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Variation in Expression of Sex Factor Genes in the *Proteus-Providencia* Group Relative to *Escherichia coli*

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Several instances of anomalous expression of genes introduced from *Escherichia coli* K-12 into *Proteus mirabilis* have been described. It is shown here that control of sex pilus synthesis directed by the F-like R factor R1 and its derepressed derivatives R1-16 (o^c) and R1-19 (i^-) is also anomalous in *P. mirabilis*. Piliation in cells bearing the derepressed plasmids is expressed at a lower level than in *E. coli* K-12, and repression is absent in R1-carrying cells. Preliminary results show a similar effect in *Providencia*. In *Proteus morganii*, a similarly reduced level of piliation in R1-16$^+$ or R1-19$^+$ cultures is observed, but an intermediate level of repression occurs in R1$^+$ cultures. Less extensive data suggest that expression of the sex factor genes of an R factor of the N incompatibility group differs far less between *E. coli* and *P. mirabilis* hosts. Possible bases for these effects are discussed.

Exogenous genes have been reported to show altered expression in *Proteus* as compared with other enterobacteria. For instance, Colby and Hu (10), extending earlier observations (18), demonstrated that: (i) β-galactosidase encoded by F*lac*-carrying *P. mirabilis* shows a roughly 10-fold higher uninduced specific activity than in *Escherichia coli* K-12, but the effect of inducers is relatively weak, raising the activity by a factor of only 5; (ii) the relative efficiencies of various inducers differ as between the two hosts; and (iii) although, as in *E. coli*, i^s mutants behave as super-repressed, i^- mutants as constitutive, and certain o^c mutants as semi-constitutive, the intermediate levels determined by the latter are not increased by inducer. The enzyme is, however, very similar or identical to that formed in *E. coli* (11). These authors suggested that the anomalous regulation observed results from a conformational alteration of the *lac* repressor in *P. mirabilis* cytoplasm. Similar results, including also galactoside permease, have been reported by Stubbs et al. (41). By contrast, inducibility of β-galactosidase in *Serratia marcescens* is the same as in *E. coli* (26).

Some antibiotic resistance markers carried by R factors behave similarly. The specific activity of β-lactamase encoded by R1818 (= R46) is similar in *E. coli*, *S. marcescens*, *Klebsiella aerogenes*, and *Alkalescens* sp., but in *P. mirabilis* is reduced almost 20-fold (40). The enzymes produced in *E. coli* and *P. mirabilis* are the same as regards substrate specificity, pH optimum, K_m, and molecular weight (13). Franklin and Rownd (20) have shown that the expression of inducible tetracycline resistance determined by R factors NR1 and FR1 is lower, in both uninduced and induced states, in *P. mirabilis* than in *E. coli*. This effect did not apply to NR1-determined chloramphenicol resistance (34).

We wished to investigate how generally control systems encoded by "foreign" deoxyribonucleic acid (DNA) behave anomalously in *Proteus* species. We chose to study control of fertility in view of its probable importance from ecological and epidemiological standpoints.

We therefore studied the frequencies of cells infectable with the male-specific phages MS2 and IKe in strains of *Proteus mirabilis*, *Proteus morganii*, and *Providencia* bearing R factors that sensitize their hosts to these phages. MS2 is a ribonucleic acid (RNA) phage that adsorbs to F-type pili; the frequency of cells bearing such pili, in an *E. coli* population that contains a plasmid encoding them, is determined by a complex control system containing negatively (19, 33) and possibly also positively (1) acting components. IKe is a filamentous single-stranded DNA phage (28) that adsorbs to cells carrying plasmids only of incompatibility group N. N$^+$ populations of *E. coli* K-12 contain a high frequency of IKe-infectable cells, indicating that IKe sensitivity is constitutively expressed at a high level (S. Dennison and S. Baumberg, in press). Although the sighting of N pili has not yet been reported (4), it is probable

that such N-encoded analogues of F pili exist (R. H. Olsen, personal communication) and that they bear the IKe receptor (Dennison and Baumberg, in press).

We show here that control of sex piliation of an F-like R factor is anomalous, similar to that of other plasmid-linked genes in *Proteus* species. Control of N-directed IKe sensitivity differs less markedly as between *E. coli* and *P. mirabilis* hosts. A preliminary account of this work has appeared (S. Dennison, D. R. Paton, and S. Baumberg, Heredity **29**:123, 1972).

