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Is there any role for cAMP–CRP in carbon catabolite repression of the *Escherichia coli lac* operon? Reply from Görke and Stülke

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We are appreciative of the correspondence on our Review article (Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. *Nature Rev. Microbiol.* **6**, 613–624 (2008))[1], by Crasnier-Mednansky (Is there any role for cAMP–CRP in carbon catabolite repression of the *Escherichia coli lac* operon? *Nature Rev. Microbiol.* 20 Oct 2008 (doi:10.1038/nrmicro1932-c1))[2] . When discussing carbon catabolite repression (CCR) in *E. coli*, we must take three different factors into account. First, there is no doubt that cAMP–CRP is essential for the expression of most catabolic genes, including the *lac* operon. It is the actual regulatory role of this complex that is important. Second, different operon-specific mechanisms contribute to CCR, but these mechanisms and the levels of their implication differ from system to system. And third, because of these two factors, we need to study each catabolic system individually to understand how CCR is exerted.

The traditional view, as supported by Crasnier-Mednansky, places cAMP–CRP at the centre of CCR. This view is plausible, as it links glucose availability, the phosphorylation state of $EIIA^{Crr}$ and the activity of adenylate cyclase to the transcription activation of catabolic genes. In addition, inducer exclusion was considered to be an auxiliary mechanism. Unfortunately, only the cAMP model found its way into the textbooks. In our opinion, the classic model was most brilliantly and convincingly described in a review by M. H. Saier Jr[3].

More recent work showed, however, that we need to re-adjust our view on CCR in *E. coli*. Several impressive studies from the laboratory of Hiroji Aiba provided compelling evidence that inducer exclusion is the main factor that determines CCR of the *E. coli lac* operon and thus the glucose–lactose diauxie. This conclusion is supported by a systematic analysis of the components of CCR and the mathematical modelling of CCR. This model also predicts that CCR of the *E. coli lac* operon results mainly from inducer exclusion. However, it is important to note that the same model predicts a major role for cAMP–CRP for CCR of glycerol utilization genes[4]. This observation is crucial, as it shows that it is imperative to look at different catabolic systems individually.

The first important result is related to the cAMP levels. Previously, it was thought and generally accepted that cAMP concentrations are low in the presence of glucose and high in its absence. However, both the studies by Inada *et al.*[5] and Bettenbrock *et al.*[6] show that the cAMP levels during growth with glucose and lactose are similar. There is a transient increase of cAMP after the exhaustion of glucose and the concomitant transition from glucose to lactose use. Again, it is important to look at each condition individually. If *E. coli* grows with glycerol, maltose or succinate, the cAMP pool is significantly increased compared with glucose- or lactose-growing cells[6]. Because the determination of intracellular cAMP levels involves many steps and may give rise to artefacts, Bettenbrock *et al.* used an elegant approach to circumvent this problem: they used a reporter construct that depends exclusively on the cAMP–CRP system but not on any other regulator. As expected, expression of the reporter was indistinguishable (low) in the presence of glucose or lactose. By contrast, increased expression was observed during growth with those carbon sources that give rise to higher cAMP levels, such as glycerol, maltose and succinate[6]. Taken together, the cAMP levels cannot be the cause for the CCR of the lactose operon, but they may play a key part in CCR of other sugars (for example, glycerol or maltose).

E. coli strains that express a cAMP-independent CRP variant (CRP*) and lack adenylate cyclase still exhibit CCR of the *lac* operon, and the glucose–lactose diauxie is indistinguishable from the wild type. This is a second important observation that is even strengthened by the determination of CRP* pools, which are constant throughout the diauxic life cycle[5]. This finding shows that mechanisms other than cAMP–CRP are sufficient to exert CCR of the *lac* operon.

A third simple experiment identifies this mechanism: if the Lac repressor gene (*lacI*) is inactivated by a mutation or by induction with isopropyl-β-D-thiogalactopyranoside (IPTG) (which does not depend on the lactose permease for transport), expression of the *lac* operon becomes independent of the availability of any external inducer. However, the cAMP–CRP system is still operative in such a mutant. Inada *et al.* determined β-galactosidase synthesis and growth behaviour of such a *lacI* mutant. Interestingly, expression of the *lac* operon was constitutive even in the presence of glucose, and diauxic growth was abolished[5]. This unambiguously shows that the operon-specific induction mechanism is the key player in CCR of the *lac* operon, whereas the cAMP–CRP system is not directly involved.

Finally, as mentioned by Crasnier-Mednansky, the addition of exogenous cAMP to cultures of *E. coli* abolishes the glucose–lactose diauxie. This is a suggestive observation. However, the abolition of diauxie is not paralleled by an abolition of glucose repression of *lacZ* expression[5]. This obvious contradiction can be explained by an increased basal level of the lactose operon proteins (including lactose permease) after the addition of cAMP. This would lead to an enhanced uptake of lactose and eliminate the lag phase between the two growth phases.

Now we must face the question: why is the traditional model of CCR so suggestive and successful that it seems difficult to replace it by a more balanced model. First, this model is based on experimental observations that suggest a stringent logic. Unfortunately, most experiments that established this model used glycerol or succinate as non-repressing carbon sources and IPTG as the inducer for the operon. As mentioned above, the cAMP concentration is indeed significantly increased when *E. coli* uses these two carbon sources, but not with lactose. Another suggestive observation is the inability of *E. coli* mutants that lack cAMP or CRP to grow with lactose as the only carbon source. This clearly suggests that cAMP–CRP is essential for expression of the *lac* operon, and indeed it is, but it is not directly involved in CCR or in the glucose–lactose diauxie! Kimata and colleagues have shown that control of the glucose permease gene, *ptsG*, by cAMP–CRP gives rise to an indirect involvement of cAMP–CRP in glucose repression of the *lac* operon[7].

In conclusion, the relative roles of cAMP–CRP and operon-specific regulatory systems differ from operon to operon. In the case of

the paradigm of catabolite repression, the *E. coli lac* operon, inducer exclusion is the major player in CCR, whereas cAMP–CRP has an indirect regulatory role.

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