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Bacteriophage T4 Development in *Escherichia coli* is Growth Rate Dependent

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Three independent parameters (eclipse and latent periods, and rate of ripening during the rise period) are essential and sufficient to describe bacteriophage development in its bacterial host. A general model to describe the classical "one-step growth" experiment [Rabinovitch *et al.* (1999a) *J. Bacteriol.* **181,** 1687–1683] allowed their calculations from experimental results obtained with T4 in *Escherichia coli* B/r under different growth conditions [Hadas *et al.* (1997) *Microbiology* **143,** 179–185]. It is found that all three parameters could be described by their dependence solely on the culture doubling time τ before infection. Their functional dependence on τ, derived by a best-fit analysis, was used to calculate burst size values. The latter agree well with the experimental results. The dependence of the derived parameters on growth conditions can be used to predict phage development under other experimental manipulations.

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Development of T4 bacteriophages inside an *Escherichia coli* B/r cell under varying well-defined physiological states of the host (Fishov *et al.*, 1995) upon infection has lately been described in a series of "one-step growth" experiments (Hadas *et al.*, 1997). In such an experiment, a phage that infects a bacterium starts to multiply after an eclipse period (v). The rate at which new phages are created (α) is considered to be a constant during the period between the eclipse and the latent (or lysis time, μ) leading to a burst size (number of released phages, B) (Rabinovitch *et al.*, 1999a). The first three independent parameters are sufficient to satisfactorily derive the fourth, $B = \alpha(\mu - v)$. To obtain well-defined quantitative values, they were rigorously defined and a complete time dependence of the process was calculated, with due care taken of the statistical distributions within populations of both phages and bacteria of the time parameters.

It is demonstrated here that all three "operating" parameters are related to just one variable of the bacterium, namely the doubling time τ of the culture. Results of various experiments (Hadas *et al.*, 1997; Rabinovitch *et al.*, 1999a and unpublished data) were analysed by this model; the ensuing "experimental" values of the eclipse and latent periods were plotted against τ for a wide range (23–136 min) (Fig. 1), and values of phage assembly rate were plotted (Fig. 2) against its reciprocal $1/\tau$ (i.e. bacterial growth rate). Since the accuracy in fitting a

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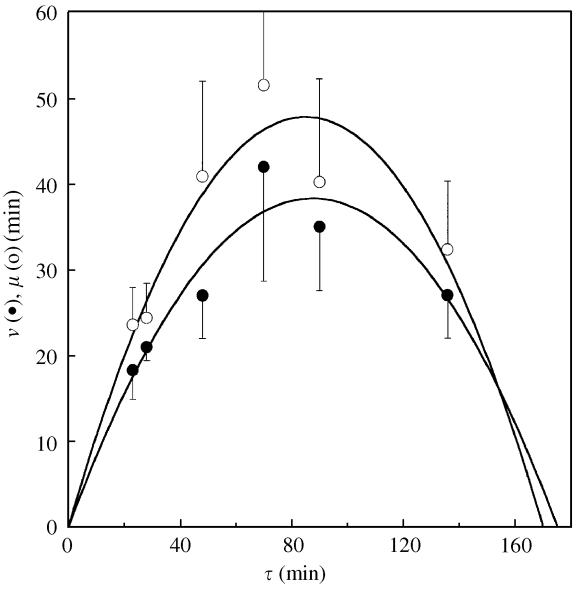

FIG. 1. Eclipse ν and latent μ periods as functions of the culture doubling time τ. The lines represent theoretical fits according to eqn (1) (see text and Fig. 3).

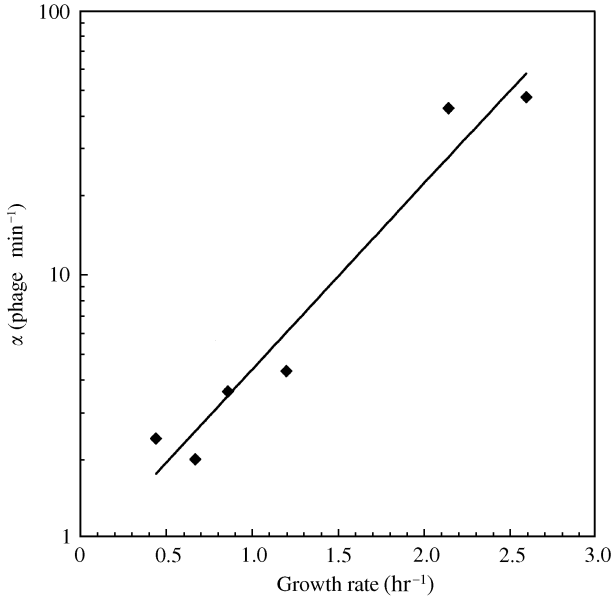

FIG. 2. Rate of intracellular phage development during the rise period (α) as a function of bacterial growth rate ($1/\tau$). (——) curve—from eqn (1). The line represents the best linear fit obtained by standard function of KaleidaGraph (Abelbeck Software).

