The term “Rex phenotype” connotes generalized plaque exclusion by λ lysogens, a process that restricts plaque formation by λl mutants of T4 (4), certain T7 and T5 mutants, and particular variants of lambdoid phages (12, 17). The rex locus of coliphage λ encoded by genes rexA and rexB (11) is cotranscribed as part of the prexA-rexA-rexB-timm operon expressed by a repressed λ prophage (8). The model of Parma et al. (13) predicts that RexB protein forms an inner membrane pore that is opened upon direct interaction with at least two RexA proteins, resulting in a cellular apoptotic response termed altruistic cell death. The degree of apoptosis was unreported. We asked if the Rex phenotype confers a protective or a cell-killing response to phage attack.

We utilized derivatives of Escherichia coli K-12 strains R594 [F− lac-3350 galK2 galT22 rpsL179 in(rrnrD-rrnE)1 λ−] (3), W3350A [F− lac-3350 galK2 galT22 in(rrnrD-rrnE)1 λ−] (3), and SA500 [F− his-87 relA1 strA181 tsx-83 λ−] to prepare lysogens. The λ wild type was from our stock (no. 271), and λrexA5A and λrexA30A were from G. Gussin (11) via W. Szybalski. The phages T4ΔII (point mutation in the hIIA gene of T4), T4ΔIIΔ1589 (deletion spanning the hIIA and hIB genes), and T4ΔD were obtained from G. Mosig.

Cellular viability was determined following T4ΔII infection (multiplicity of infection, 10) of the Rex− lysogen R594(λ), the Rex− lysogens R594(λrexA30A) and R594(λrexA5A), and nonlysogenic R594 cells. Optimal infection occurred at temperatures between 37 and 43°C, with a reduction of >103-fold infectivity at 30°C, in agreement with the results of earlier studies (2, 6). The examination of spread plates from mock infections without T4 showed that virtually 100% of the CFU arose during the first 24 h of plate incubation at temperatures between 30 and 43°C. No survivors were seen among the T4ΔII-infected cells during the same interval. We continued incubation for an additional 24 h, during which the CFU from mock infections increased in size, and tiny surviving CFU appeared between 36 and 48 h among the T4ΔII-infected Rex− lysogens, revealing a prolonged growth arrest. The CFU that survived T4ΔII infection were examined for retention of the Rex− phenotype and sensitivity to T4. All CFU tested remained Rex− and T4 sensitive. The Rex− R594(λ) lysogens survived T4ΔII infections with ≥40% viability at temperatures between 37 and 43°C. In contrast, we found that the viability of Rex+ R594 culture cells infected at temperatures between 37 and 43°C was <0.001%. Similar results were found for R594(λrexA) and R594(λrexB) lysogens. Identical infections of the Rex− lysogens SA500(λ) and W3350A(λ) yielded the same level of survivors as that of R594(λ), whereas the viability of their Rex− derivatives was <0.01%. This experiment revealed that the Rex+ phenotype can confer an enormous (>103-fold) protective advantage to infected λ lysogenic cells. We also monitored the viability of Rex+ and Rex-defective lysogenic and nonlysogenic cells infected in solution with T4ΔII at a multiplicity of infection of 5. Both R594 and R594(λrex) culture cells were reduced in titer by more than 103-fold (assay minimum) within the first hour, and surviving CFU were not subsequently detected. By contrast, infected R594(λ) cells showed a 10-fold drop in cell titer within the first hour of infection, a lag in cell growth, and a subsequent increase in CFU. None of the surviving R594(λ) CFU tested were found to be resistant to T4. In all of the infection experiments, we observed that the surviving cells in aliquots removed from cultures appeared as CFU after a prolonged lag in cell growth and were considerably smaller than the CFU arising from parallel mock infections.

Our findings suggest that the rex genes of λ confer symbiotic protection to the lysogenic host against secondary infection. Previous studies have shown that high cellular levels of Rex expression restricts plaque formation by phages T2, T4, T5, T6, and T7 (15); thus, the advantage of the Rex phenotype in the wild may be more widespread than is appreciated. However, mechanistically, it is far from clear that the Rex phenotype evolved, is maintained, or functions in the wild for the purpose of host protection against secondary lytic infection. It is our view that the cellular manifestations of Rex exclusion that are triggered upon infection may be severe enough to result in cell death but may also provide the intolerable environment necessary to eliminate invading phage DNA. We found that both the infection of λrex− lyogenic cells with T4ΔII and their transformation with a rexA multicopy plasmid (data not shown) delayed the emergence of CFU. The prolonged arrest in cell growth of rexA transformants of R594(λ) helped us to account for the results of Snyder and McWilliams (16).

The question of how a Rex− cell avoids lethal gene expression from infecting T4ΔII remains unanswered. The stationary physiological phase of E. coli host cells has been shown to prevent the growth of T4 phage (7, 9), and these cells maintain...
a lower proton motive force (10). Furthermore, during starvation, the stringent response prevents macromolecular synthesis (5, 14) and may lead to bacterial apoptosis (1). The Rex system acquired by lambda can channel lysogenic cells into an arrested growth phase resembling the stationary phase or the stringent response, both of which have levels of cell killing associated with them; however, on the whole, the responses of this system exhibit mutualism, conferring a protective ability to the host.

The hypothesis that the λ Rex phenotype triggers an altruistic response in excluding the plating of T4rII requires that the rexB-rexA genes function as a suicide module. Our study does not support this model but rather suggests that Rex exclusion of invading phage is a protective mechanism which results in the increased survival of infected cells and in turn defends the cell population as a whole from subsequent phage exposure.

This study was supported by an NSERC operating grant to S. H. Roderick Slavcev received teaching fellowships from the Colleges of Medicine and Graduate Studies and Research, University of Saskatchewan.

REFERENCES

8. Hayes, S., H. Bull, and J. Tuloch. 1997. The rex phenotype of altruistic cell death following infection of a λ lysogen by T4rII mutants is suppressed by plasmids expressing OOP RNA. Gene 189:35–42.