MATERIALS AND METHODS

Bacterial and phage strains and R factors. These are listed in Table 1. R factors were introduced into *Proteus* or *Providencia* by overnight mating with the R^+ *E. coli*, followed by selection on MacConkey agar containing the appropriate antibiotic(s) and polymyxin to kill the *E. coli* present. The R factors were shown to be genetically unchanged on transfer back to *E. coli* K-12.

Media and chemicals. MacConkey agar (Oxoid), with or without antibiotic, was used except where phages were to come into contact with the medium, in which case Oxoid nutrient broth no. 2 solidified with 1.25% Davis New Zealand agar was generally used. For certain experiments, Lennox broth (28) solidified with 1.25% agar was used. MacConkey agar had the advantage of suppressing swarming of *Proteus mirabilis*; when nutrient agar had to be used, this was achieved by the addition of ethanol to 5% final concentration (vol/vol).

The concentrations and sources of antibiotics were: ampicillin, 25 μg/ml (Penbritin, Beecham); chloramphenicol, 25 μg/ml (Chloromycetin, Parke-Davis); kanamycin, 25 μg/ml (Kanasyn, Winthrop); polymyxin, 100 μg/ml (polymyxin B sulfate, Sigma); streptomycin, 15 μg/ml (streptomycin sulfate B.P., Glaxo); and tetracycline, 10 μg/ml (Terramycin, Pfizer). Antibiotic resistance of cultures was routinely tested by use of Multodisks (Oxoid no. 3790E).

To standardize starting inocula, bacterial strains containing R factors were maintained as cultures grown from single colonies and characterized for antibiotic resistance and phage sensitivity as appropriate, at −20 C in nutrient broth containing glycerol at 50% final concentration (wt/vol).

Phage techniques. High-titer MS2 suspensions were prepared by propagation in liquid nutrient broth, and after centrifugation and membrane filtration were kept over chloroform at 4 C. High-titer IKe suspensions were prepared by confluent lysis in soft agar on a Lennox agar base, plates being left at room temperature for 2 days; they were centrifuged and heated to 60 C for 30 min, ether was added to maintain sterility, and the suspensions were stored at 4 C. *E. coli* K-12 clones carrying R1-16, R1-19, or R269N-1 were checked for spot lysis by MS2 or IKe on a lawn in soft agar or by cross-streaking. For these purposes and for all phage assays, cells exposed to MS2 or IKe were grown at 42 and 30 C, respectively, these being the optimal temperatures for plaque development.

Preparation of anti-MS2 serum. MS2, at 10^{10} plaque-forming units per ml, was mixed with an equal volume of Freund adjuvant (complete for the first injection, incomplete subsequently). Five milliliters of the mixture was injected (twice weekly for at least 3 weeks) intrascapularly into adult male New Zealand white rabbits. The animals were bled from the marginal vein of the ear. The blood was allowed to clot; the serum was clarified by centrifugation and was then dialyzed overnight against 18% (wt/vol) Na_2SO_4.

TABLE 1. *Bacterial and phage strains used*

Strain	Species	Relevant genetic markers	Source and/or reference
Bacteria			
J53	*Escherichia coli* K-12	F^- *met pro* λ^+	N. Datta; 6
WR1	*Proteus mirabilis*	*nic lac*	L. Baron; 10
2815	*Proteus morganii*	*nic cys pan lac*	R. W. Hedges ex J. N. Coetzee; 8
29	*Providencia*		
Plasmid	**Compatibility group**	**Markers**	**Source and/or reference**
R factors			
R1	F	*fi*$^+$ *amp chl kan str sul*	E. Meynell; 32
R1-16	F	*fi*$^-$ (*o*^{c} or *i*$^{-d}$) *kan*	E. Meynell; 31
R1-19	F	*fi*$^-$ (*i*$^-$) *amp chl kan str sul*	E. Meynell; 31
R269N-1	N	*amp kan str tet hspII*	N. Datta; 23
Phage		**Source and/or reference**	
Phages			
MS2		E. Meynell	
IKe		R. V. Iyer; 28	

The immunoglobulin G (IgG) precipitate was redissolved in the minimum volume of water, dialyzed at 4 C against water to remove Na_2SO_4, and finally stored at −20 C. Nonspecific IgG was adsorbed out twice with Hfr Hayes λ^- (the strain on which MS2 was propagated), the supernatant being finally sterilized by membrane filtration. The K value of the preparations produced in this way was never more than 12.