straight line (regression) is higher than that of a parabolic curve, μ/τ and ν/τ were fitted rather than μ and ν themselves (Fig. 3). Both ν and μ have been assumed (Rabinovitch *et al.*, 1999a) to

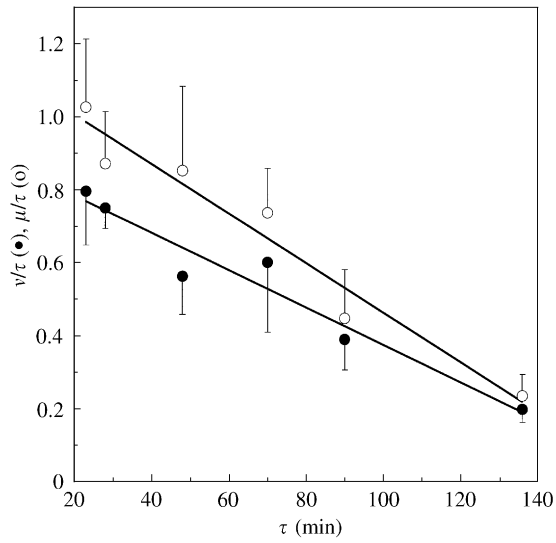

FIG. 3. Relative values of ν/τ and μ/τ vs. τ (regression). (——) represents values calculated by eqn (1). The lines represent best linear fits obtained by a standard function of KaleidaGraph (Abelbeck Software).

have Gaussian distributions with standard deviations σ and β, respectively; the values of these latter parameters were therefore used to estimate their standard errors (bars in Figs. 1 and 3).

From the figures, the following relations were obtained:

$$\mu = 1.1407\tau - 0.006778\tau^2 \quad (R \sim 0.97),$$

$$\nu = 0.8865\tau - 0.005122\tau^2 \quad (R \sim 0.98),$$

$$\alpha = \exp(92.1/\tau) \quad (R \sim 0.93). \tag{1}$$

The numerical values of eqn (1) were used to derive the functional relationship between the burst size B and τ:

$$B = \alpha(\mu - \nu) = (0.254\tau - 0.00166\tau^2)\exp(92.1/\tau). \tag{2}$$

Figure 4 depicts B as a function of both τ [Fig. 4(a)] and the growth rate ($1/\tau$) [Fig. 4(b)] of the bacterium. As is seen, the agreement with the experimental results is quite good.

Several points should be noted:

1. μ/τ and ν/τ are monotonous in τ, while μ and ν themselves are highly peaked around $\tau =$ 80 min.

