lac operon induction in *Escherichia coli*: Systematic comparison of IPTG and TMG induction and influence of the transacetylase LacA

Anja Marbach, Katja Bettenbrock *

Max-Planck-Institute for Dynamics of Complex Technical Systems, Sandtorstr. 1, 39106 Magdeburg, Germany

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ABSTRACT

Most commonly used expression systems in bacteria are based on the *Escherichia coli lac* promoter. Furthermore, *lac* operon elements are used today in systems and synthetic biology. In the majority of the cases the gratuitous inducers IPTG or TMG are used. Here we report a systematic comparison of *lac* promoter induction by TMG and IPTG which focuses on the aspects inducer uptake, population heterogeneity and a potential influence of the transacetylase, LacA. We provide induction curves in *E. coli* LJ110 and in isogenic *lacY* and *lacA* mutant strains and we show that both inducers are substrates of the lactose permease at low inducer concentrations but can also enter cells independently of lactose permease if present at higher concentrations. Using a *gfp* reporter strain we compared TMG and IPTG induction at single cell level and showed that bimodal induction with IPTG occurred at approximately ten-fold lower concentrations than with TMG. Furthermore, we observed that *lac* operon induction is influenced by the transacetylase, LacA. By comparing two *Plac-gfp* reporter strains with and without a *lacA* deletion we could show that in the *lacA*+ strain the fluorescence level decreased after few hours while the fluorescence further increased in the *lacA*− strain. The results indicate that through the activity of LacA the IPTG concentration can be reduced below an inducing threshold concentration—an influence that should be considered if low inducer amounts are used.

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1. Introduction

Based on the detailed knowledge about the *Escherichia coli lac* operon and the easy controllability by chemical inducers, *lac* promoter driven expression systems are favored in prokaryotes (Donovan et al., 1996; Martin et al., 2008; Olaofe et al., 2010; Xie et al., 2003). Moreover, the *lac* regulation system is a subject of systems biological studies (Dreisigmeyer et al., 2008; Mettetal et al., 2006; Ozbudak et al., 2004) and serves today as a tool in synthetic biology (Kaern et al., 2003). The *lac* operon comprises the genes *lacZ*, *Y* and *A*. *lacZ* encodes the β-galactosidase, which cleaves lactose into glucose and galactose and is responsible for the production of allolactose, the natural inducer of the *lac* operon. *lacY* encodes the lactose permease responsible for lactose uptake (as a review see Adhya, 1996). The last gene of the operon, *lacA*, encodes a transacetylase that seems to have a function in detoxification (Roderick, 2005). The transcription of the *lac* operon is dependent on repression by the lactose repressor LacI, activation by cAMP-CRP and inducer exclusion by the PEP-dependent phosphotransferase system (Deutscher et al., 2006).

The most commonly used inducers in molecular biology and biotechnology are IPTG and TMG. These thio-galactosides inhibit the action of the lactose repressor LacI. Although induction kinetics with respect to IPTG and TMG are available (Boezi and Cowie, 1961; Herzenberg, 1959; Maloney and Rotman, 1973) there is a growing demand for experimental data concerning *lac* regulation. Previous work was mostly targeted towards the understanding of the design of the system. Results were obtained from a variety of strains, most of them not used anymore and in the majority of the cases derived from UV or spontaneous mutagenesis. Because *lac* operon elements are used today as tools for cloning (Makrides, 1996; Terpe, 2006), construction of biological switches (Gardner et al., 2000) or modeling approaches (Mettetal et al., 2006; Ozbudak et al., 2004) a more detailed knowledge about induction behavior is required. For this data are still lacking or have not been compiled in a systematic way. For example, the transport of IPTG across the bacterial membrane is not sufficiently characterized (Dreisigmeyer et al., 2008). Beckwith (1987) and Hansen et al. (1998) reported that IPTG can pass the cell membrane independently of the lactose permease but Jensen et al. (1993) showed that IPTG is actively transported by LacY. The entry of IPTG into the cell is an important factor in modeling studies. Furthermore, a lot of publications deal with the optimal inducer

* Corresponding author. Tel.: +49 391 6110249; fax: +49 391 6110510.
E-mail address: bettenbrock@mpi-magdeburg.mpg.de (K. Bettenbrock).

and the inducer concentration for heterologous gene expression (Donovan et al., 1996; Olaofe et al., 2010; Xie et al., 2003). This is certainly influenced by the ability of the inducer to penetrate the cells and by toxic effects that can occur in dependence of the inducer concentration, something which has not been thoroughly investigated so far.

Another less characterized element of *lac* operon induction is the transacetylase LacA. It is known that IPTG can be acetylated by LacA (Herzenberg, 1961) but it is not reported if this enzyme activity has an influence on *lac* operon induction. As a result, LacA is not considered in probably all works with *lac* operon elements. A potential action of the transacetylase could decrease the inducer concentration and therefore change the course of the experiment especially if small amounts of inducer are used. A further important aspect of *lac* based expression systems is cell heterogeneity. It was shown that under certain conditions the *lac* operon exhibits bimodal induction behavior (Maloney and Rotman, 1973; Mettetal et al., 2006; Novick and Weiner, 1957; Ozbudak et al., 2004). The reason for this is a positive feedback loop. In the absence of inducer the operon is very weakly transcribed and either no or only few permease molecules are built. At subsaturating inducer concentrations cells which possess by chance some permease molecules will induce *lac* operon transcription whereas those having no lactose permease will remain uninduced. Most studies about bimodality dealt with TMG induction. Information about bimodal induction by using IPTG is less abundant and furthermore inconsistent. Maloney and Rotman (1973) reported about bimodal induction with IPTG using a fluorogenic assay but they did not compare these data to TMG induction. Ozbudak et al. (2004) used a *Plac-gfp* reporter strain to observe single cell behavior. They focused on TMG induction but also reported that the fluorescence level of the uninduced cells at subsaturating IPTG concentrations was higher than with TMG and explained this by influx of IPTG independently of LacY. Unfortunately they did not mention the IPTG concentrations tested. Khlebnikov and Keasling (2002) reported that they did not observe bimodality with IPTG by using a *Plac-gfp* fusion on a plasmid. Detailed knowledge about the potential of the system to evoke bistability is necessary for example to tune the expression of a gene to a certain level. Western-blot analysis will only reflect the population-averaged expression but the amount of the protein in individual cells could be different. Because no study exists which compares the characteristics of both IPTG and TMG under the aspects of cell entry, heterogeneity and a potential influence of LacA, we conducted experiments in a systematic approach. We compared the induction behavior between both inducers and could show by using a *lacY* deletion strain that TMG as well as IPTG are mainly transported via the lactose permease at low inducer concentrations but can also enter the cell independently of lactose permease at higher concentrations. By using a *gfp* reporter strain we showed that both inducers evoke similar bimodal induction behavior but at different concentrations and that this bimodal behavior changes into a unimodal response if the lactose permease is lacking. Furthermore, we demonstrated that in dependence of the cell density the action of the transacetylase can be high enough to reduce the external IPTG concentration and therefore to reduce the expression level of *lac* promoter controlled genes.