Determination of the frequency of MS2-infectable cells in populations carrying R1 and its derivatives. A 0.2-ml amount of the culture to be tested was added to 0.2 ml of MS2 suspension (titer about 10^{10} plaque-forming units/ml) at 37 C. Adsorption was usually for 7 min. The mixture was diluted 10-fold (0.1 ml → 0.9 ml) in broth; according to whether the expected frequency of MS2-infectable cells was low or high, 0.1 ml of anti-MS2 IgG was either added to the adsorption mixture or to 0.8 ml of buffer to make up the 0.9 ml into which this was diluted. Five minutes was allowed for neutralization of unadsorbed phage particles by the IgG. Further 10-fold serial dilutions were made in broth. Where very high frequencies of MS2-infectable cells were expected, 0.1 ml of anti-MS2 IgG was added also to 0.8 ml of buffer to make up the 10^{-3} dilution. Finally, 0.2-ml aliquots were assayed in duplicate by pouring in soft agar with the indicator strain Hfr P4X, and 0.1-ml aliquots of suitable dilutions were plated in duplicate on MacConkey agar to determine viable count. An R^- culture otherwise isogenic with the R^+ culture and at the same stage of growth was always treated in parallel to control for antiserum efficiency. Plaques arising from incomplete neutralization of the MS2 amounted to about one-third of all infective centers when MS2-infectable cells were at the very low frequency of 10^{-6}; at the higher frequencies much more commonly found, their contribution was negligible.

Control experiments also showed that the MS2 multiplicity of infection was always sufficient (i.e., nonlimiting) under the conditions used, and that residual IgG, even though not removed, had no effect on the number of infective centers developing. It may be noted that the latter differs from the method of Meynell and Datta (32); these authors filtered antibody-treated MS2-infected cells to remove excess antibody, whose K value (10,000) far exceeded that of the IgG used here.

Determination of the frequency of IKe-infectable cells in populations carrying R269N-1. A 0.2-ml amount of the culture to be tested was added to 0.2 ml of IKe suspension (titer about 10^{12} plaque-forming units/ml), and 15 min was allowed for adsorption. The cells were then centrifuged, washed twice with broth, and finally resuspended in 0.2 ml of broth. The suspension was then diluted in buffer and 0.1-ml aliquots were plated on nutrient agar; the washing procedure effectively eliminated free phage from the dilutions used. After overnight incubation at 30 C, the colonies were exposed to ether vapor for 5 min to kill the cells, and the plates were then ventilated for 5 min. Finally, the plates were carefully overlaid with 0.5 ml of indicator strain, usually J53 (R269N-1), in 5 ml of soft agar, and the plates were reincubated overnight at 30 C. IKe-infected cells in the original mixture were detected as having formed colonies surrounded by a halo of inhibited growth of the indicator. Such colonies were usually, though not invariably, much smaller (1 to 2 mm in diameter) than the colonies not surrounded by a halo. In general, plates containing 100 to 200 well-separated colonies were chosen for overlay; however, in the case of *P. mirabilis* the colony number had to be reduced to 20 to 30 because of the tendency to swarm, despite the inclusion of ethanol in the nutrient agar (control experiments showed that ethanol had no effect on the recovery of IKe-infected clones in this way).

RESULTS

Variation in frequencies of MS2-infectable R^+ E. coli cells during growth. This is shown in Fig. 1 for J53 cultures carrying R1, a repressed R factor of compatibility group FII, and two derepressed variants, R1-16 (either a genuine o^c mutant or of the i^{-d} type [31; S. Dennison, Ph.D. thesis, University of Leeds, Leeds, England, 1974] and R1-19 (i^- [17, 31]). It is seen that the frequency of MS2-infectable, that is, F-piliated, cells rose during the first hour for J53 (R1) and J53 (R1-19) but remained constant for J53 (R1-16). The frequencies 3 h after diluting 50-fold into fresh medium when the cultures had reached viable counts of 10^8 to 4×10^8/ml were: J53 (R1), 1.6×10^{-4}; J53 (R1-16), 5.2×10^{-1}; and J53 (R1-19), 2.7×10^{-1}. The frequency of F-piliated cells dropped considerably (about two orders of magnitude) during stationary phase for J53 (R1-16) and J53 (R1-19), but not so greatly for J53 (R1). To ensure that reduction in MS2-infectable cells was not due to R factor loss, 10 colonies each from the 7-h and overnight viable count plates were tested for donor ability. All except two J53 (R1) overnight clones could transfer kanamycin resistance.

