*)	0·013	i.e. 4·3 tubes or 7·4% of the producers
Average burst on (*c*)		
B374	23	
JM260	8·5	

The single burst analysis was performed as described in material and methods.
(*a*) the numbers given are the total numbers obtained from 3 independent experiments.
(*b*) these values ($P > 1$) were calculated from the Poisson distribution.

$$P > 1 = 1 - p(0) - p(1)$$
$$\left.\begin{aligned} p(0) &= e^{-m} \\ p(1) &= me^{-m} \end{aligned}\right\}$$ where m is the proportion of producers.

(*c*) The culture used for the single burst analysis was induced and the lysate obtained was titred on JM260 and B374.

We looked at the bursts of individual bacteria in cultures of JA112 (as described in Material and Methods). The results are summarized in Table 4: 69% of the yielders produced only Mu(G−), 26% produced only Mu(G+) and only 5% produced both types of phages. As shown in Table 5, the calculated number of aliquots which should contain more than one producer is higher than the number found (7·4% vs. 5%). Thus JA112 probably does not produce any mixed bursts and the asymmetry between the two types of phages produced is due to the number of bacteria which produce G(+) or (G(−) phage alone.

Search for conditions increasing the number of Mu(G−) phage in induced lysates.

The titre of Muc*ts*62(G−) particles in most Muc*ts*62 lysates grown by induction, including those prepared from B374 lysogens, is low (Table 2). Since most of the existing mutants of Mu which are of great help in using it as a genetic tool have been isolated from Muc*ts*62, we searched for growth conditions favouring Muc*ts*62(G−) particles so that such lysates could be used to transduce Mu(G−)

Table 5. *Efficiencies of plating on non-restricting and restricting bacteria of Mu lysates grown lytically and by induction*

Phage	Host	Efficiency of plating on 2379 (*a*)	RH6001 (*b*)	20D3 (*b*)
	Lysates grown by infection			
Mu Vir3051 *mom*3452	B374	nd	3×10^{-3}	8×10^{-4}
Muc$^+$	B374	nd	$\leqslant 10^{-4}$	10^{-3}
Muc*ts*61	B374	nd	4×10^{-4}	2×10^{-4}
Muc*ts*62	B374	nd	4×10^{-4}	2×10^{-4}
	Lysates grown by induction			
Muc*ts*61	JA112	0·4	0·25	3×10^{-3}
Muc*ts*62	DM1	0·5	1	2×10^{-3}
Muc*ts*62 *mom*3452	N100	2×10^{-3}	10^{-2}	3×10^{-4}
Muc*ts*62	B374	nd	0·1	2×10^{-2}
Muc*ts*62 *mom*3452	B374	3×10^{-3}	10^{-3}	3×10^{-5}
MPh1	B374	0·16	0·5	nd
MP11	B374	1	0·2	nd

Lysates were prepared as described in material and methods. They were titrated at 36° on the different indicator bacteria.

(*a*) The efficiency of plating (E.O.P.) is given by the ratio between the titre obtained on 2379 (restricting strain) and on JM260 (the non-restricting strain). (*b*) the E.O.P. is given by the ratio between the titre on RH6001 or 20D3 (the restricting strains) and B374 (the non-restricting strain).

sensitive bacteria. We induced B374 derivatives lysogenic for different Mu prophages, in the presence of different concentrations of either sodium citrate of EDTA and found that EDTA, added to a final concentration of $10^{-4}M$ at the time of induction (see Material and Methods for detailed procedure), allows recovery of up to 10^9 Mu(G−) particles/ml, although it slightly decreases the number of Mu(G+) phages.

Expression of mom *by Mu(G−) phages*

In *E. coli K*12 the *mom* function is repressed in Mu prophages but is expressed at a higher level after induction than after infection. This has only been shown for Mu(G+) phages, since titration on restricting and non-restricting strains (to measure the level of modification of Mu DNA) have always been performed on strains resistant to Mu(G−). The fact that Mu DNA extracted from induced lysates,

which contain phages of both types, is resistant to digestion by several restriction enzymes (Allet & Bukhari, 1975; Kahman, Kamp & Zipser, 1977) already indicated that both types of DNA were modified to the same extent. We tested the level of modification of lytically grown and induced Mu(G−) particles, by titrating them on B374 (non-restricting) and RH6001 and 20D3 (restricting strains).

