Bacteriophage T4 development depends on the physiology of its host *Escherichia coli*

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Several parameters of phage T4 adsorption to and growth in *Escherichia coli* B/r were determined. All changed monotonously with the bacterial growth rate (μ), which was modified by nutritional conditions. Adsorption rate was faster at higher μ values, positively correlated to cell size, and increased by pretreatment with low penicillin (Pn) concentrations; it was directly proportional to total cellular surface area, indicating a constant density of T4 receptors on cell envelopes irrespective of growth conditions. Parameters of phage development and cell lysis were μ-dependent. The rate of phage release and burst size increased, while the eclipse and latent periods decreased with increasing μ. Differentiation between the contribution of several physiological parameters to the development of T4 was performed by manipulating the host cells. A competitive inhibitor of glucose uptake, methyl α-D-glucoside, was exploited to reduce the growth rate in the same effective carbon source. Synchronous cells were obtained by the 'baby-machine' and large cells were obtained by pretreatment with low Pn concentrations. Lysis was delayed by superinfection, and DNA content and concentration were modified by growing a *thy* mutant in limiting thymine concentrations. The results indicate that burst size is not limited by cell size or DNA composition, nor directly by the rate of metabolism, but rather by the rates of synthesis and assembly of phage components and by lysis time. The rates of synthesis and assembly of phage components seem to depend on the content of the protein-synthesizing system and lysis time seems to depend on cellular dimensions.

INTRODUCTION

Studies on bacteriophage growth and development played a vital role in the history of molecular biology (Cairns *et al.*, 1966; Karam, 1994; Mathews *et al.*, 1983). The information which was accumulated during the 1940s, particularly with the model species T4, laid the basics for the evolving field (Cairns *et al.*, 1966). The classical one-step growth experiment performed by Ellis & Delbrück (1939) defined the latent period, rise time and burst size, and the eclipse period was discovered by the effective procedure devised to disrupt infected bacteria before their spontaneous lysis and without damage to the mature phages (Doermann, 1948).

Phage–host interactions begin by a highly specific attachment to one of the cell envelope layers; the phage tail fibres bind to specific receptors in the bacterial envelope (Goldberg *et al.*, 1994). Adsorption depends on environmental factors as well, and is followed by penetration of the phage DNA through the bacterial wall, which is weakened by the lysozymic activity of the tail baseplate. The infected cell then becomes dedicated to the phage's needs for efficient multiplication by expressing phage-encoded proteins. Early proteins govern DNA replication and synthesis of coat components; late proteins induce cell lysis thus releasing phage progeny. All time and kinetic parameters differ with interacting species and vary over a wide range.

By the time bacterial physiology was established as a discipline (Kjeldgaard *et al.*, 1958; Schaechter *et al.*,

1958), molecular biology became so promising that some unsolved questions in phage–host cell interactions have been ignored and not seriously looked at since. The vast amount of knowledge gained during the last 35 years on the biochemistry, genetics and physiology of bacteria (Ingraham *et al.*, 1983; Koch, 1971; Neidhardt *et al.*, 1987) enables a fresh look on these interactions, which may shed light on various cell properties (Eddy, 1992; Hadas *et al.*, 1994; Kutter *et al.*, 1994).

Most previous studies of bacteriophage development reported in the literature were carried out under optimal conditions, such as under high aeration, at 37 °C and in rich media. The question thus arises whether the physiological state of *Escherichia coli* affects the kinetic parameters of T4 growth. Development of T4 is studied here as a function of cell size, age and shape, rates of metabolism and chromosome replication, and time of lysis.

METHODS

Phage and bacterial strains. Wild-type T4 phage, *E. coli* B/r (H266) (Zaritsky *et al.*, 1979) and *E. coli* K-12 (CR34, *thr leu thy drm*) (Zaritsky & Woldringh, 1978) were used throughout this study.

