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효모균에서의 Plasmid 번식체계와 혼성유전자 발현

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Plasmid Propagation and Heterologous Gene Expression in Recombinant Yeast

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ABSTRACT

The effects of genetic and environmental factors on productivity of a cloned protein were studied in recombinant *Saccharomyces cerevisiae*. Plasmid stability and copy level were very high for a *REP*$^+$ system(at ca. 10 generations, stability: 65-90%, plasmid copy number per cell:40-200), whereas these were very low for a *rep*$^-$ system(at ca. 10 generations, stability: 30%, plasmid copy number per cell: 20). In plasmids containing the 2 μm circle genome, a [cir^o] strain was a preferred host cell since the plasmid stability and the copy number in a [cir^o] strain were higher than in a [cir$^+$] strain. Cloned gene expression was dependent on plasmid copy number and stability. The inducer(galactose) level played a very important role in cloned *lacZ* gene expression, showing that a galactose concentration of 0.8% was sufficient for induction of gene expression. Induction rate was very fast in the case of plasmids exhibiting high stability and copy number by a factor of 4 to 25. The time to reach the peak value of gene expression was longer when galactose was added at the start of fermentation(ca. 26 hours) than at the mid-exponential phase(ca. 6 hours). Glucose repression was reduced by a factor of 2 to 5 as the relative inducer level increased.

INTRODUCTION

This research has focused on the mechanism of plasmid propagation and the enhancement of heterologous gene expression in recombinant *Saccharomyces cerevisiae*, from the view point of plasmid stability and induction of gene expression. The yeast 2 μm circle is a 6318bp double-stranded DNA plasmid present in most *Saccharomyces cerevisiae* strains at 60-100 copies per haploid genome(1). The yeast 2 μm circle plasmids and 2 μm-based hybrid plasmids are maintained stably and at high copy number in yeast cells. Plasmid stability rests on the *REP* system governing plasmid equipartitioning to both progeny cells, i.e., mother and daughter, at cell division(2, 3). An equipartitioning process requires the *trans*-acting gene proteins, *REP1* and *REP2*, through interaction with the *REP3* locus which is active only in *cis*(4, 5). This equipartitioning yields very high probability of plasmid transmission at

mitosis, resulting in high stability. This work has shown that, in *REP*$^+$ host-vector systems, probability of plasmid transmission at cell division and stability are higher in a strain [cir^o] than in a strain [cir$^+$] regardless of whether the *REP* system is available from a hybrid plasmid or a 2μm circle plasmid. In addition, this interaction has been confirmed by culturing strains [cir^o] and [cir$^+$] harboring plasmid pSI4 and pSI5, respectively, for the long term culture.

Cloned gene expression generally depends on the plasmid stability and the copy number. A plasmid exhibiting higher stability leads to high levels of gene expression. If the stability is maintained at a high level, the large fraction of the population can produce a cloned gene product. On the other hand, when the stability is low, only a small fraction of the population can produce the cloned gene product. Stability should be maintained at a high level to yield high cloned gene productivity. Plasmid copy number also plays an important role in gene expression since copy number can affect the stability of the vector plasmid(1, 6). High copy number yields high expression levels which can be reduced by glucose repression. The inducer level is important for the cloned gene expression by changing the ratio of galactose/glucose concentrations(7, 8). Thus, it is desirable to reduce glucose repression and to maintain high plasmid stability and copy number in order to increase the cloned gene expression level.

MATERIALS AND METHODS

Strains and Plasmids

Strains S150-2B[cir$^+$] and [cir^o] (*MATa leu 2-3 leu2-112 his3 ura3-52 trp1*) are isogenic yeast strains containing and lacking the endogenous 2μm circle plasmids, respectively. All of the plasmids used in this work are shuttle vectors which contain origins of replication and selectable markers. Plasmids, pSI5, pSI4, and XHO3(provided by Dr. J. R. Broach, Princeton University, NJ, U. S. A.) contain pBR322 plus *LEU2* sequences(Table 1). Plasmids pSI4 and XHO3 have the entire 2μm circle regulatory sequences. Plasmid pSI5 contains only the *REP3* locus(*Eco*RI fragment of the 2μm circle plasmid spanning the origin of replication) (9).

