LINK TO ORIGINAL ARTICLE

cAMP does not have an important role in carbon catabolite repression of the *Escherichia coli lac* operon

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In a recent exchange of correspondences on the review article by Görke and Stülke (Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. *Nature Rev. Microbiol.* **6**, 613–624 (2008))[1], the authors make different conclusions from almost identical data[2,3]. Specifically, Görke and Stülke[3] note that because diauxic growth was abolished in *lac*-constitutive mutants, "the cAMP–CRP system is not directly involved" in the carbon catabolite repression of the *lac* operon. By contrast, Crasnier-Mednansky[2] states that "constitutive β-galactosidase synthesis (which does not require the inducer for synthesis) was repressed by glucose in the absence of cAMP[4] and that the rate of β-galactosidase synthesis in fully induced cells growing on glucose was less than in cells growing on less preferred carbon sources. Both observations indicate that glucose transport by the phosphotransferase system (PTS) affect β-galactosidase by reducing the cAMP level[5]." Here, I show that the contradiction arises because the authors implicitly assume that only two mechanisms affect *lac* expression: cAMP-mediated repression and inducer exclusion. The contradiction is clarified if the data are analysed by taking account of enzyme dilution. This analysis shows that the repression of fully induced or *lac*-constitutive cells noted by Crasnier-Mednansky exists, but is almost entirely due to dilution rather than cAMP-mediated repression. Consequently, the conclusion of Görke and Stülke[1] remains valid: cAMP does not play an important part in the glucose–lactose diauxie.

FIGURE 1 shows the data obtained during exponential growth of fully induced or *lac*-constitutive cells of *Escherichia coli* on various carbon sources. The β-galactosidase activity during growth on glucose is certainly smaller than the activities observed during growth on less-preferred carbon sources, such as glycerol and succinate. However, this does not imply that the rate of β-galactosidase synthesis varies substantially with the carbon source. Indeed, the mass balance for β-galactosidase is provided by Equation 1, in which e is the β-galactosidase activity (units per mg protein), μ is the specific growth rate (per hour) and r is the β-galactosidase synthesis rate (units per hour per mg protein).

$$\frac{de}{dt} = r - \mu e \qquad (1)$$

Because the β-galactosidase activity of exponentially growing cells is at steady state, the corresponding β-galactosidase synthesis rate is derived from Equation 2.

$$r = \mu e \qquad (2)$$

The curves in FIG. 1 show that to a first approximation, the β-galactosidase activity of exponentially growing cells is inversely proportional to the specific growth rate. Thus, the β-galactosidase synthesis rate, r, is essentially constant regardless of the carbon source.

Although r is independent of the carbon source, the intracellular cAMP level changes significantly with the carbon source. It follows that r is essentially independent of the intracellular cAMP level. This is probably because the intracellular cAMP is already at near-saturating levels in carbon-limited cultures, as the addition of 5 mM cAMP to the carbon-limited cultures in FIG. 1a increases the β-galactosidase activity less than twofold. This small change cannot account for the several 100-fold difference of the β-galactosidase activities during the first and second exponential growth phases of the glucose–lactose diauxie[6].

The weak effect of cAMP in carbon-limited cultures does not imply that cAMP has no affect whatsoever on *lac* expression. In nitrogen-limited cultures, the intracellular cAMP levels are much smaller than those observed in carbon-limited cultures[7,8]. The addition of 2–5 mM cAMP to such cultures increases the β-galactosidase activity by 40–50-fold[4,7].

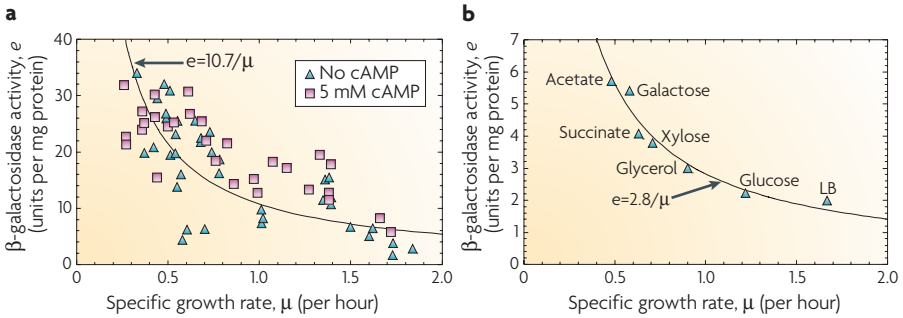

Figure 1 | **Variation of the steady-state β-galactosidase activity with the specific growth rate.** **a** | The β-galactosidase activity observed during exponential growth of fully induced or *lac*-constitutive *Escherichia coli* NC3 on various carbon sources is inversely proportional to the specific growth rate[9]. The curve shows the best fit to the data obtained when no cAMP is added to the medium. The addition of 5 mM cAMP to the medium increases the β-galactosidase activities less than twofold. One unit of β-galactosidase refers to the number of micromoles of ortho-nitrophenol-β-galactoside (ONPG) hydrolyzed per minute. **b** | The same inverse relationship is also observed during exponential growth of the *lac*-constitutive strain *E. coli* MC4100 λCPT100 on various carbon sources[10] and Luria–Bertani (LB) medium.

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