Frequencies of MS2-infectable R^+ P. mirabilis cells during growth. This is illustrated in Fig. 2 for WR1 cultures carrying R1, R1-16, and R1-19. It is apparent that for all three strains the frequency of F-piliated cells varied little up to the end of exponential growth but dropped considerably on overnight incubation. In another experiment, although WR1 (R1) and WR1 (R1-19) behaved as in Fig. 2, WR1 (R1-16) showed a fall in frequency of F-piliated cells throughout exponential growth. In that instance, 10 colonies from 9-h viable count plates were tested for R factor segregation (note that even at high cell densities the actual segregation frequency was not masked by reinfection, since the cross was relatively infertile). All except one WR1 (R1-16) clone could transfer kanamycin resistance; however, of the nine WR1 (R1-16)

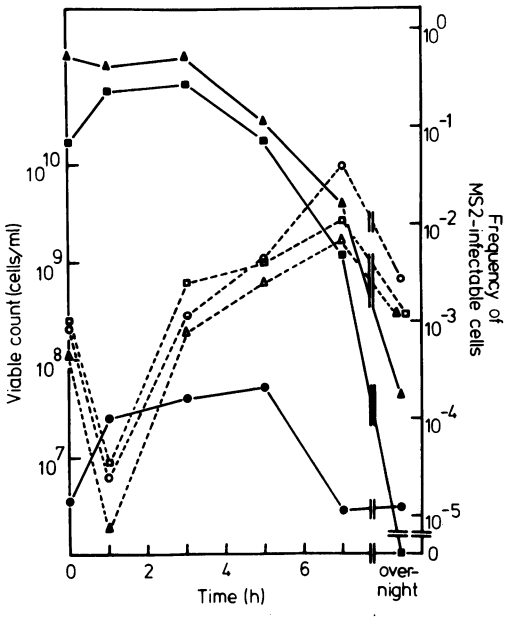

FIG. 1. *Variation in frequency of MS2-infectable J53 cells carrying R1, R1-16, or R1-19 during the growth cycle. Stock cultures of J53 (R1), J53 (R1-16), or J53 (R1-19) were streaked on MacConkey + kanamycin plates, which were grown overnight at 37 C. Several colonies from each were then resuspended in glycerol broth and part of the suspension was kept at −20 C for future reference. The suspension was assayed for viable count on MacConkey plates and for MS2-infectable cells (see Materials and Methods), diluted 50-fold into fresh prewarmed kanamycin broth, and incubated at 37 C with shaking. Further samples were taken for assay at the times indicated. Viable counts: ○, J53 (R1); △, J53 (R1-16); □, J53 (R1-19). Frequencies of MS2-infectable cells: ●, J53 (R1); ▲, J53 (R1-16); ■, J53 (R1-19).*

clones that did transfer this resistance, none also transferred a positive response to an MS2 spot test. Examination of the original inoculum showed that it still carried the typical R1-16, so any variants must have arisen in the course of the experiment. Similar behavior was noted in WR1 (R1-16) in a third experiment. The reason for this behavior is not known.

The most important conclusion from Fig. 2, however, is that the frequencies of F-piliated cells in the three cultures are similar throughout. The frequencies after 5 h of growth following dilution into fresh medium, at which point the cultures had attained viable counts similar to those reached by the *E. coli* cultures cited above after 3 h, were: WR1 (R1), 1.2×10^{-2}; WR1 (R1-16), 5.3×10^{-3}; WR1 (R1-19), 1.7×10^{-2}. Higher frequencies than these were observed sporadically. An example of this is the result for WR1 (R1-19) after 7 h in Fig. 2. In another similar experiment, in which the initial viable counts were much higher, frequencies of MS2-infectable cells 1 h after dilution were 5.8 $\times 10^{-2}$ for WR1 (R1) and 8.7 $\times 10^{-2}$ for WR1 (R1-19); however, in all succeeding examples, the frequencies recorded were no greater than those shown in Fig. 1. We therefore consider the 5-h values from Fig. 1 to be typical of these strains. The fact that higher frequencies have not been observed for WR1 (R1-16) may reflect the unexplained instability of this particular mutant R factor in *P. mirabilis*.

