Genet. Res., Camb. (1971), **18**, *pp.* 29–32
With 1 *plate*
Printed in Great Britain

Transduction mechanisms of bacteriophage ϵ^{15}

III. A new class of mutations affecting the conversion of *Salmonella anatum* by bacteriophage ϵ^{15}

BY R. W. HEDGES

Bacteriology Department,
Royal Postgraduate Medical School,
Du Cane Road, London, W. 12

(*Received* 8 *September* 1970)

SUMMARY

Salmonella anatum lysogenized by a doubly mutant strain of bacteriophage ϵ^{γ} shows a markedly rough colonial phenotype on tetrazolium agar. Mutants that abolish the roughness are reported.

1. INTRODUCTION

Normal cell walls of *Salmonella anatum* contain, in their lipopolysaccharide layers, polymers composed of mannosylrhamnosylgalactose subunits. These polymers constitute the somatic antigen. The repeating units are joined by α-galactosyl linkages whose formation is catalysed by the enzyme α-polymerase (Wright, Dankert & Robbins, 1965). The galactosyl residues are acetylated (Robbins & Uchida, 1962).

If *S. anatum* is lysogenized by bacteriophage ϵ^{15} a remarkable complex of alterations to the somatic antigen is induced (Iseki & Sakai, 1953; Losick & Robbins, 1969).

(1) A new enzyme, β-polymerase, is produced. This catalyses the synthesis of a somatic antigen in which the trisaccharide subunits are joined by β-galactosyl linkages (Losick & Robbins, 1967).

(2) The enzyme responsible for the acetylation of the galactosyl residues is repressed (Robbins *et al.* 1965).

(3) A protein capable of specifically inhibiting the α-polymerase is produced (Losick, 1969).

Bacteriophage mutants with altered conversion properties have been isolated (Uetake & Uchida, 1959). These fall into two classes (Uetake & Uchida, 1959; Bray & Robbins, 1967; Robbins & Uchida, 1965); $\epsilon^{15}a$ mutants have lost the ability to repress the synthesis of the acetylating enzyme and $\epsilon^{15}b$ mutants have lost the ability to produce β-polymerase. Cells lysogenic for a doubly mutant phage, known as ϵ^{γ}, produce a somatic antigen qualitatively very similar to that of the uninfected bacteria. But the presence of the inhibitor of the α-polymerase ensures that the lysogen produces less somatic antigen than does the non-lysogenic

https://doi.org/10.1017/S0016672300012386 Published online by Cambridge University Press

cell (Robbins & Uchida, 1965). These lysogens are, thus, partially 'rough' but on ordinary media do not show marked differences from non-lysogenic cells in colonial morphology.

This paper reports a phenotypic pecularity of strains of *S. anatum* lysogenic for phage ϵ^{γ} and the way in which this can be exploited to obtain a new class of mutation of the conversion pathway.

2. MATERIALS AND METHODS

Bacteria: S. anatum strain *A 1* (Uetake, Luria & Burrows, 1958) and an F*lac* derivative of this strain (Hedges, 1971).

Bacteriophage; ϵ^{γ} (Uetake & Uchida, 1959).

Techniques: described in Hedges (1971).

3. RESULTS

Lactose-fermenting transductants of *S. anatum* were isolated after infection with phage ϵ^{γ} propagated on a strain of *S. anatum* carrying an F*lac* episome (Hedges, 1971). These transductants carried defective ϵ^{γ} prophages (ϵd*lac*). When streaked on lactose tetrazolium indicator agar (Lederberg, 1948) the large majority produced colonies quite unlike those formed by uninfected bacteria (Fig. 1). A smooth colony of non-lysogenic *S. anatum* is shown in Fig. 2.

The peculiar phenotype of the lysogenic strains was not due to some peculiarity of lactose metabolism since wild-type (galactose positive) *S. anatum* carrying an ϵ^{γ} prophage formed rough colonies on galactose tetrazolium indicator agar.

Over 100 independent lactose-positive transductants were isolated carrying defective prophages (and hence unable to produce phage particles) (Hedges, 1971). About half were immune to superinfection whilst half were susceptible. All but one gave rise to the peculiar rough colonies on tetrazolium plates and only one produced normal smooth colonies.

When large numbers of rough transductants are spread on lactose tetrazolium indicator plates it is occasionally possible to observe a colony with a smooth sector. Such a colony is shown in Fig. 1. From these sectors, bacterial clones uniformly producing smooth colonies may be isolated. These are not strains that have been delysogenized, because they retain the lactose genes and when the original transductant was immune the smooth segregants retain the immunity.

4. DISCUSSION

Although a significant fraction of ϵd*lac* prophage genomes lacked the gene(s) conferring immunity, only one of more than 100 such prophages lacked the gene(s) responsible for roughness. This shows that the gene responsible for the roughness (presumably the gene that codes for the inhibitor of the polymerase) occupies a site on the phage genome that is almost invariably included in any ϵd*lac* genome

https://doi.org/10.1017/S0016672300012386 Published online by Cambridge University Press