2. Materials and methods

2.1. Bacterial strains

E. coli strains used in this study are listed in Table 1. *E. coli* LJ110 (Zeppenfeld et al., 2000) was used as parental strain. The appropriate *lacY* and *lacA* deletion mutants were constructed as described by Datsenko and Wanner (2000) using plasmid pKD13. The primers

Table 1
Strains used in this study.

Strain	Relevant genotype	Reference
LJ110	W3110, F⁻, Fnr⁺	Zeppenfeld et al. (2000)
AM1	LJ110 attΦ80::pAM7	This study
AM2	LJ110 ΔlacY	This study
AM4	LJ110 ΔlacY attΦ80::pAM7	This study
AM5	LJ110 ΔlacA attΦ80::pAM7	This study

used were designed in accordance to the Keio collection (Baba et al., 2006) and are listed in Table 2. The kanamycin resistance cassettes were removed as described (Datsenko and Wanner, 2000) and the remaining scar sequences were confirmed by sequencing.

To detect *lac* operon expression at single cell level a translational fusion of the *lac* promoter and *gfp* was constructed and integrated into the *E. coli* chromosome. The *lac* promoter sequence was PCR amplified with primers 5 and 6 (Table 2) using chromosomal DNA of *E. coli* LJ110 as template. The GFPmut3.1 encoding fragment was PCR amplified from plasmid pGFPmut3.1 (Clontech) with primers 7 and 8 (Table 2). The two PCR fragments were digested with *Kpn*I, ligated with T4-DNA ligase and reamplified with the outside lying primers 5 and 8. The resulting 1.4 kb fragment was digested with *Bam*HI and *Eco*RI and integrated into pAH162 (Haldimann and Wanner, 2001). The obtained plasmid pAM7 was verified by sequencing and integrated into the genome of LJ110 and in isogenic *lacY* and *lacA* deletion strains as described by Haldimann and Wanner (2001).

2.2. Media and growth conditions

For β-galactosidase analysis cells were first grown in LB medium (10 g/l Trypton, 5 g/l yeast extract, 5 g/l NaCl) for 3–4 h until the solution became turbid. Afterwards the cell suspension was diluted 1:100 in minimal medium (Tanaka et al., 1967) with 0.2% L-arabinose (w/v) as carbon source and incubated overnight. This preculture was washed once with minimal medium without carbon source and transferred into fresh minimal medium containing 0.2% L-arabinose (w/v) to an optical density at 420 nm (OD$_{420}$) of 0.1 corresponding to about 5×10^7 cells per ml. TMG or IPTG respectively was added when cell growth reached the exponential phase. Cultivation for GFP expression analysis was conducted as described but 0.2% succinate was used as carbon source and the starting OD$_{420}$ of the batch cultures was 0.03 corresponding to about 1.5×10^7 cells per ml.

2.3. β-Galactosidase assay

β-Galactosidase activity was determined in accordance to Miller (1992) with toluenized cells. To stop β-galactosidase synthesis during sampling and centrifugation, chloramphenicol was added to

Table 2
Primers used in this study.

Nr	Sequence (5′–3′)	Application
1	TGTCTGCCCG TATTTCGCGT AAGGAAATCC ATTATGATTC CGGGGATCCG TCGACC	*lacY* deletion
2	TCCGACATTG ATTGCTTAAG CGACTTCATT CACCTGTGTA GGCTGGAGCT GCTTCG	*lacY* deletion
3	ATCCGACCAA CATATCATAA CGGAGTGATC GCATTGATTC CGGGGATCCG TCGACC	*lacA* deletion
4	GGCAATTTTT ATAATTTAAA CTGACGATTC AACTTTTGTA GGCTGGAGCT GCTTCG	*lacA* deletion
5	TATGGATCCC CAATACGCAA ACCGCCTCT	P*lac* amplification
6	TATGGTACCG TCTGGCCTTC CTGTAGCCA	P*lac* amplification
7	TATGGTACCT AGAATTAAAG AGGAG	*gfp* amplification
8	TATGAATTCA CGGCCGACTA GTAGGTCAG	*gfp* amplification

a final concentration of 25 µg/ml. Subsequently, cells were harvested by centrifugation and the cell pellet was resuspended in 50 mM phosphate buffer (34 mM Na_2HPO_4, 16 mM NaH_2PO_4, pH 7.2). The OD was measured at 650 nm and aliquots were frozen at $-20\,°C$ until use. To determine the β-galactosidase activity, samples were thawed and 10 µl of toluene was added to 500 µl of the sample. After incubation for 5 min in a thermomixer at 37 °C and 1000 rpm the reaction was started by adding 25 µl of 20 mM o-nitrophenyl-β-D-galactopyranoside (ONPG). The reaction was stopped by the addition of 750 µl 0.2 M Na_2CO_3 as soon as the sample became yellow. Cell debris was removed by centrifugation and the intensity of the coloration was determined by measuring the absorbance at 420 nm. The β-galactosidase activity was calculated by the following equation: β-galactosidase activity (U per mg protein) $= (OD_{420\,nm} \times V_A)/(\varepsilon \times d \times t \times A_P)$, with $OD_{420\,nm}$ as the absorbance at 420 nm, V_A as the reaction volume, ε as the extinction factor of o-nitrophenol at 420 nm (4500/M cm), d as the length of the light path, t as the reaction time and A_P as the amount of protein used. The amount of protein was estimated by the absorbance at 650 nm (1 OD at 650 nm corresponds to 0.25 mg/ml protein).