Media and cell growth. *E. coli* B/r was cultivated as previously described (Woldringh *et al.*, 1977; Zaritsky *et al.*, 1979) in the following media: Luria–Bertani broth containing glucose (0·4%) (LBG; doubling time, $\tau = 23$ min); M9 minimal medium supplemented with (a) casein hydrolysate (1%, w/v) and tryptophan (50 μg ml^{-1}), alone (Casa; $\tau = 28$ min), with glucose (0·4%; GC; $\tau = 30$ min), and with yeast extract (1·25%, w/v) and glucose (GCY; $\tau = 23$ min) or (b) 0·4% of either glucose (Glu; $\tau = 48$ min), glycerol (Gly; $\tau = 70$ min), succinate (Suc; $\tau = 90$ min) or acetate (Acet; $\tau = 136$ min).

Cells were cultivated in a New Brunswick gyratory water-bath shaker with vigorous aeration (250 r.p.m.) at 37 °C. Overnight cultures were appropriately diluted into prewarmed fresh medium and growth was monitored by cell number (using Coulter Counter model ZM) and OD$_{450}$ (using an LKB Ultraspec II spectrophotometer). Steady-state growth (Fishov *et al.*, 1995) was maintained by further, appropriate dilutions (below OD$_{450}$ = 0·4) and demonstrated by the Kolmogorov–Smirnov test for size distributions in successive generations (Woldringh *et al.*, 1993).

Phage infection and growth determination. Unless otherwise stated, experiments were initiated by infecting a steady-state (or appropriately treated) culture of 10^8 cells ml^{-1} by phage at a multiplicity of 0·5 (to guarantee single infection) in the presence of 2 mM KCN (to synchronize the infective process).

Adsorption rate was detected during the first 5 min of infection by titrating unadsorbed phage particles (using chloroform to kill infected bacteria). Phage development was initiated 4 min after phage addition by dilution (10^{-4}) in the same medium to cease infections and eliminate the bacteriostatic effect of the cyanide. Samples were withdrawn periodically and plated, immediately and through chloroform (after evaporation) after appropriate dilutions, with soft agar using *E. coli* B/r (H266) as indicator. The number of plaques was counted on each plate after overnight incubation at 37 °C, and the number of phages at each time was calculated. (For additional details,

consult Adams, 1959; Brown, 1956; Eisenstark, 1967; Stent, 1963.)

Calculations. The raw data (p.f.u. ml^{-1}) were transformed to derive the number of phages per infected cell as function of time. A p.f.u. value obtained in the chloroform series (total phage; C1) reflects all phage, both free (C1$_0$) and newly matured inside an infected bacterium (C2; which is equal to C1 − C1$_0$). A p.f.u. value obtained without chloroform (total infective centres; C3) stems from either a ripe phage or an infected bacterium ('infective centre') whether or not it harbours mature phages. The number of unadsorbed phages (C1$_0$) is C1 during the eclipse period; it is considered the background p.f.u. value and is thus subtracted from all raw data (C1 and C3). C4 is the sum of the net number of infected bacteria and of naturally released phages (C3 − C1$_0$); during the latent period, it reflects the number of infected bacteria (C4$_0$), and is used to divide each datum (C2 and C4) to yield the number of mature phages per cell during the infective process: normalized intracellular ripe phages (through chloroform; C5; which is equal to C2/C4$_0$) and normalized mature phages naturally appearing outside the cells (C6; which is equal to C4/C4$_0$).

RESULTS

Adsorption kinetics

When *E. coli* cells grow faster in richer media (not at higher temperatures), they are larger with proportionally more surface area, to which phage are adsorbed. Rates of adsorption increased with decreasing τ in richer media, and ranged under these conditions (see Methods) from 0·71 × 10^7 phage ml^{-1} min^{-1} in Acet to 3·36 × 10^7 phage ml^{-1} min^{-1} in LBG. A selected series of such adsorption curves is displayed in Fig. 1.