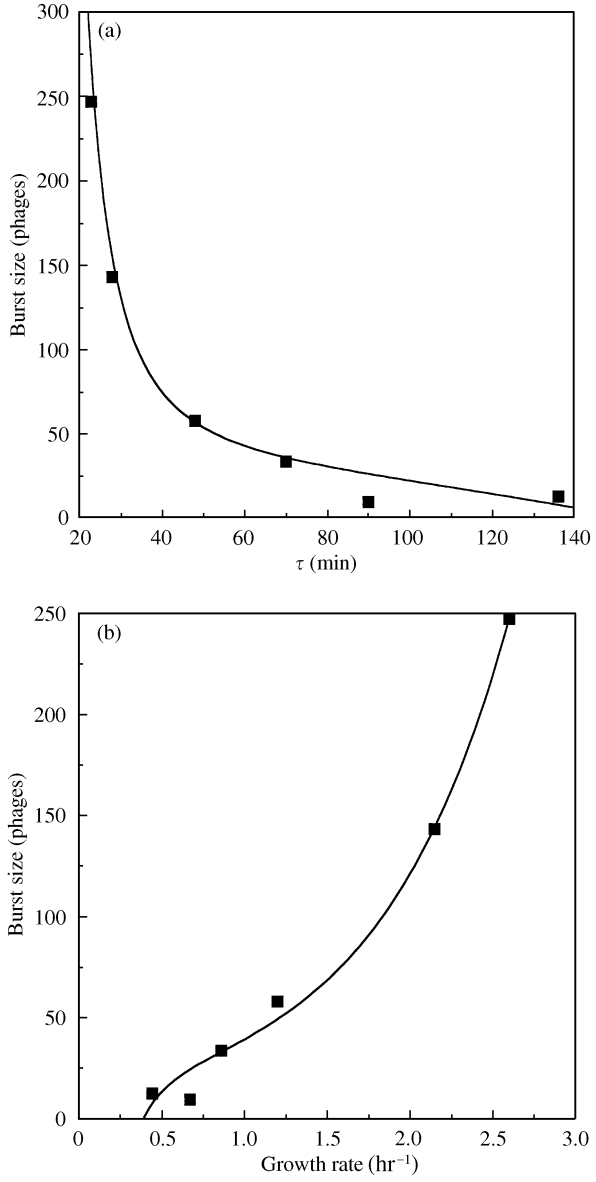

FIG. 4. Burst size B as a function of τ (a) and $1/\tau$ (b): (■)—"experimental" values obtained in Hadas *et al.* (1997); (◡)—calculated values from eqn (4).

2. Around τ of 160 min, $\mu = \nu$, thus $(\mu - \nu)$ together with B go to zero. Hence, it would be interesting to find out whether any bacterium can release phages when its doubling time is longer than 160 min. The small number (six) of experiments does not exclude the possibility that both μ and ν level off for $\tau > 100$ min. To distinguish between the two possibilities, chemostat-growing bacterial cultures with $\tau > 160$ min should be exploited.

3. For very short bacterial doubling times, $\tau < 20$ min, $(\mu - \nu) \to 0$. In this range, due to the enormously high phage production rates α, a very short $(\mu - \nu)$ can produce a large burst size B.

4. The increase of the eclipse time ν with τ for $20 < \tau < 80$ is partly due to the decrease in the rate of synthesis of the new materials needed for phage, a rate that is dependent on the bacterial metabolism reflected by $1/\tau$. Against this, however, is the decrease of the eclipse time ν for $\tau > 80$, which implies that metabolism is not a limiting factor in the synthesis of phage materials. In other words, if the concentration of ribosomes, etc. (see below) is limiting for $\tau < 80$, it should be limiting for $\tau > 80$. It is not. This implies that there is another reason. It is perhaps advantageous for the phages to wait for a longer period than necessary inside the bacterium in this situation ($\tau \sim 80$ min) so that μ could also increase (the mechanism of increase of μ seems to be similar to that of ν) such that $(\mu - \nu)$ would increase.

5. The relationship between the rate α of phage maturation and the culture growth rate $1/\tau$ is satisfactory (Fig. 2). The latter is known to correlate closely with the *percentage* of cell mass devoted to the protein synthesizing system (PSS) under steady-state exponential growth conditions (Bremer & Dennis, 1996). It should be noted that, when cell size (hence, PSS *content*) was extended by specific inhibition or delay of the division process by low penicillin concentrations (Hadas *et al.*, 1995) or thymine limitation of *thyA* mutants (Zaritsky & Pritchard, 1973), α, and consequently B, are higher (Figs 3 and 5, respectively, in Hadas *et al.*, 1997). Similarly, manipulating the cell to delay its lysis by super-infection (Bode, 1967; Abedon, 1999) that increases μ and hence $(\mu - \nu)$, results in a significant rise of the burst size (Fig. 4 in Hadas *et al.*, 1997).

6. The number (six) of experiments is small because reproducible experiments are not easy to perform. All statistical inferences are therefore lacking. On the other hand, the good agreement between the calculated and the experimental B values lends support to the hypothesis.

The results reported here and elsewhere (Hadas *et al.*, 1997) clearly indicate that burst

size of bacteriophage T4 is not limited by cell size or DNA composition, nor directly by the rate of metabolism, but rather by the rates of synthesis/assembly of phage components, and by lysis time. The rates of synthesis and assembly of phage components seem to depend, in turn, on the contents of the PSS, while lysis time depends on cell dimensions (Rabinovitch *et al*., 1999b).

The recent finding, that burst size of the temperate bacteriophage of *E. coli*, λ, is larger in faster growing host cells (Gabig *et al*., 1998), extends the validity of our conclusions to a wider range of phage–host interacting systems.

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