2.4. Fluorescence microscopy

For single cell analysis 2.5×10^7 to 5×10^7 cells were harvested by centrifugation and resuspended in 35 µl minimal medium with 25 µg/ml chloramphenicol to stop protein synthesis. The samples were applied to microscope slides coated with 1% agarose and fluorescence microscopy was performed with an AxioImager M1 microscope equipped with an AxioCam MRm CCD Camera (Zeiss). Monochrome photographs of 600 cells on average were taken in the fluorescence channel (excitation: BP 470/40, beamsplitter: FT 495, emission: BP 525/50) and with phase contrast. Cell boundaries were determined from the phase contrast images by using Axiovision software of Zeiss. The mask generated was used for measuring GFP fluorescence in the fluorescence image. Fluorescence values were determined as the average fluorescence of the whole cell in arbitrary units. After subtracting the average background fluorescence of regions without cells fluorescence values were normalized. For normalization only fluorescence values of one experimental run were compared. The average fluorescence value of the induced cells at the latest point of induction was calculated, set to 100% and used as reference for all other measurements of the same day.

3. Results

3.1. Comparison of IPTG and TMG induction in LJ110 and in a lacY deletion strain

To characterize *lac* operon expression with the gratuitous inducers TMG and IPTG, different concentrations were added to exponentially growing cultures and β-galactosidase activity was determined before and after induction. Fig. 1 shows that the effect of both inducers is comparable. At subsaturating inducer concentrations it took a significant amount of time before β-galactosidase activity rose above the basal activity. In the further time course the measured values increased slowly before reaching a plateau. At higher inducer concentrations the β-galactosidase-activity increased immediately, except for a minor lag phase during the first 5 min, most probably due to protein production. Similar induction curves with increasing inducer concentrations indicated that the maximal rate of β-galactosidase synthesis was reached. In comparison to TMG the inducer molecule IPTG can be used at lower concentrations. Similar β-galactosidase activities were obtained if approximately ten-fold lower IPTG concentrations were used. Interestingly, we also observed differences between both
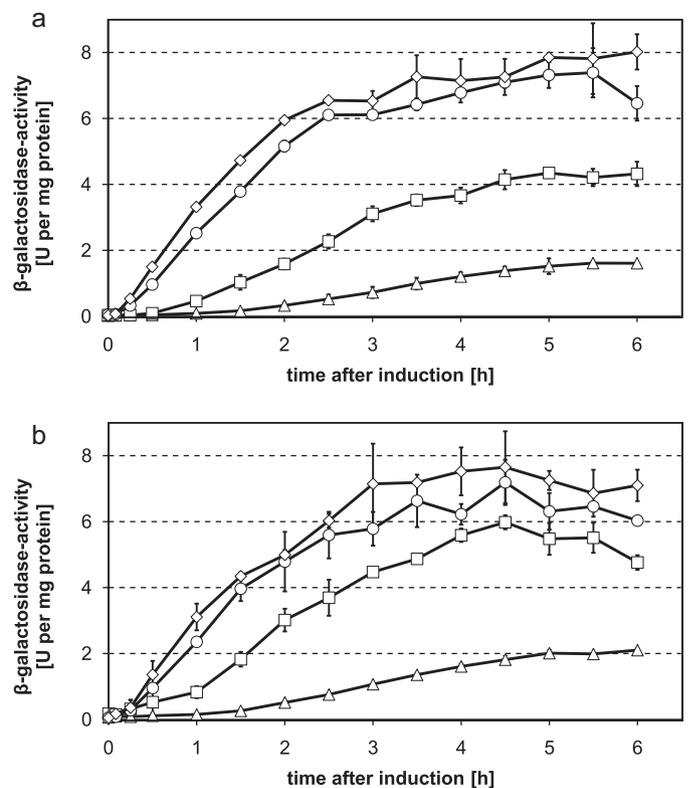

Fig. 1. Comparison of TMG and IPTG induction in *E. coli* LJ110. The figure shows time courses of β-galactosidase activity after the addition of TMG (a) and IPTG (b) at different concentrations. Data represent the average of two independent experiments. Error bars represent the standard deviation. (a) (△) 50 µM TMG, (□) 100 µM TMG, (○) 250 µM TMG, (◇) 500 µM TMG; (b) (△) 5 µM IPTG, (□) 10 µM IPTG, (○) 15 µM IPTG, (◇) 20 µM IPTG.

inducers with respect to the cell growth rate. The growth rate of LJ110 dropped with increasing TMG concentrations from $0.53\,h^{-1}$ with 50 µM TMG to $0.31\,h^{-1}$ with 1000 µM TMG. This corresponds to a drop in growth rate of about 40%. This effect was not observed for IPTG. In our experiments with IPTG the growth rate varied in the range of 0.46–$0.52\,h^{-1}$ without an obvious correlation between amount of inducer and growth inhibition.