To distinguish between the possible effects of medium composition and those of cell size, low penicillin (Pn) concentrations were used, which specifically block cell division (Hadas *et al.*, 1995). Adsorption rate was indeed
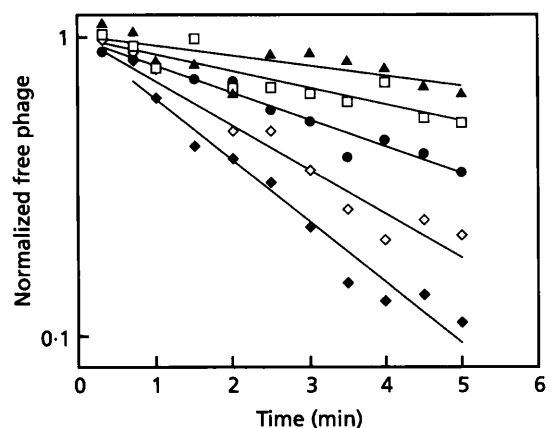
Fig. 1. Rates of adsorption of T4 phage to *E. coli* B/r cells grown on the following carbon sources: Acet (▲); Gly (□); Glu (●); LBG (◇); LBG+Pn (◆). Normalized free phage = C1, normalized to 10^8 bacterial cells ml^{-1} at time zero, addition of phage.
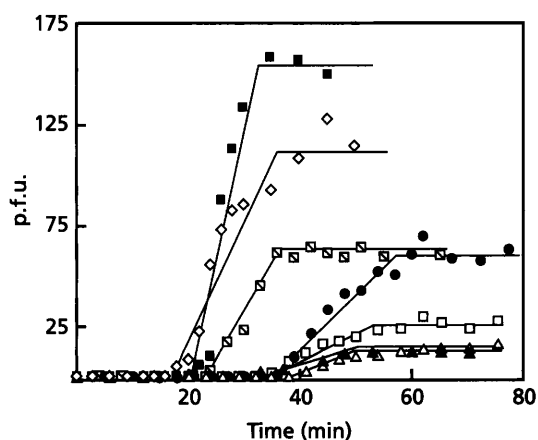
Fig. 2. Phage development in single infections of steady-state *E. coli* B/r H266 cells grown on the following carbon sources: Acet (▲); Suc (△); Gly (□); Glu (●); Casa (◨); GC (■); LBG (◇). p.f.u. = C6.
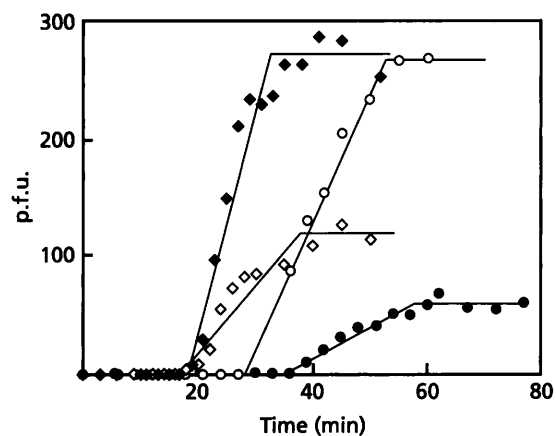
Fig. 3. Phage development in single infections of *E. coli* B/r H266 cells either grown under steady-state conditions in LBG (◇) or Glu (●), or pretreated with Pn for two τs before infection. ◆, LBG+Pn (75 U ml⁻¹); ○, Glu+Pn (30 U ml⁻¹). p.f.u. = C6.

significantly higher: 4.68×10^7 phage $\text{ml}^{-1}\,\text{min}^{-1}$ in the Pn-pretreated LBG culture, for example (Fig. 1).

One growth cycle

Kinetic parameters of intracellular T4 growth were determined under different physiological states of *E. coli* obtained by using different carbon sources to change the growth rate (μ), cell size and macromolecular composition. Larger cells obtained in richer media grow faster because a higher proportion of their mass is included in the protein-synthesizing system (PSS) (Bremer & Dennis, 1987; Ingraham *et al.*, 1983). Rates of phage development and cell lysis were indeed τ-dependent (Fig. 2): latent periods increased from 21 min in GC to 35 min in Acet, and burst sizes decreased from 150 to 12, respectively.