For comparison the same lysates were also titrated on JM260 (non-restricting) and 2379 (restricting strain). As mentioned earlier, in *E. coli K*12 expression of *mom* requires expression of the host *dam* methylase. Since it is not known whether B374 expresses an equivalent enzyme, we wondered how *mom* would be expressed in that strain. Results are shown in Table 5. Phages grown upon induction plate with a higher efficiency on RH6001 than those grown by infection. This is the case whether the phage were grown in *E. coli* or in B374. Moreover the efficiencies of plating (E.O.P.) on 2379 (Mu(G+) phages) and on RH6001 (MuG(−) phages) are almost the same. Thus both types of phages seem to be modified to the same extent under the same growth conditions in both types of hosts. On 20D3 all lysates have about the same E.O.P. This situation is similar to that found for Mu(G+) phages on *E. coli* strains carrying the *B* restriction–modification system and suggests that *mom* is expressed after induction in B374, although the modification protects against RH6001 restriction but not against 20D3 restriction enzymes.

DISCUSSION

Thanks to the fact that we found a strain of *Erwinia carotovora* which is naturally sensitive to Mu(G−) phage particles and restrictionless, we could easily measure the amounts of the two types of phages in a variety of Mu lysates. Using one strain of *Erwinia herbicola* and one of *Erwinia uredovora* (which are also naturally sensitive to Mu(G−) phages but do carry host specific restriction systems) we could also measure the level of modification of Mu(G−) phages grown under different conditions. Mu lysates grown by infection of JM260 (*E. coli K*12) contain very few Mu(G−) particles ($<2\times10^{-4}$) while lysates grown by infection of B374 (*E. carotovora*) contain very few Mu(G+) particles ($<5\times10^{-4}$). This was expected since only Mu(G+) can infect and multiply in JM260 while only Mu(G−) can infect and multiply in B374. The proportion of the two types of phages is variable in lysates grown by induction of strains of either *E. coli K*12 or *Erwinia*.

Among the strains we have tested, the Mu*cts*62 lysogens tended to produce more Mu(G+) phages while the Mu*cts*61 and Mu-P1 hybrid lysogens had a tendency to produce more Mu(G−) phages. In the one case where a Mu*cts*61 single lysogen was analysed, the ratio was the same in 3 independent lysates and single burst analysis showed that in the culture of the lysogen a majority (69%) of bacteria carried a Mu(G−) prophage. In that case the majority of Mu(G−) phages in the lysate is correlated with a majority of bacteria carrying a Mu(G−) prophage in the induced culture. The reason for this assymmetry remains to be elucidated.

The final Mu(G+)/Mu(G−) ratio in the Mu*cts*62 lysates is not only a con-

sequence of the ratio between Mu(G+) and Mu(G−) prophages in the induced culture, since addition of EDTA at the time of induction increased the number of Mu(G−) particles recovered. This suggests that Mu*cts*62(G−) particles are inactivated in the absence of EDTA, maybe due to their adsorption on bacterial debris. Indeed Mu*cts*62(G−) phages have been found to be inactivated by the Mu(G+) receptor (Sandulache *et al.* to be published). It seems that Mu*cts*61(G−) and Mu-P1(G−) particles are more stable than the Mu*cts*62(G−) particles. This may be due to an unknown mutation in the *S′* gene produced by the mutagenesis used to isolate the mutant (Howe, 1973). The proportion of Mu(G+) and Mu(G−) phages in an induced lysate seems therefore to depend on both the strain of bacteria and phage used.

There are two important conclusions to be drawn from these data: (i) an experiment in which one titrates Mu phages on *E. coli* only should be interpreted very cautiously, since it might lead one to ignore the majority of the phages present in a lysate; (ii) in cases where Mu(G−) are desired, Mu*cts*61 might be a better choice than the widely used Mu*cts*62. We assayed for the expression of the *mom* gene in lytically grown and induced Mu(G−) phages. Induced Mu(G−) phages are almost unrestricted on RH6001 compared to those grown lytically; this shows that *mom* modification protects Mu DNA from the host-specific restriction of that particular *Erwinia herbicola* strain. *mom* modification does not seem to protect against 20D3 host-specific restriction since all lysates are restricted to the same extent on that strain. A similar situation was found earlier for Mu(G+) phages on *E. coli*, where *mom* modification does not protect against the *E. coli* B host specific restriction whereas it does protect against A and K restrictions (Toussaint, 1976). It seems therefore that *mom* is expressed at a higher level after induction than after infection regardless of G orientation. It has been proposed that methylation by *dam* of a Mu sequence located proximal to the *mom* gene might be a regulating factor in *mom* expression (Hattman, submitted for publication). This would be independent of G inversion and orientation. The *mom* gene is also expressed at a higher level after induction than after infection of B374, and since it seems unlikely that the *mom* gene would be regulated by different mechanisms leading to the same phenotype in B374 and *E. coli K*12, this suggests that B374 carries an equivalent of the *E. coli dam* function. This hypothesis is currently being tested. Since induced Mu(G−) phages are modified, it is most likely that, as in the case for Mu(G+) particles, MuG(−) lysates grown by induction of a strain with one type of host specific modification could efficiently infect strains with a different type of host specific restriction.

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