Table 1. Characteristics of 2μm-based hybrid plasmids.

plasmid	size(kb)	Selectable marker		2μm gene sequences
		yeast	*E. coli*	
pSI5	8.08	*LEU2*	*Amp*^{R},	*ori, REP3*
pSI4	11.9	*LEU2*	*Amp*^{R},	*ori, REP1, REP2, REP3*
XHO3	12.6	*LEU2*	*Amp*^{R}, *Tet*^{R}	*ori, REP1, REP2, REP3*

Plasmids pRY131, LR1△20B, and pRY171 (Table 2) are provided by Dr. M. Ptashne(Harvard University, MA, U. S. A.). Plasmid pRY131, which is derived from plasmid pRY121 (8), has the *lacZ*(β-galactosidase) gene fused at the GAL1 site of *GAL1-GAL10* hybrid promoter which controls the transcription of the *lacZ* gene. This plasmid contains a 2μm fragment including the origin of replication and the *REP3* site. Plasmid LR1△20B is exactly the same as pRY131 except for deletions between UAS_G (*GAL* upstream activating sequence) and the *GAL1* TATA box. These deletions which are 158 base pairs long, allow constitutive synthesis of cloned gene product and reduce glucose repression(10). Plasmid pRY171 does not contain the 2μm fragment. That is, pRY171 does not have the origin of replication, hence, this plasmid should be inserted into the chromosome in order to be replicated(8). These three plasmids have a *URA3* gene providing a selective advantage to plasmid-harboring cells.

Media

The complete, non-selective medium for yeast was YPD containing bacto-yeast extract(10g/ℓ), bacto-peptone(20g/ℓ), and glucose(20g/ℓ). An optimized C-limited SD minimal medium contained glucose (4g/ℓ), bacto-yeast nitrogen base without amino acids(6.7g/ℓ); L-tryptophan(100mg/ℓ), L-histidine-HCl(100mg/ℓ), L-leucine(150mg/ℓ), and uracil(100mg/ℓ) were added when required. For shake-flask cultivation, all media were buffered to pH 5.5 with citrate buffer(0.1M). YPD plates and supplemented SD minimal plates contained bacto-agar(20g/ℓ) in addition to the constituents above. Supplemented SD minimal plates were of two types, leucine-lacking(*leu*$^-$) and uracil-lacking(*ura*$^-$) SD

Table 2. Characteristics of plasmids with *GAL* promoter.

Plasmid	Size(kb)	Selectable marker		Promoter	Fusion type	Plasmid type
		Yeast	*E. coli*			
pRY131	10.64	*URA3*	Amp^R	*GAL1-10*	*GAL1*	2 μm
LR1△20B	10.5	*URA3*	Amp^R	*GAL1-10*	*GAL1*	2 μm
pRY171	8.8	*URA3*	Amp^R	*GAL1*	*GAL1*	Integrated at *GAL1*

media plates. YPG plates for plasmid stability assays contained bacto-yeast extract(10g/ℓ), bacto-peptone(20g/ℓ), galactose(20g/ℓ), and 40mg/ℓ of X - Gal(4-bromo-5-chloro-3-indolyl- β-D-galactoside)dissolved in dimethylformamide buffered to pH 7.0 by the addition of Na_2HPO_4 and KH_2PO_4 to 70mM of final concentration(8).

Culture Conditions

Batch experiments were performed in shake flasks and in a 2L fermentor (Multigen, Model F-2000, New Brunswick Scientific, NJ, U. S. A.). In the case of shake flask culture, 250mℓ Erlenmeyer flasks containing 50mℓ medium buffered to pH 5.5 were inoculated from the overnight culture at an inoculum size of 1%(v/v) and cultured in a gyrotory shaker (Model G25, New Brunswick Scientific, NJ, U. S. A.) at 30℃ and 250rpm. For the long term culture in non-selective YPD medium, a 1%(v/v) inoculum was transferred every 12 hours to a new flask containing fresh medium in order to maintain the exponential growth for long generations by serial shake-flask cultures, while there were no limiting nutrients. In the case of the Multigen fermentor, batch cultivations were performed at 30℃, 400rpm, and pH 5.5 with an air flow rate of 1vvm while the working volume was 1.5ℓ. The induction of cloned *lacZ* (β-galactosidase) gene expression in batch culture was performed by the addition of galactose either at the exponential phase or at the start of the culture.