Frequencies of MS2-infectable R⁺ P. morganii cells during growth. *P. morganii* differs from other *Proteus* species in possessing DNA of guanine plus cytosine percentage above 50%, whereas the *Proteus* norm is around 40% (24); it also differs from other *Proteus* species in certain biochemical characteristics (7). It therefore seemed of interest to determine the frequencies of MS2-infectable cells in strains of this species carrying R1, R1-16, and R1-19 (Fig. 3). As with these R factors in *E. coli*, the frequency of MS2-infectable cells rose initially and then

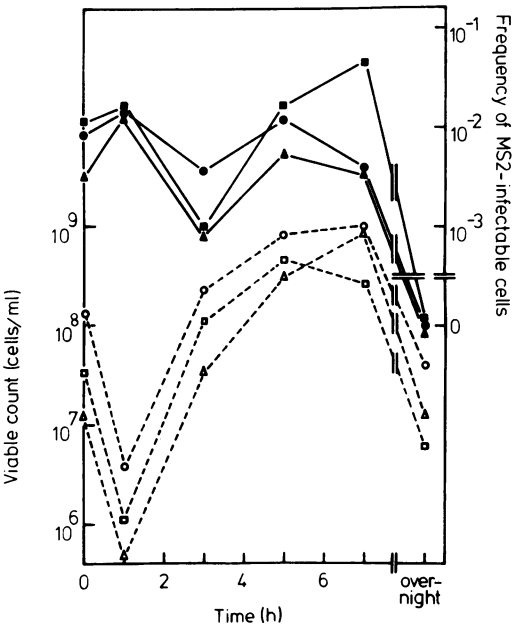

FIG. 2. *Variation in frequency of MS2-infectable Proteus mirabilis WR1 cells carrying R1, R1-16, or R1-19 during the growth cycle. Cultures of WR1 (R1), WR1 (R1-16), and WR1 (R1-19) were grown and assayed for viable count and MS2-infectable cells as described in the legend to Fig. 1. Viable counts: ○, WR1 (R1); △, WR1 (R1-16); □, WR1 (R1-19). Frequencies of MS2-infectable cells: ●, WR1 (R1); ▲, WR1 (R1-16); ■, WR1 (R1-19).*

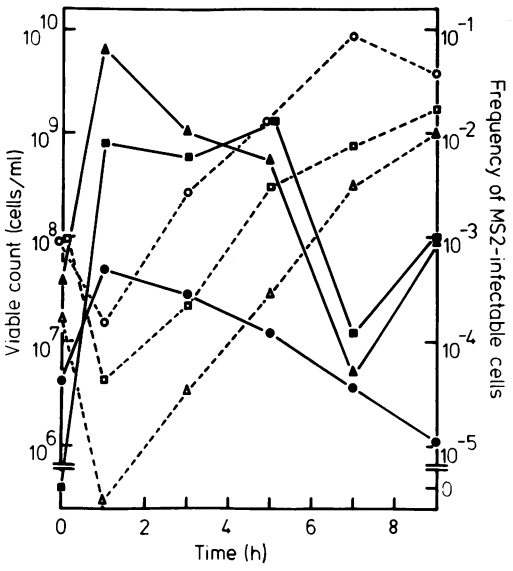

FIG. 3. *Variation in frequency of MS2-infectable P. morganii 2815 cells carrying R1, R1-16, or R1-19 during the growth cycle. Cultures of 2815 (R1), 2815 (R1-16), and 2815 (R1-19) were grown and assayed for viable count and MS2-infectable cells as described in the legend to Fig. 1, except that the inoculum suspension was diluted 10-fold rather than 50-fold. Viable counts:* ○, *2815 (R1);* △, *2815 (R1-16);* □, *2815 (R1-19). Frequencies of MS2-infectable cells:* ●, *2815 (R1);* ▲, *2815 (R1-16);* ■, *2815 (R1-19).*

diminished. The greater fall-off for 2815 (R1-16) than for 2815 (R1-19) may indicate the same kind of instability of R1-16 as in *P. mirabilis*. Due to the very different growth curves of the three strains, it seems reasonable to consider as typical for these strains the frequencies from the 3-h sample of 2815 (R1) and the 5-h samples of the other two cultures. With cell densities roughly standardized in this way, the frequencies of MS2-infectable cells were then: 2815 (R1), 2.8×10^{-4}; 2815 (R1-16), 5.4×10^{-3}; 2815 (R1-19), 1.3×10^{-2}. It is interesting that though the frequencies for 2815 (R1-16) and 2815 (R1-19) were similar to those for the R$^+$ *P. mirabilis* strains, that for 2815 (R1) was about 50-fold lower, though this repression ratio did not approach the 2,000-fold found for the R$^+$ *E. coli* strains. In this, as in the variation in frequencies during the growth cycle, *P. morganii* seemed to show a tendency away from the *P. mirabilis* anomaly.