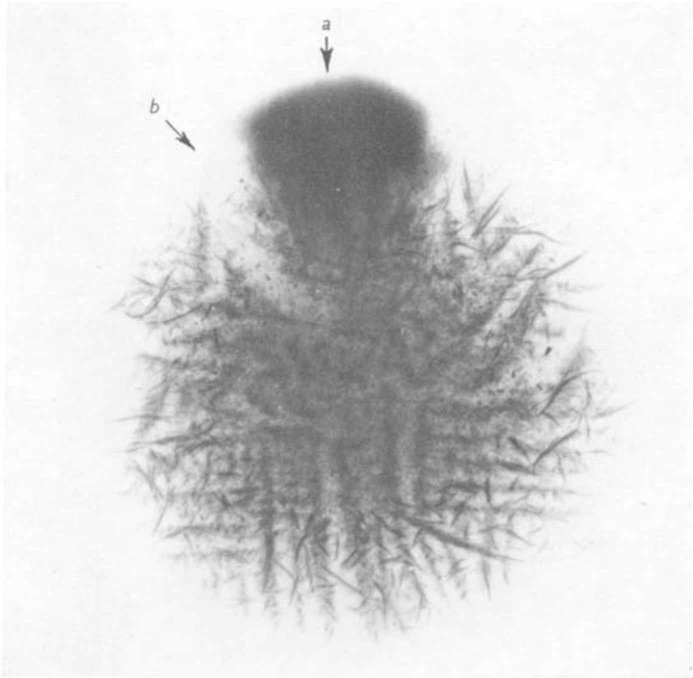

Fig. 1. A 'rough' colony of *S. anatum* lysogenic for ε*dlac* on lactose tetrazolium indicator agar, showing a lactose-negative sector (marked (*a*)) and a 'smooth' sector (marked (*b*)).

Fig. 2. Colony of non-lysogenic *S. anatum* F*lac* on lactose tetrazolium indicator agar. A lactose-negative sector is shown.

R. W. HEDGES

(*Facing p.* 30)

https://doi.org/10.1017/S0016672300012386 Published online by Cambridge University Press

capable of transducing. It also suggests that the site must be rather distant from that occupied by the prophage repressor gene (which has been lost by so many $\epsilon dlac$ genomes). Thus, although both genes are expressed constitutively in the lysogenic bacterium they can hardly belong to the same operon. Perhaps, as in phage ϵ^{34} (Ikawa, Toyama & Uetake, 1968) the gene(s) responsible for conversion are located very close to the attachment site of the phage genome, perhaps in the lysogenic state being transcribed as part of an operon whose promoter is located on the bacterial chromosome.

The defective lysogenic strains produce variants which form smooth colonies on lactose tetrazolium indicator agar. These do not seem to be the result of spontaneous delysogenization. Probably these are mutants of the prophage which have lost the ability to produce the inhibitor of the α-polymerase, but it is also possible that some are mutants in which that enzyme has been so modified as to be less readily inhibited than in wild-type cells. In either case, the technique described permits the isolation of mutants modified in a third aspect of the conversion process and hence may provide material for the more complete understanding of the mechanisms of conversion.

I would like to thank Mr N. Asquith (Leicester University) for the photographic work and Dr N. Datta for help in writing this paper. I am especially grateful to Dr R. Rosenberg, who was the first to notice the 'roughness' of the transductants.

REFERENCES

Bray, D. & Robbins, P. W. (1967). Mechanism of ϵ^{15} conversion studied with bacteriophage mutants. *Journal of Molecular Biology* **30**, 457.

Ikawa, S., Toyama, S. & Uetake, H. (1968). Conditional lethal mutants of bacteriophage ϵ^{34}. I. Genetic map of ϵ^{34}. *Virology* **35**, 519.

Iseki, S. & Sakai, T. (1953). Artificial transformation of O antigens in *Salmonella* E group. II. Antigen transforming factor in bacilli of subgroup E 2. *Proceedings of the Japanese Academy* **29**, 127.

Hedges, R. W. (1971). Transduction mechanisms of bacteriophage ϵ^{15}. I. General properties of the system. *Genetical Research* **18**, 9–19.

Lederberg, J. (1948). Detection of fermentative mutants with tetrazolium. *Journal of Bacteriology* **56**, 695.

Losick, R. (1969). Isolation of a trypsin-sensitive inhibitor of O antigen synthesis involved in lysogenic conversion by ϵ^{15}. *Journal of Molecular Biology* **42**, 237.

Losick, R. & Robbins, P. W. (1967). Mechanism of ϵ^{15} conversion studied with a bacterial mutant. *Journal of Molecular Biology* **30**, 445.

Losick, R. & Robbins, P. W. (1969). The receptor site for a bacterial virus. *Scientific American* **221** (5), 121.

Robbins, P. W., Keller, J. M., Wright, A. & Bernstein, R. L. (1965). Enzymatic and kinetic studies on the mechanism of O antigen conversion by bacteriophage ϵ^{15}. *Journal of Biological Chemistry* **240**, 384.

Robbins, P. W. & Uchida, T. (1962). Studies on the chemical basis of the phage conversion of O antigen in E group *Salmonellas*. *Biochemistry* **1**, 323.

Robbins, P. W. & Uchida, T. (1965). Chemical and macromolecular structure of O antigen from *Salmonella anatum* carrying mutants of bacteriophage ϵ^{15}. *Journal of Biological Chemistry* **240**, 375.

Uetake, H., Luria, S. E. & Burrous, J. W. (1958). Conversion of somatic antigens in Salmonella by phage infection leading to lysis or lysogeny. *Virology* **5**, 68.

https://doi.org/10.1017/S0016672300012386 Published online by Cambridge University Press

UETAKE, H. & UCHIDA, T. (1959). Mutants of *Salmonella* phage ϵ^{15} with abnormal conversion properties. *Virology* **9**, 495.

WRIGHT, A., DANKERT, M. & ROBBINS, P. W. (1965). Evidence for an intermediate stage in the biosynthesis of the *Salmonella* O antigen. *Proceedings of the National Academy of Sciences, Washington* **54**, 235.

https://doi.org/10.1017/S0016672300012386 Published online by Cambridge University Press