In addition, a competitive inhibitor of glucose uptake, methyl α-D-glucoside (α-mG; 1·25%, with 0·05% D-Glu), which lowers μ (Zaritsky & Helmstetter, 1992), was used to obtain small cells (at $\tau = 90$ min) similar to those obtained in Suc (with a similar τ) but without changing the effective carbon source (Zaritsky *et al.*, 1993). The smaller α-mG-grown cells yielded fewer phages (burst size of 36; data not shown) than control Glu cells (burst size of 60). However, they yielded more phages than the Suc cells (burst size of 14). Thus, the ability to support phage maturation is not proportional to cell size as such.

Cell size and macromolecular composition

To distinguish between the effects of cell size and those of metabolic rate, which are correlated under steady-state growth in different media (Schaechter *et al.*, 1958), two regimes were employed.

(i) Synchronous Glu cells, obtained by the 'baby-machine' (Helmstetter, 1969), were infected either upon collection ('babies') or after 40 min of growth (almost one mass doubling). The latter, larger synchronous cells, supported a slightly faster phage assembly and yielded more phages (burst size of 60) than the 'babies' (burst size of 35).

(ii) Low Pn concentrations were used to specifically block division without affecting mass growth-rate (Hadas *et al.*, 1995). Exposure during about two τs before infection resulted in approximately fourfold larger cells. The rate of phage assembly was faster and the yield was fourfold larger (burst size of 260) in Pn-pretreated Glu cells than in control Glu cells (Fig. 3). In LBG cells, this treatment resulted in a 2·5-fold increase in burst size only, from 100 to 270, despite the faster rate of phage synthesis (Fig. 3).

Lysis inhibition

The question thus arises whether the capacity of an *E. coli* cell to support phage multiplication is limited to a burst size of about 300 under all circumstances or whether this capacity is limited by the lysis, which is quicker in faster growing cells (Fig. 2). Superinfection, which is known to delay cell lysis (Bode, 1967), was used to distinguish between the two possibilities. Pn-pretreated LBG-grown cells were superinfected at 10 min extending the latent period from 20 to 28 min (Fig. 4). This delay raised the burst size further, from 270 to about 700 (Fig. 4). The rate of T4 formation was only slightly higher with superinfection than without, but both rates were significantly higher than in the steady-state cells. Lysis was delayed by superinfection of steady-state LBG cells as well; an increase in burst size from 100 to 200 was obtained in cells superinfected at 6 min. Superinfection at 10 min did not increase the burst size (Fig. 4).
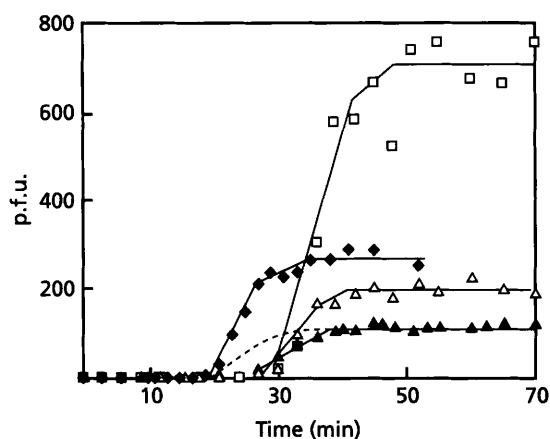
Fig. 4. Phage development in single infections of LBG-grown *E. coli* B/r H266 cells. ----, Control; △, superinfected at 6 min; ▲, superinfected at 10 min; ◆, Pn-pretreated (75 U ml⁻¹ for 1 h); □, superinfected at 10 min after pretreatment with Pn. Multiplicity of superinfection = 10. p.f.u. = C6.
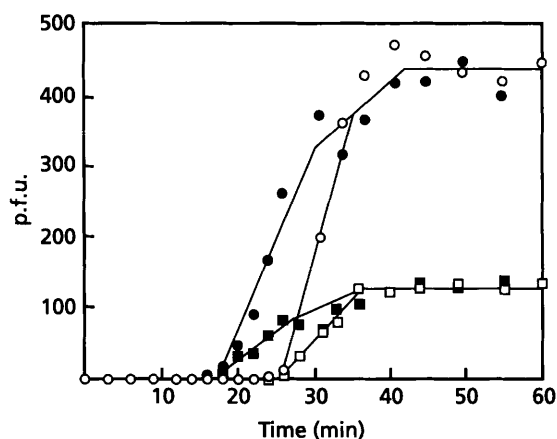
Fig. 5. Phage development in single infections of *E. coli* K-12 (CR34; *thy*) cells grown on GC augmented by 2·5 μg thymine ml⁻¹, with (□, ■) and without (○, ●) deoxyguanosine (100 μg ml⁻¹). Open symbols represent total phages (C6). Closed symbols represent mature phages in cells artificially lysed by chloroform (C5).