Preliminary experiment with MS2-infectable R$^+$ Providencia. Single colonies of 29 (R1), 29 (R1-16), and 29 (R1-19) were inoculated into kanamycin broth and incubated overnight. They were then diluted 10-fold in fresh kanamycin broth, incubated at 37 C for 2 h, and assayed for viable count and MS2-infectable cells. This particular procedure has been found (S. Dennison, Ph.D. thesis) to give frequencies of piliated cells for R$^+$ *P. mirabilis* cultures of the same order as the maximum frequencies obtained in the more definitive experiments quoted above. The frequencies found for 29 (R1), 29 (R1-16), and 29 (R1-19) were 8.5×10^{-4}, 2.3×10^{-3}, and 4.3×10^{-3}, respectively, suggesting a similarity between the strain carrying the repressed R factor and those carrying its derepressed variants that is reminiscent of *P. mirabilis* rather than *P. morganii*.

Frequencies of IKe-infectable cells of R$^+$ E. coli and P. mirabilis. Due to the "antiserum effect" typical of filamentous single-stranded DNA phages, whereby antisera to the phages kill infected cells (30), frequencies of IKe-infectable cells in a population could not be determined in the same way as those of MS2-infectable cells. A method was therefore developed (see Materials and Methods) based on the detection of an IKe-infected cell by the release of IKe phage from the colony derived from such a cell, with the resulting appearance of a zone of partial "clearing" or "halo" around the colony in a lawn of IKe-sensitive cells overlaid in soft agar. This technique, using F-specific filamentous phage M13, yielded similar estimates of frequencies of cells bearing F-type pili in cultures of J53 (R1) and J53 (R1-19) to those obtained by the MS2 method (results not shown). Because of the laboriousness of this technique, especially for *P. mirabilis* (see Materials and Methods), estimates of frequencies of IKe-infectable cells were not made on samples taken at various times during the growth cycle of a culture, as was done for MS2-infectable cells, but on only one sample taken during midexponential growth. Results for the frequencies of IKe-infectable cells of J53 (R269N-1) or J53N (R269N-1), WR1 (R269N-1), and 2815 (R269N-1) are shown in Table 2.

It is clear that the results obtained for both *E. coli* and *P. mirabilis* were rather variable from one experiment to another. However, the difference between the average frequencies of IKe-infectable cells in a WR1 (R269N-1) population, 5.2×10^{-2}, and in a J53 (R269N-1) population, 2.0×10^{-1}, was less than that between the frequencies of MS2-infectable cells in a WR1 (R1-19) population, 1.7×10^{-2}, and in a J53 (R1-19) population, 2.7×10^{-1}. To the extent that the difference in each case between the WR1 and J53 cultures reflects a difference in expression of piliation genes (the nature of the

TABLE 2. *Frequencies of IKe-infectable cells in cultures of J53 or J53N, WR1, and 2815 carrying the N incompatibility group R factor R269N-1*[a]

Expt	Strain	Proportion of IKe-infectable cells	Frequency of IKe-infectable cells
1	J53 (R269N-1)	308/1,329	2.3×10^{-1}
	WR1 (R269N-1)	4/81	5×10^{-2}
	2815 (R269N-1)	0/882	$<10^{-3}$
2	J53N (R269N-1)[b]	339/1,887	2.1×10^{-1}
	WR1 (R269N-1)	1/151	7×10^{-3}
	J53N (R269N-1)[c]	399/1,559	2.6×10^{-1}
	2815 (R269N-1)	0/3,322	$<3 \times 10^{-4}$
3	J53 (R269N-1)	5/454	10^{-2}
	WR1 (R269N-1)	30/435	7×10^{-2}
Mean	J53 or J53N (R269N-1)	1,051/5,229	2.0×10^{-1}
	WR1 (R269N-1)	35/667	5.2×10^{-2}

[a] Techniques are described in Materials and Methods. No halos were ever observed around colonies derived from R⁻ cultures tested in the same way.

[b] Obtained by transfer of R269N-1 from WR1 (R269N-1) in a mating with selection for K^R Nal^R transconjugants.

[c] Obtained by transfer of R269N-1 from 2815 (R269N-1) in a mating with selection for K^R Nal^R transconjugants.

IKe receptor is discussed by Dennison and Baumberg [in press]), these results suggest that the anomaly in expression affects such genes in R269N-1 less than those in R1-19. Since the measured frequencies are erratic, this suggestion can only be tentative. However, the tendency of the *P. mirabilis* colonies to spread after soft-agar overlay probably resulted in failure to score some IKe-infected clones, leading to underestimated frequencies.