The inhibitory effect of TMG on the growth rate was abolished in the *lacY* mutant strain AM2 indicating that in a permease negative background TMG was not accumulated in such amounts, that it disturbed cell metabolism. By inducing the *lacY* mutant strain with different concentrations of TMG or IPTG we showed that both inducers can evoke β-galactosidase production but significantly higher inducer concentrations were needed compared to the parental strain (Fig. 2). To reach maximal activities similar to the *lacY*+ strain a twenty-fold higher TMG and a ten-fold higher IPTG concentration were required. The shapes of the induction curves in the *lacY*+ and the *lacY*− strains differed at low inducer concentrations.

3.2. lac operon induction under the aspect of cell heterogeneity

Our previous experiments revealed that both IPTG and TMG are substrates of the lactose permease at low inducer concentrations and therefore have the potential to evoke bimodal induction behavior. To compare both inducers at single cell level we constructed a strain expressing GFP under control of the *lac* promoter similar to Ozbudak et al. (2004) with the exception that the *lac* promoter fragment contained all three known operator sites (Oehler et al., 1990). Due to the inclusion of operator-2,
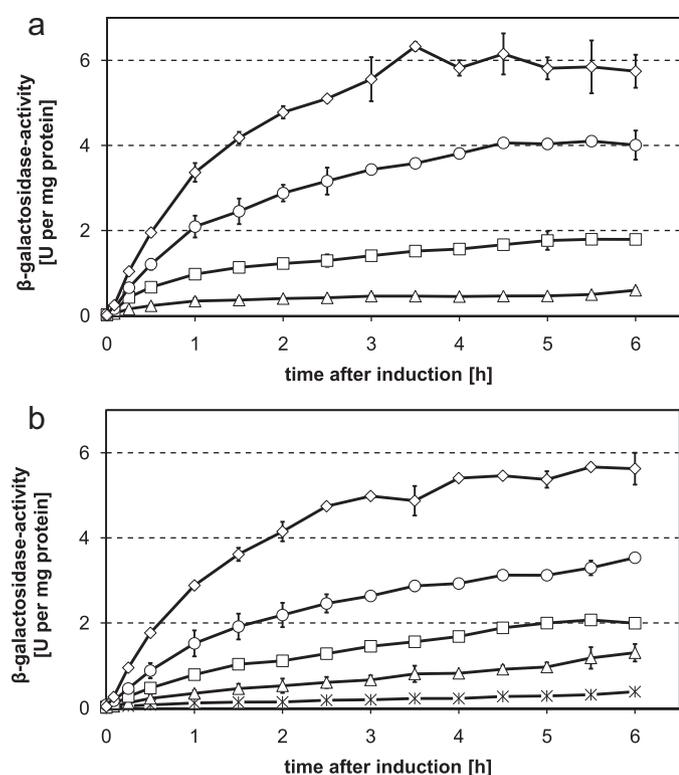
Fig. 2. Comparison of TMG and IPTG induction in a $\Delta lacY$ background (AM2). The figure shows β-galactosidase production after the addition of different concentrations of the inducers TMG (a) and IPTG (b). Data represent the average of two independent experiments. Error bars represent the standard deviation. (a) (△) 500 μM TMG, (□) 1000 μM TMG, (○) 2000 μM TMG, (◇) 4000 μM TMG; (b) (∗) 20 μM IPTG, (△) 30 μM IPTG, (□) 45 μM IPTG, (○) 60 μM IPTG, (◇) 120 μM IPTG.

a chromosomally encoded translational fusion was created consisting of the first 140 amino acids of LacZ fused to GFPmut3.1. We wanted to test if the additional operator sequence in the resulting strain AM1 changed the induction behavior in comparison to Mettetal et al. (2006). Therefore we conducted experiments with succinate as carbon source and TMG as inducer. Bimodal induction behavior was observed for 10 and 20 μM TMG, similar to the published results (Mettetal et al., 2006). Fig. 3a shows the time course of fluorescence distribution of cells induced with 20 μM TMG. The majority of the cells (82 respectively 84% in a second experiment) became fluorescent within this period whereas about 18% of the cells remained non-fluorescent over the whole period of the experiment (fluorescence ≤ 10%). A similar behavior was observed for 10 μM TMG but in this case a smaller percentage of cells became fluorescent (31 respectively 34%, data not shown). Subsequently, we investigated induction behavior in response to IPTG. Based on the results at the population level, we deduced that bimodal induction behavior should occur at a ten- to twenty-fold lower concentration than with TMG. Several concentrations were tested and bimodal behavior could be detected within the predicted concentration range. In Fig. 3b a time course of the fluorescence distributions of cells after the addition of 2.5 μM IPTG is shown. During the time course parts of the population began to express the *lac* operon whereas some cells remained non-fluorescent. Similar to the experiments with TMG, the percentage of non-fluorescent cells dropped within 8 h to 24% (respectively 28%). For 1.5 μM IPTG a major part of the cells remained non-fluorescent (data not shown).