Host DNA

Faster-growing larger cells contain proportionally more PSS (which may enable faster phage production and larger burst size) and more DNA; however, the DNA concentration is lower (Pritchard & Zaritsky, 1970; Zaritsky & Pritchard, 1973). Does one of these parameters affect phage development? This question can be answered by slowing down chromosome replication without affecting τ, achieved by growth of *thy* mutants in limiting thymine concentrations (Pritchard & Zaritsky, 1970). Such cells are larger with more DNA but with a lower DNA to mass ratio (Zaritsky & Pritchard, 1973), similarly to faster-growing cells. The control was the same cells (*E. coli* K-12, strain CR34) grown with supplemented deoxyguanosine, simulating a Thy⁺ phenotype (Zaritsky & Woldringh, 1978). The rate of phage assembly (Fig. 5) can be accounted for by cell size alone, as in the case of Pn-treated cells, apparently due to the larger number of ribosomes (Bremer & Dennis, 1987; Ingraham *et al.*, 1983). Burst size increased from 100 to 400, in parallel with DNA content but not with DNA concentration, despite the unchanged rate of metabolism. In this experimental system (Fig. 5), the chloroform data (artificially released phages) are also displayed; they were omitted from previous graphs to simplify presentation. In each culture (not shown), the linear rate of rise in artificially released phages was similar to that in spontaneously released phages.

DISCUSSION

Most of the previous studies on bacteriophage development have been performed under optimal conditions for the host cell but these conditions may not be optimal for the phage. In nature, *E. coli* faces unfavourable growth conditions such as those prevailing in the human gut (Koch, 1971). How do they affect phage develop-

ment? This study characterizes the influences of well-defined physiological conditions on T4 growth and its interactions with the host.

Adsorption kinetics

Adsorption is the first event of the infective process. It has been divided into two stages (Goldberg *et al.*, 1994; Stent & Wollman, 1952): reversible and irreversible attachment. The rate of the irreversible step was determined in this study and found (Fig. 1) to increase with μ (inversely proportional to τ). To determine its dependence on μ, the two interacting components (phage particles and cell receptors) must be well-quantified. Under steady-state growth, cell size is correlated with μ (Woldringh *et al.*, 1977). The rate of T4 adsorption per unit surface area was calculated according to an experimental equation (Zaritsky *et al.*, 1979), derived from the known dimensions of the same substrain (H266) of *E. coli* B/r (Woldringh *et al.*, 1977), and found to be constant (Fig. 6). This result indicates that the density over cell surface area of the receptor for the irreversible stage does not change with cell dimensions, which are modified by nutritional conditions. The μ-dependence of lipopolysaccharide (LPS) density in the *E. coli* envelope (Zaritsky *et al.*, 1979) precludes the possibility that the irreversible step in T4 adsorption occurs on LPS. The constant surface density of peptidoglycan (Zaritsky *et al.*, 1979), on the other hand, suggests that this layer may be the major anchor for T4 baseplates. This implication is supported by existence of lysozyme activity in the hub of the baseplate, the product of the T4 gene *g5* (Goldberg *et al.*, 1994). It is only after perforation of the peptidoglycan layer and attachment of the tail tube to the cellular cytoplasmic membrane, which is sometimes considered as the third stage of adsorption, that phage DNA is injected.
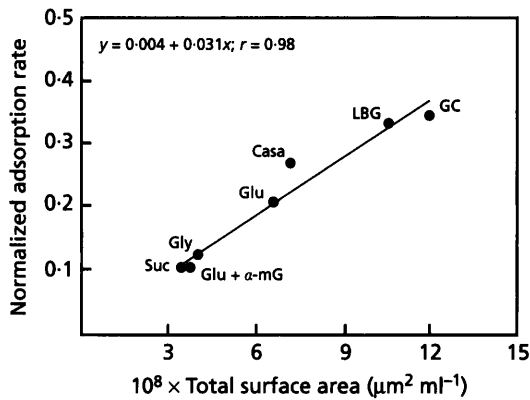
Fig. 6. Adsorption rate as a function of total surface area of infected cells in the indicated media.