The failure to observe IKe-infected *P. morganii* colonies could have been due either to failure of the phage to adsorb to 2815 (R269N-1) or to its inability to enter or to propagate in that host. We have described (Baumberg and Dennison, in press) experiments demonstrating that IKe does in fact adsorb to R269N-1⁺ (but not R⁻) cells of *P. morganii* strain 2815 but fails to multiply. The basis for this phenomenon is unknown.

DISCUSSION

The results obtained for frequencies of piliated cells in cultures of various hosts carrying R1 or its derepressed mutants R1-16 and R1-19 are shown in Table 3. They indicate that the control of R1-determined sex pilus formation is indeed anomalous in *P. mirabilis* by comparison with *E. coli*, and in ways that resemble the anomalies noted by Colby and Hu (10) and Stubbs et al. (41) for control of F*lac* in *P. mirabilis*. In what follows, it is assumed that the frequency of male-specific phage-infectable cells in a population is a measure of the expression of at least some plasmid-encoded genes determining the phage receptors, i.e., that it is not limited merely, for instance, by the rate of assembly of pilin subunits into functional pili.

(i) The frequency of phage-infectable cells in genetically derepressed cells is less in *P. mirabilis* than in *E. coli* [compare 1.7×10^{-2} for WR1 (R1-19) with 2.7×10^{-1} for J53 (R1-19)]. The comparison can only be made for IKe-infectable cells if we assume that R269N-1 does not possess a functional repression system, which seems reasonable in view of the high frequency of 2.0×10^{-1} found for J53 (R269N-1); the corresponding figure for WR1 (R269N-1) is 5.2×10^{-2}. The apparent difference may be in part attributed to the poor resolution of the technique in the case of WR1 (R269N-1). This aspect of the control anomaly in *P. mirabilis* is analogous to the low specific activities of β-galactosidase in induced or i^- *P. mirabilis* carrying F*lac* as compared to *E. coli* (10, 41) and R1818-encoded penicillinase in the former as contrasted to the latter host (40).

(ii) The ratio of derepressed to repressed frequencies of MS2-infectable cells (cultures carrying the R1-16 or R1-19 as against those carrying R1) is less for *P. mirabilis* than for *E.*

TABLE 3. *Summary of results on frequency of piliated cells in cultures of various hosts carrying R1 or derepressed mutants thereof*

Host	R factor	Frequency of piliated cells[a]	Repression ratio[b]
J53	R1	1.6×10^{-4}	
	R1-16	5.2×10^{-1}	3,250
	R1-19	2.7×10^{-1}	1,700
WR1	R1	1.2×10^{-2}	
	R1-16	5.3×10^{-3}	approx. 1
	R1-19	1.7×10^{-2}	1.4
2815	R1	2.8×10^{-4}	
	R1-16	5.4×10^{-3}	19
	R1-19	1.3×10^{-2}	46
29	R1	8.5×10^{-4}	
	R1-16	2.3×10^{-3}	3
	R1-19	4.3×10^{-3}	5

[a] In cultures at approximately the same stage of growth, containing 10^8 to 4×10^8 cells/ml.

[b] (Frequency of piliated R1-16⁺ or R1-19⁺ cells) ÷ (frequency of piliated R1⁺ cells).

coli, the ratio being of the order of 1 for the former and of the order of 2,000 for the latter. This is more extreme than the corresponding difference between derepressed and repressed levels of β-galactosidase, where Colby and Hu's data suggest ratios of 5 and >1,000 (10) and the data of Stubbs et al. suggest ratios of 60 and 800 (41). It may be noted that the difference between these two sets of results, although possibly reflecting just strain differences, culture conditions, etc., may also be affected by differences in rate of loss of F*lac* from *P. mirabilis* cultures, which is reported as having been checked in the latter but not in the former (one of us [S. Dennison, Ph.D. thesis] has found, in concurrence with references 18 and 41, that F*lac* is exceedingly hard to maintain in *P. mirabilis*, even under selective conditions).

We suggest that anomaly (ii) (i.e., the reduced repression ratio) may be merely a reflection of (i) (the low level constitutivity). It could result from reduced expression of "foreign" genes in *P. mirabilis* applying to repressor genes as well as the structural genes they control. The *lac* repressor is present in only about 10 copies per *E. coli* cell (21); the equivalent figures for the various components of the R1 repression system are unknown, but it seems probable, from the fact that repressible plasmids of the F and I groups are phenotypically derepressed for several generations after transfer to a new host (33), that at least one component is present in a small number of copies also. Presumably, reduced expression of a gene(s) encoding such a limiting component, as of the *i* gene in the *lac* case, greatly increases the proportion of cells completely lacking repressor molecules due to random apportionment of these among daughter cells at division, such cells being in effect derepressed. Repression would thus necessarily be less effective in *P. mirabilis* than in *E. coli*. It cannot be ruled out, however, that these anomalies reflect some characteristic in control of *P. mirabilis* that applies even to its own genes: the arginine biosynthetic pathway in this organism shows very limited potentiality for derepression by comparison to *E. coli* (37).