General Procedures

Yeast transformation and plasmid DNA isolation were performed as previously described (11, 12). β-galactosidase assays were performed as described by Miller(13). Plasmid copy number was measured as described by Broach(9). Stability was measured by the replica plate method in duplicate.

RESULTS AND DISCUSSION

Host-Vector Interactions

For a recombinant cell process employing plasmids, plasmid stability is very important in maintaining a large population of plasmid-harboring cells. Fig. 1 shows the probability of plasmid transmission at cell division in both strains [cir$^+$] and [cir^o]. In the case of plasmid pSI5, which contains only the *REP3* locus (*Eco*RI fragment spanning origin), the equipartitioning by the *REP* function occurs in a [cir$^+$] strain due to the presence of the endogenous 2 μm circle, but it does not occur in a [cir^o] strain lacking *REP1* and *REP2* gene products. As expected, the probability of plasmid transmission at cell division

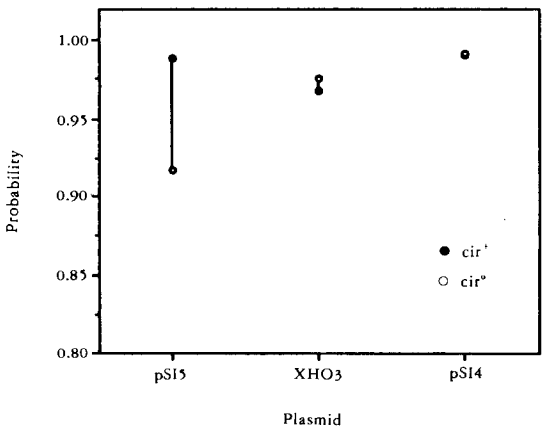


Fig. 1. Difference in probability of plasmid transmission between a [cir$^+$] and a [cir^o] strain. Strain S150-2B[cir^o]: pSI5 (designated by open circle(o) in the first column pSI5) cannot confer the *REP* system (that is, *rep*$^-$). In the other strains, *REP*$^+$ system is available. Here, strains [cir$^+$] and [cir^o] refer to the isogenic yeast strains containing and lacking the endogenous 2 μm circle plasmids, respectively.

(segregational stability) was much higher in a [cir$^+$] strain than in a [cir^o] strain. In other words, the rate of plasmid loss at cell division(segregational instability) in a [cir$^+$] strain approximately 8-10 times less than a [cir^o] strain. This result is compatible with the fact that *trans*-acting *REP1* and *REP2* gene products from the endogenous 2 μm circle play an important role in partitioning at cell division when the hybrid plasmid does not contain these two gene sequences.

The stability is defined as the percentage of plasmid-harboring cells present in the population under non-selective conditions. As shown in Fig. 2, the plasmid stability in strain S150-2B[cir$^+$]:pSI5 was maintained at very high level due to the high probability of plasmid transmission at cell division. However, the plasmid in strain [cir^o] behaves essentially like an *ARS* plasmid, which is mitotically unstable due to a strong bias to segregate the plasmid to the mother cell at mitosis rather than the daughter cell. The low probability of plasmid transmission yields low stability, resulting from poor partitioning in which *REP* function is not applicable. The high copy number of 2 μm-based plasmids is dependent in part on 2 μm circle *REP* functions. Plasmid pSI5 is present at about 175 copies per cell in a [cir$^+$] strain, but is present at only 20 copies per cell in its isogenic [cir^o] strain. Thus, the *REP* function enhancing stability may also play a role in maintaining high copy level.

The *REP* functions can be provided by the hybrid plasmid itself when the plasmid contains the entire 2 μm circle sequences. Hybrid plasmid XHO3 containing the entire 2 μm circle sequences can provide the *REP* functions regardless of the presence or the absence of the endogenous 2 μm circle plasmid in the yeast cells. Fig. 1 shows that plasmid XHO3 has reasonably high probability of plasmid transmission in both [cir$^+$] and [cir^o] strains. Although strain [cir^o] does not contain the endogenous 2 μm circle plasmid, XHO3 confers the *REP* functions which affect positively the equipartition at cell division. Thus, the probability of plasmid transmission could be maintained at a high level in both strains.