Already in 1957 it was proposed that bimodal induction behavior at subsaturating TMG concentrations is due to the autocatalytic process caused by lactose permease (Novick and Weiner, 1957). Deletion of *lacY* hence was expected to shift the induction behavior from a bimodal to a unimodal course. We integrated plasmid pAM7 carrying the *lac* promoter *gfp* fusion into a *lacY* deletion strain resulting in strain AM4 (Table 1) and compared the induction behavior of AM4 with the *lacY*[+] strain AM1 (Table 1). As expected much more inducer had to be added to induce GFP fluorescence
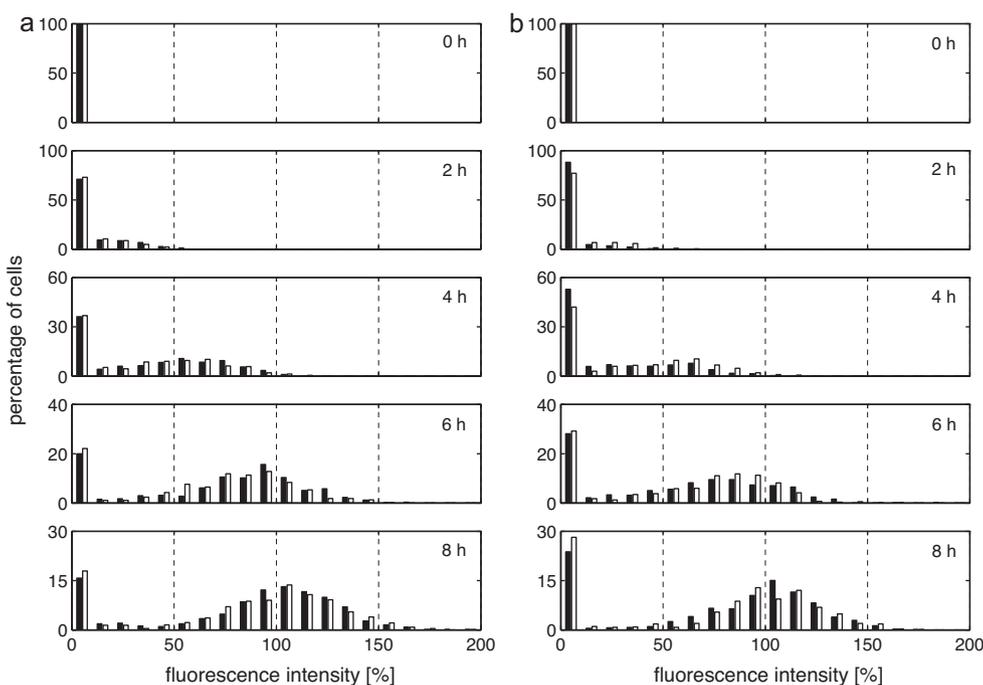

Fig. 3. Cell heterogeneity after induction with TMG and IPTG in AM1. (a) Fluorescence distributions of cells at various hours after the addition of 20 μM TMG. (b) Fluorescence distributions after addition of 2.5 μM IPTG. The average fluorescence value of induced AM1 cells 8 h after addition of TMG (a) respectively IPTG (b) was set to 100%. Although the comparison of IPTG and TMG induction was not done in the same experimental run the absolute fluorescence values of both time courses were comparable. *Legend*: The black and white bars represent two independent experimental repeats.
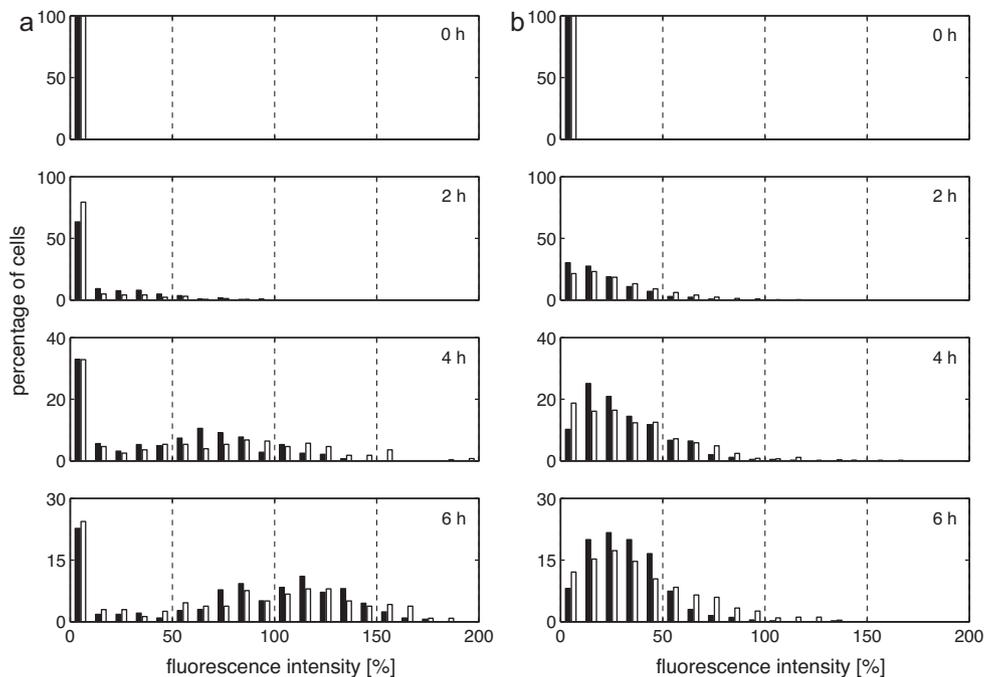
Fig. 4. Unimodal induction behavior after deletion of *lac* permease. (a) Addition of 20 μM TMG to *E. coli* AM1 led to a bimodal induction with cells expressing the *lac* operon and cells not expressing the *lac* operon. (b) Unimodal fluorescence time course of the *lacY* deletion mutant AM4 after induction with 500 μM TMG. The average fluorescence value of induced AM1 cells 6 h after TMG addition was set to 100% fluorescence and compared to fluorescence values of AM4. *Legend*: The black and white bars represent two independent experimental repeats.

(Fig. 4). To observe fluorescence signals readily distinguishable from the background level about 500 μM TMG had to be used in AM4. No bimodal distribution of GFP expression could be detected. Instead, all cells of the population responded equally. The average fluorescence distribution of both experimental repeats 6 h after inducer addition was Gaussian (average: 27.2, standard deviation: 22.5, adjusted R^2: 0.983).