The results for *P. morganii* are not immediately in accord with this explanation, since maximal expression of piliation [1.3×10^{-2} for 2815 (R1-19)] is very similar to that in *P. mirabilis*, yet some degree of repression is apparent. That expression of repression though not of derepressed pilus (28) synthesis differs as between *P. mirabilis* and *P. morganii* raises the possibility that the negative control functions evolved relatively late in the case of FII group R factors. In this context it may be significant that some plasmid types commonly found in soil bacteria lack such control. The expression of other foreign genes, such as *E. coli*'s *lac* (on F*lac*) or penicillinase (on R factors) genes, has not been studied for these hosts, so it is not known whether the suggestion that control of R1-determined piliation in *P. morganii* is to some degree intermediate between *P. mirabilis* and *E. coli* would accord with these other systems.

It has been assumed throughout that (i) all cells sensitive to a phage at any given time will have adsorbed at least one effective phage particle by the end of the adsorption period, and (ii) adsorption invariably leads to the appearance of an infective center. These assumptions appear to be justified for *E. coli* K-12 in view of the very high measured frequencies of infectable cells; however, this may not be true for *Proteus*. Nevertheless, even if these assumptions are not wholly justified for *Proteus*, the repression ratio (derepressed to repressed) should be unaffected, although the derepressed frequencies themselves may be artifactually low.

As to causes of these anomalies of foreign gene expression in *Proteus*, perhaps the most appealing hypothesis is that of Dale and Smith (13), who suggest that the *Proteus* RNA polymerase may have reduced affinity for exogenous promoters. This idea suggests the possibility of selecting, after mutagenesis, for mutant R factors with more efficient promoters for *Proteus* RNA polymerases; preliminary experiments, however, have not revealed any such R factors. A second hypothesis involving a transcriptional effect is that the anomalies reflect an involvement of cyclic 3′,5′-adenosine monophosphate in control of piliation which functions differently in *E. coli* and *Proteus*. Cyclic 3′,5′-adenosine monophosphate may in certain cases play a role in control of pilus production (22), though its effect here differs from that on expression of some other plasmid-encoded functions (16, 25). Although it has been stated that cyclic 3′,5′-adenosine monophosphate can act in controlling gene expression in *P. mirabilis* (36), the full evidence has not been published. Colby et al. (12) found that catabolite repression of β-galactosidase synthesis in *P. mirabilis* carrying F*lac* was abnormal.

An alternative type of explanation involves a translational effect; here the report (2) that in vitro translation in systems prepared from *P. vulgaris* is poor and limited by availability of transfer RNA may be relevant. Perhaps assay of plasmid-specific messenger RNA might help to resolve the matter.

It would have been interesting to look at

plasmids from other groups; however, of those for which group-specific phages exist, the I group are unable to infect *Proteus* species (14), whereas phages for the potentially interesting P group (see reference 35) were only reported when this work was nearly complete. In any case, it cannot yet be concluded that the control anomalies described here are of ecological or evolutionary significance.

It is tempting to speculate that the tendency of some R factors that exist as co-integrates in *E. coli* to dissociate in *P. mirabilis*, and the differing control of replication of plasmid DNAs as between these hosts (5, 9, 27, 38, 39), as well as the instability of F*lac* in *P. mirabilis* referred to above, reflect these same regulatory anomalies. It is also possible that the complex pattern of control of replication of resistance transfer factor and r-determinants in *Proteus* (27, 38, 39) might itself affect sex pilus production—note that in our experiments R^+ *Proteus* cultures were always grown in the presence of antibiotic, which alters the number and association of these replicons. The results already cited on anolmalous control of β-galactosidase (10, 41), β-lactamase (40), and tetracycline resistance (2) may all be explained on the basis of the mechanism suggested. That expression of NR1-encoded chloramphenicol resistance does not follow this pattern (34) might be due to the cancelling-out of lowered gene expression by multiplication of resistance determinants under relaxed control.

Such anomalies may well be found in other hosts. For instance, it is reported (3) that P group R factor-determined carbenicillin resistance is poorly expressed in *Rhizobium leguminosarum* in comparison with *E. coli*.

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