Phage multiplication

The first serious attempt to relate growth parameters of a virulent phage to the state of its host was performed by Delbrück (1945), who found the distribution of burst sizes to be wider than that of cell sizes. This result was obtained under optimal conditions for *E. coli* growth. The largest burst size obtained in steady-state growing cultures (about 150 in GC; Fig. 2) is similar to values documented in the literature (e.g. Bode, 1967; Delbrück, 1945; Doermann, 1948; Eddy, 1992; Ellis & Delbrück, 1939; Karam, 1994; Mathews *et al.*, 1983). Table 1 summarizes the changes of phage growth parameters with bacterial μ under steady-state conditions, gleaned from Fig. 2 and data not shown. With a few exceptions, the rate of phage release and the burst size increased with μ while lengths of the eclipse and latent periods decreased.

Several regimes were employed to dissociate between the effects on phage development of cell size, macromolecular composition and metabolic rate; all change with μ (Bremer & Dennis, 1987; Ingraham *et al.*, 1983; Kjeldgaard *et al.*, 1958; Neidhardt *et al.*, 1987; Schaechter *et al.*, 1958; Woldringh *et al.*, 1977; Zaritsky *et al.*, 1979). Under the same nutritional conditions (Glu minimal medium), smaller cells obtained with α-mG

(Zaritsky & Helmstetter, 1992) or with the 'baby-machine' (Helmstetter, 1969) yielded fewer phages than larger cells obtained by pretreatment with low Pn concentrations (Hadas *et al.*, 1995; Fig. 3) or after 40 min growth of 'babies' (data not shown). It seems that cell size rather than metabolic rate is one of the major factors that affects T4 development. The question thus arises, what cellular parameter related to cell size determines the rate of synthesis of phage components and their assembly to mature particles? A possible role of DNA was eliminated by modifying its content and concentration using thymine limitation of a *thy* mutant (Zaritsky & Pritchard, 1973; Zaritsky & Woldringh, 1978; Fig. 5). Excluding the case of LBG, the data (Figs 2, 3; Table 1) are consistent (qualitatively, at least) with the hypothesis that the rate of phage production is proportional to the amount per cell of the PSS at the time of infection. Indeed, larger cells, either Pn-pretreated (Fig. 3) or thymine-limited (Fig. 5), which contain more PSS, produced phage quicker than untreated or unlimited cells, respectively, in rough proportion to the cellular PSS content. The increased rate of phage production resulted in larger burst sizes in the bigger cells. This hypothesis was further confirmed by phage growth kinetics, in cells shifted-up (Kjeldgaard *et al.*, 1958) upon infection, which were identical to the kinetics in unshifted cells (data not shown).

Several factors other than PSS seem to be involved simultaneously in T4 development. For example, the slower-than-expected rate of phage multiplication and the lower final burst size in the exceptional LBG case (Fig. 2; Table 1) seem to result from an inhibitory effect of the yeast extract component in this medium. This is supported by decreased burst size in GC when supplemented with yeast extract (from 130 to 50; data not shown) without any change in the other parameters.