3.3. Influence of the cell density and transacetylase activity on the induction behavior in single cell studies

It is known from literature that LacA is able to modify IPTG and TMG and that the modified form of IPTG does not act as inducer anymore (Herzenberg, 1961). But no study showed an influence of this action on induction behavior and therefore LacA is omitted in systems biological studies. In our experiments we noticed that after induction with IPTG the percentage of the non-fluorescent cells between 6 and 8 h dropped only slightly (induction with IPTG, see Fig. 3b). This could have two possible explanations: (i) the inducer was depleted or (ii) the culture reached a steady state between uninduced and induced cells. To test these two hypotheses the induction experiment was repeated with a higher starting cell density (OD 0.1 corresponding to 5×10^7 cells per ml) to strengthen the effect if the cells take up all inducer or modify them to a certain extent. In Fig. 5a the time course of induction is shown. Throughout the first 6 h the percentage of the non-fluorescent cells declined steadily and the fluorescence intensity of the induced cells rose. But 8 h after induction the percentage of the non-fluorescent cells remained constant. Moreover, the fluorescence intensity of the induced cell population decreased, indicating that the GFP concentration in the cells dropped. The decreasing fluorescence level can be attributed to previously induced cells which switched off GFP expression. The GFP fluorescence is diluted due to cell growth as a consequence. Obviously, IPTG was depleted throughout the experiment. To test if LacA activity was responsible for the observed effect, we constructed a *lacA* deletion mutant AM5 carrying the *lac*

promoter *gfp* fusion (Table 1). We repeated the batch experiments with the higher cell densities with AM5. Within the first 6 h the mutant strain showed no relevant differences in comparison to AM1 (Fig. 5b). The percentage of non-induced cells dropped continuously and the fluorescence intensity of the induced cells increased. But in contrast to AM1 the percentage of the non-induced cells dropped continuously over the whole period of the experiment and the fluorescence intensity of the induced cells rose continuously, too. This resulted in a higher fluorescence intensity of the *lacA* deletion strain AM5 in comparison to AM1. This experiment clearly demonstrated that the transacetylase activity has an influence on the induction of cells by IPTG.

4. Discussion

Today, *lac* operon regulation is a research object in systems biology (Dreisigmeyer et al., 2008; Mettetal et al., 2006; Ozbudak et al., 2004) and *lac* operon elements are used as tools in biotechnology (Makrides, 1996) and synthetic biology (Kaern et al., 2003). For this purpose detailed knowledge about *lac* operon induction is required. In this study the induction behavior of the *E. coli lac* operon was investigated in a systematic manner. We focused on the differences of the two gratuitous inducers IPTG and TMG with respect to the transport into cells, the ability to evoke bimodal induction behavior and the influence of the transacetylase.

By analyzing *lac* operon induction in LJ110 with different inducer concentrations it could be demonstrated that TMG and IPTG induction are very similar except for an approximately ten-fold difference in the required inducer concentration which can easily be explained by different affinities of the inducers for the lactose repressor (Riggs et al., 1970). Taking into account the induction behavior of the isogenic *lacY* mutant strain revealed that at low inducer concentrations IPTG as well as TMG are mainly transported by the lactose permease while at ten- to twenty-fold higher concentrations also a lactose permease independent entry is possible. The lactose permease catalyzed uptake of inducers at low
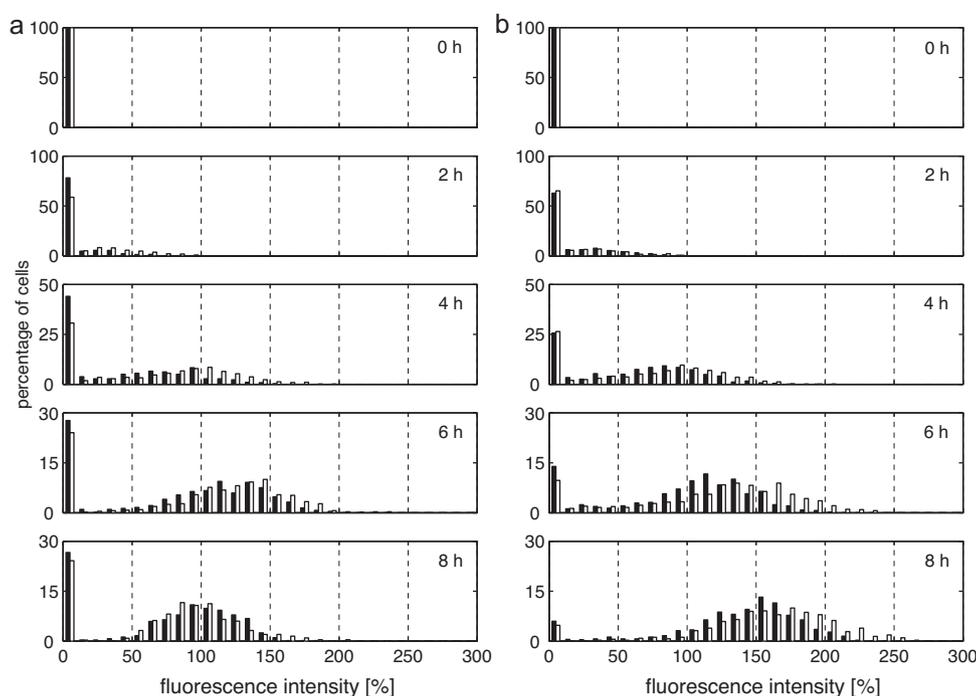
Fig. 5. Influence of the cell density and LacA on the bimodal induction behavior. To demonstrate a potential activity of LacA we conducted induction experiments at the single cell level with cultures growing at a 3.3-fold higher cell density as in the experiments shown in Fig. 3 and compared the strains *E. coli* AM1 with the wild-type background (a) and *E. coli* AM5 with a deletion of *lacA* (b). Both strains were induced with 2.5 μM IPTG. The average fluorescence level of induced cells of AM1 8 h after IPTG addition was set to 100% and compared to fluorescence values of AM5. *Legend*: The black and white bars represent two independent experimental repeats.