Burst size is a function of the rate of phage synthesis, dictated by the cellular content of PSS, but is significantly affected by cell lysis time as well: delay of cell lysis, obtained by superinfection, yielded much more phage (Fig. 4). Lysis time is related to lysozyme synthesis (time and rate) and its effective concentration, which, in turn, depends on cell dimensions (volume and surface area). Increased burst size by lysis inhibition was maximized

Table 1. Parameters of T4 phage development in *E. coli* B/r cultured under steady-state growth in different media

Growth medium	τ (min)	μ (h^{-1})	Burst size (p.f.u. per cell)	Eclipse period (min)	Latent period (min)	End of lysis (min)	Slope (p.f.u. per cell min^{-1})
LBG	23	2·60	110	14	18	36	13·7
GC	28	2·14	152	20	21	33	30·4
Casa	33	1·81	63	22	24	36	10·5
Glu	50	1·20	60	26	36	56	6·0
Gly	68	0·88	27	32	35	54	3·9
Suc	96	0·62	14	35	37	58	3·5
Acet	136	0·44	12	27	35	53	1·3

by superinfection at 6 min in steady-state LBG-grown cells and at 10 min when such cells were pretreated with low Pn concentrations (Fig. 4). The rate of T4 formation was only slightly higher with superinfection than without in both cases, supporting the conclusion (above) that the total amount of cellular PSS is the major factor which determines the rate of phage maturation. Superinfection of steady-state LBG cells at 10 min did not affect burst size, indicating an intense schedule for intracellular synthesis of phage components and their assembly to mature phages.

The results indicate that the rates of phage synthesis and assembly are directly proportional to the amount of PSS per cell, but the burst size is limited by the time of lysis which, in turn, is dependent on cellular surface area. The optimal parameters are now being derived by a numeric procedure (A. Rabinovitz, H. Hadas, M. Einav, Z. Melamed & A. Zaritsky, unpublished) and will be used to analyse this hypothesis.

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REFERENCES

Adams, M. H. (1959). *Bacteriophages*. New York: Interscience Publishers.

Bode, W. (1967). Lysis inhibition in *Escherichia coli* infected with bacteriophage T4. *J Virol* 1, 948–955.

Bremer, H. & Dennis, P. P. (1987). Modulation of chemical composition and other parameters of the cell by growth rate. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 1527–1542. Edited by F. C. Neidhardt, J. L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington, DC: American Society for Microbiology.

Brown, A. (1956). A study of lysis in bacteriophage-infected *Escherichia coli*. *J Bacteriol* 71, 482–490.

Cairns, J., Stent, G. S. & Watson, J. D. (editors) (1966). *Phage and the Origins of Molecular Biology*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Delbrück, M. (1945). The burst size distribution in the growth of bacterial viruaes (bacteriophage). *J Bacteriol* 50, 131–135.

Doermann, A. H. (1948). The intracellular growth of bacteriophage. *Carnegie Inst Wash Year Book* 47, 176–182.

Eddy, S. (1992). *Introns in the T-even bacteriophages*. PhD thesis, University of Colorado, USA.

Eisenstark, A. (1967). Bacteriophage techniques. *Methods Virol* 1, 449–524.

Ellis, E. L. & Delbrück, M. (1939). The growth of bacteriophage. *J Gen Physiol* 22, 365–384.

Fishov, I., Zaritsky, A. & Grover, N. B. (1995). On microbial states of growth. *Mol Microbiol* 15, 789–794.

Goldberg, E., Grinius, L. & Letellier, L. (1994). Recognition, attachment, and injection. In *Molecular Biology of Bacteriophage T4*, pp. 347–356. Edited by J. D. Karam. Washington, DC: American Society for Microbiology.

Hadas, H., Einav, M., Fishov, I. & Zaritsky, A. (1994). Dependence of T4 bacteriophage development on the physiology of its host *Escherichia coli*. EMBO Workshop *The Bacterial Cell Cycle*, pp. 142–144. Sandhamn, Sweden: EMBO.

Hadas, H., Einav, M., Fishov, I. & Zaritsky, A. (1995). Division-inhibition capacity of penicillin in *Escherichia coli* is growth-rate dependent. *Microbiology* 141, 1081–1083.

Helmstetter, C. E. (1969). Methods for studying the microbial division cycle. *Methods Microbiol* 1, 327–364.

Ingraham, J. L., Maaløe, O. & Neidhardt, F. C. (1983). Growth rate as a variable. In *Growth of the Bacterial Cell*, pp. 267–315. Sunderland, MA: Sinauer Associates.

Karam, J. D. (editor-in-chief) (1994). *Molecular Biology of Bacteriophage T4*. Washington, DC: American Society for Microbiology.

Kjeldgaard, N. O., Maaløe, O. & Schaechter, M. (1958). The transition between different physiological states during balanced growth of *Salmonella typhimurium*. *J Gen Microbiol* 19, 607–616.

Koch, A. L. (1971). The adaptive responses of *Escherichia coli* to a feast and famine existence. *Adv Microb Physiol* 6, 147–217.

Kutter, E., Kellenberger, E., Carlson, K., Eddy, S., Neitzel, J., Messinger, L., North, J. & Guttman, B. (1994). Effects of bacterial growth conditions and physiology on T4 infection. In *Molecular Biology of Bacteriophage T4*, pp. 406–418. Edited by J. D. Karam. Washington, DC: American Society for Microbiology.

Mathews, C. K., Kutter, E. M., Mosig, G. & Berget, P. B. (editors) (1983). *Bacteriophage T4*. Washington, DC: American Society for Microbiology.

Neidhardt, F. C., Ingraham, J. L., Brooks Low, K., Magasanik, B., Schaechter, M. & Umbarger, H. E. (editors) (1987). *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*. Washington, DC: American Society for Microbiology.

Pritchard, R. H. & Zaritsky, A. (1970). Effect of thymine concentration on the replication velocity of DNA in a thymineless mutant of *Escherichia coli*. *Nature* 226, 126–131.

Schaechter, M., Maaløe, O. & Kjeldgaard, N. O. (1958). Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. *J Gen Microbiol* 19, 592–606.

Stent, G. S. (1963). *Molecular Biology of Bacterial Viruses*. San Francisco: W. H. Freeman.

Stent, G. S. & Wollman, E. L. (1952). On the two step nature of bacteriophage adsorption. *Biochim Biophys Acta* 8, 260–269.

Woldringh, C. L., de Jong, M. A., van den Berg, W. & Koppes, L. (1977). Morphological analysis of the division cycle of two *Escherichia coli* substrains during slow growth. *J Bacteriol* 131, 270–279.

Woldringh, C. L., Huls, P. G. & Vischer, N. O. (1993). Volume growth of daughter and parent cells during the cell cycle of *Saccharomyces cerevisiae* a/α as determined by image cytometry. *J Bacteriol* 175, 3174–3181.

Zaritsky, A. & Helmstetter, C. E. (1992). Rate maintenance of cell division in *Escherichia coli* B/r: analysis of a simple nutritional shift-down. *J Bacteriol* **174**, 8152–8155.

Zaritsky, A. & Pritchard, R. H. (1973). Changes in cell size and shape associated with changes in the replication time of the chromosome of *Escherichia coli*. *J Bacteriol* **114**, 824–837.

Zaritsky, A. & Woldringh, C. L. (1978). Chromosome replication rate and cell shape in *Escherichia coli*: lack of coupling. *J Bacteriol* **135**, 581–587.

Zaritsky, A., Woldringh, C. L. & Mirelman, D. (1979). Constant peptidoglycan density in the sacculus of *Escherichia coli* B/r growing at different rates. *FEBS Lett* **98**, 29–32.

Zaritsky, A., Woldringh, C. L., Helmstetter, C. E. & Grover, N. B. (1993). Dimensional rearrangement of *Escherichia coli* B/r cells during a nutritional shift-down. *J Gen Microbiol* **139**, 2711–2714.