concentrations is a prerequisite for bimodal induction behavior as it is part of the positive feedback loop of the *lac* operon (Novick and Weiner, 1957). By using the *Plac-gfp* reporter strain AM1 we showed bimodal induction behavior with TMG. The inducer concentrations required for bimodality were similar to Mettetal et al. (2006). Therefore it might be suggested that the presence or absence of *lac* operator-2 does not have an important effect on *lac* operon expression. The similar characteristics of IPTG and TMG induction at the β-galactosidase assays (Fig. 1) led us to propose that IPTG should cause bimodal distribution of cells at an approximately ten-fold lower concentration than TMG. The single cell measurements revealed that similar bimodal distributions were obtained at 20 and 10 μM TMG respectively 2.5 and 1.5 μM IPTG confirming the predicted concentration range. The fact that both IPTG and TMG can induce bimodal induction behavior is important considering that they are often used to control gene expression of *lac* promoter controlled genes in biotechnology and molecular biology. In a *lacY*⁺ strain there is the potential for bimodal induction at low inducer concentrations. This should be considered if the gene expression level is to be controlled by the amount of inducer. In order to achieve homogeneous gene expression, experiments hence should be performed in a *lacY*⁻ strain. We showed for the first time the single cell induction behavior of a *lacY* mutant strain by using *gfp* as reporter gene. The results presented demonstrate that in the absence of a functional lactose permease the bimodal induction behavior changed into a graded response. This was shown for TMG induction but it should be the same with IPTG. On the other hand, a *lacY*⁺ background could be an advantage if the goal is maximal protein production. The comparison between the β-galactosidase induction in the parental strain and the *lacY* deletion strain showed that lower inducer amounts were necessary to obtain maximal protein synthesis. If lower amounts of inducer can be used to obtain high expression of heterologous genes the production process becomes cheaper. Additionally, it is known that IPTG can influence cell growth and induces a kind of stress response

(Andrews and Lin, 1976; Kosinski et al., 1992). However, in our experiments we could not detect a repression of growth by IPTG. But we did not use as much IPTG as in the publications mentioned. Interestingly, we observed that TMG reduced the growth rate in LJ110 but not in the *lacY* deletion mutant indicating that a functional permease accumulates high amounts of TMG that disturb cell metabolism.

The examinations of bimodality using IPTG further revealed an influence of the transacetylase on induction behavior. It was already shown in the early years of *lac* operon investigation that IPTG and TMG are substrates of the transacetylase but no particular function in the metabolism of lactose could be allocated to the transacetylase (Herzenberg, 1959, 1961). Therefore, this enzyme activity has not been considered in most *lac* operon expression studies and in mathematic models of the lactose operon. The data presented (Fig. 5) show that the transacetylase activity can influence *lac* operon induction behavior at least for induction with IPTG. At a constant inducer concentration the positive feedback loop of the lactose system guarantees that an induced cell maintains this state. Only a drastic drop in the external IPTG concentration could account for a reduced expression of the *lac* operon in strain AM1. Repeating the induction experiment with a *lacA* deletion mutant demonstrated that the fluorescence level of the cells increased and that the percentage of the non-induced cells decreased over the whole period of incubation (Fig. 5). This suggested that IPTG was modified by the action of transacetylase. Herzenberg described that IPTG is converted into isopropyl 6-O-acetyl-β-D-thiogalactopyranoside and that the modified substance, which is not able to act as an inducer anymore, is elaborated into the medium (Herzenberg, 1961). Therefore we concluded that in AM1 IPTG was acetylated in such amounts that the concentration in the medium dropped below an inducing threshold concentration. That the action of the transacetylase can lead to a reduction of the inducer concentration in the medium was already reported by Wilson and Kashket (1969). They showed that the

E. coli strain ML308 which had a *lacI⁻* phenotype and therefore constitutively expressed LacA acetylated more than 70% of IPTG and 40% of TMG in the growth medium. Our experiments now demonstrate that the transacetylase activity is also sufficient to influence induction behavior in a *lac* operon wild-type background in dependence of cell density. A higher total number of cells expressing the transacetylase in the second experiment (Fig. 5a) probably leads to a higher rate of IPTG modification and therefore to a significant reduction of the IPTG concentration. This could contribute to the dependence of IPTG induction on biomass as observed by Olaofe et al. (2010). The authors reported that the induction of a heterologous gene in *E. coli* BL21 (DE3) using a high cell density cultivation method was about half of the observed value if they used twice the cell density. On the other hand, the transacetylase could be beneficial for expression of *lac* promoter controlled genes. Andrews and Lin (1976) reported that the growth of *lacA⁻* strains was stronger influenced by the presence of IPTG than growth of *lacA⁺* strains. Wilson and Kashket (1969) reported that *lacA⁻* strains accumulate more TMG than *lacA⁺* strains. A possible function of transacetylase could be to minimize cell stress by lowering intracellular inducer concentrations. In our experiments high extracellular concentrations of TMG but not of IPTG led to reduced growth rates. This suggested that modification of IPTG by transacetylase protects cells from toxic effects. Based on the reports of Wilson and Kashket (1969), transacetylase activity obviously allows the cells to expel excess IPTG into the growth medium. As modification of TMG by transacetylase is not as efficient as for IPTG, this results in less effective protection.

Summarizing, we presented a systematic study on *lac* operon induction which will give further advice in the usage of the gratuitous inducers TMG and IPTG in systems biology, synthetic biology and biotechnology.

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References

Adhya, S., 1996. The *lac* and *gal* operons today. In: Lin, E.C.C., Lynch, A.S. (Eds.), Regulation of Gene Expression in *Escherichia coli*. Landes, pp. 181–200.

Andrews, K.J., Lin, E.C.C., 1976. Thiogalactoside transacetylase of the lactose operon as an enzyme for detoxification. J. Bacteriol. 128, 510–513.

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L., Mori, H., 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. 2.

Beckwith, J., 1987. The lactose operon. In: Neidhardt, F.C. (Ed.), *Escherichia coli* and *Salmonella typhimurium*. American Society of Microbiology, Washington, DC, pp. 1444–1452.

Boezi, J.A., Cowie, D.B., 1961. Kinetic studies of β-galactosidase induction. Biophys. J. 1, 639–647.

Datsenko, K.A., Wanner, B.L., 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. U.S.A. 97, 6640–6645.

Deutscher, J., Francke, C., Postma, P.W., 2006. How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. Microbiol. Mol. Biol. Rev. 70, 939–1031.

Donovan, R.S., Robinson, C.W., Glick, B.R., 1996. Review: optimizing inducer and culture conditions for expression of foreign proteins under the control of the *lac* promoter. J. Ind. Microbiol. 16, 145–154.

Dreisigmeyer, D.W., Stajic, J., Nemenman, I., Hlavacek, W.S., Wall, M.E., 2008. Determinants of bistability in induction of the *Escherichia coli lac* operon. IET Syst. Biol. 2, 293–303.

Gardner, T.S., Cantor, C.R., Collins, J.J., 2000. Construction of a genetic toggle switch in *Escherichia coli*. Nature 403, 339–342.

Haldimann, A., Wanner, B.L., 2001. Conditional-replication, integration, excision, and retrieval plasmid-host systems for gene structure-function studies of bacteria. J. Bacteriol. 183, 6384–6393.

Hansen, L.H., Knudsen, S., Sorensen, S.J., 1998. The effect of the *lacY* gene on the induction of IPTG inducible promoters, studied in *Escherichia coli* and *Pseudomonas fluorescens*. Curr. Microbiol. 36, 341–347.

Herzenberg, L.A., 1959. Studies on the induction of β-galactosidase in a cryptic strain of *Escherichia coli*. Biochim. Biophys. Acta 31, 525–538.

Herzenberg, L.A., 1961. Isolation and identification of derivates formed in the course of intracellular accumulation of thiogalactosides by *Escherichia coli*. Arch. Biochem. Biophys. 93, 314–315.

Jensen, P.R., Westerhoff, H.V., Michelsen, O., 1993. The use of *lac*-type promoters in control analysis. Eur. J. Biochem. 211, 181–191.

Kaern, M., Blake, W.J., Collins, J.J., 2003. The engineering of gene regulatory networks. Annu. Rev. Biomed. Eng. 5, 179–206.

Khlebnikov, A., Keasling, J.D., 2002. Effect of *lacY* expression on homogeneity of induction from the P$_{tac}$ and P$_{trc}$ promoters by natural and synthetic inducers. Biotechnol. Prog. 18, 672–674.

Kosinski, M.J., Rinas, U., Bailey, J.E., 1992. Isopropyl-β-D-thiogalactopyranoside influences the metabolism of *Escherichia coli*. Appl. Microbiol. Biotechnol. 36, 782–784.

Makrides, S.C., 1996. Strategies for achieving high-level expression of genes in *Escherichia coli*. Microbiol. Rev. 60, 512–538.

Maloney, P.C., Rotman, B., 1973. Distribution of suboptimally induced β-D-galactosidase in *Escherichia coli*—the enzyme content of individual cells. J. Mol. Biol. 73, 77–91.

Martin, R.G., Bartlett, E.S., Rosner, J.L., Wall, M.E., 2008. Activation of the *Escherichia coli marA/soxS/rob* regulon in response to transcriptional activator concentration. J. Mol. Biol. 380, 278–284.

Mettetal, J.T., Muzzey, D., Pedraza, J.M., Ozbudak, E.M., van Oudenaarden, A., 2006. Predicting stochastic gene expression dynamics in single cells. Proc. Natl. Acad. Sci. U.S.A. 103, 7304–7309.

Miller, J.H., 1992. A Short Course in Bacterial Genetics. A Laboratory Manual and Handbook for *Escherichia coli* and Related Bacteria. Cold Spring Harbor Laboratory Press, New York.

Novick, A., Weiner, M., 1957. Enzyme induction as an all-or-none phenomenon. Proc. Natl. Acad. Sci. U.S.A. 43, 553–566.

Oehler, S., Eismann, E.R., Krämer, H., Müller-Hill, B., 1990. The three operators of the *lac* operon cooperate in repression. EMBO J. 9, 973–979.

Olaofe, O.A., Burton, S.G., Cowan, D.A., Harrison, S.T.L., 2010. Improving the production of a thermostable amidase through optimising IPTG induction in a highly dense culture of recombinant *Escherichia coli*. Biochem. Eng. J. 52, 19–24.

Ozbudak, E.M., Thattai, M., Lim, H.N., Shraiman, B.I., van Oudenaarden, A., 2004. Multistability in the lactose utilization network of *Escherichia coli*. Nature 427, 737–740.

Riggs, A.D., Newby, R.F., Bourgeois, S., 1970. Lac repressor-operator interaction. II. Effect of galactosides and other ligands. J. Mol. Biol. 51, 303–314.

Roderick, S.L., 2005. The *lac* operon galactoside acetyltransferase. C. R. Biol. 328, 568–575.

Tanaka, S., Lerner, S.A., Lin, E.C.C., 1967. Replacement of a phosphoenolpyruvate-dependent phosphotransferase by a nicotinamide adenine dinucleotide-linked dehydrogenase for the utilization of mannitol. J. Bacteriol. 93, 642–648.

Terpe, K., 2006. Overview of bacterial expression systems for heterologous protein production: from molecular and biochemical fundamentals to commercial systems. Appl. Microbiol. Biotechnol. 72, 211–222.

Wilson, T.H., Kashket, E.R., 1969. Isolation and properties of thiogalactoside transacetylase-negative mutants of *Escherichia coli*. Biochim. Biophys. Acta 173, 501–508.

Xie, L., Hall, D., Eiteman, M.A., Altman, E., 2003. Optimization of recombinant aminolevulinate synthase production in *Escherichia coli* using factorial design. Appl. Microbiol. Biotechnol. 63, 267–273.

Zeppenfeld, T., Larisch, C., Lengeler, J.W., Jahreis, K., 2000. Glucose transporter mutants of *Escherichia coli* K-12 with changes in substrate recognition of IICB^{Glc} and induction behavior of the *ptsG* gene. J. Bacteriol. 182, 4443–4452.