Interestingly, the probability of plasmid transmission at cell division in strain [cir$^+$] is somewhat lower than that in strain [cir^o] in contrast to the case of plasmid pSI5. This result appears to be due to *REP1* autoregulation. In strain [cir$^+$], there is an autoregulation of the *REP1* gene product at the transcription level due to the endogenous 2 μm circle plasmid in the cell(14, 15, 16). Transcription of the *REP1* gene itself is repressed by the concerted action of the *REP1* and *REP2* gene products. *REP1* gene expression is insensitive to the presence of any single plasmid-encoded gene product, but is severely repressed by the presence of both *REP1* and *REP2* proteins. The autoregulation of *REP1* serves as a homeostatic device, which enhances sensitivity of the plasmid to deviations of copy levels from the norm. This means that the excess of *REP1/REP2* complex represses the transcription of the *REP1* gene itself. Then, the concentration of this complex will be low, resulting in low frequency in binding of this complex at the *REP3* locus. Thus, the efficiency of equipartitioning decreases, yielding a lower probability of plasmid transmission at cell division. Therefore, in strain [cir$^+$] containing more dosages of *REP1* and *REP2* genes, the probability of plasmid transmission at cell division is lower than that in strain [cir$^+$], even though the difference is small.

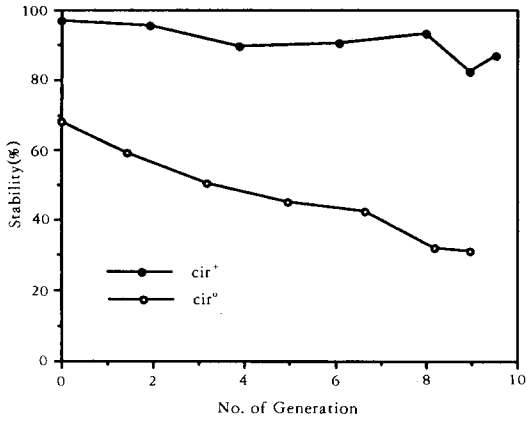


Fig. 2. Stability of strain S150-2B:pSI5. The upper line(closed circle) refers to the stability of strain S150-2B[cir$^+$]:pSI5(*REP*$^+$) and the lower line (open circle) indicates the stability of strain S150-2B[cir^o]:pSI5(*rep*$^-$).

In the case of plasmid pSI4, which contains also the entire 2 μm circle regulatory sequences, just like XHO3, the probability of plasmid transmission is very high in both strains without any significant difference. In a [cir$^+$] strain, the effect of *REP1* autoregulation by the *REP1/REP2* complex on the *REP* function is not seen. The copy number of this plasmid is approximately 200 per cell in comparison with about 40 copies of plasmid XHO3. This means that, in the case of a strain with very high plasmid copy levels, the extent of the *REP1* autoregulation effect on *REP* function is lesser than that in a strain with low copy levels. In other words, in a [cir$^+$] strain, the excess of *REP1/REP2* complex provided by the endogenous 2 μm circle plasmid represses *REP1* transcription less in a strain harboring plasmids at a relatively high copy level than in a strain with a low plasmid copy number. Hence, the concentration of *REP1/REP2* complex necessary for equipartitioning the totality of plasmids present in the cell can be maintained, yielding higher probability and stability. Therefore, the probability of plasmid transmission depends on both the extent of *REP1* autoregulation and plasmid copy number which affect the *REP* function at cell division(16, 17, 18).

Fig. 3 shows the effect of plasmid copy number on stability in *REP*$^+$ host-vector systems. Plasmid stability generally increases with plasmid copy number. That is, the higher plsamid copy level confers the higher stability. In addition, plasmid stability is higher in [cir^o] strains than in [cir$^+$] strains. The differences in stability between in [cir^o] and [cir$^+$] decrease as plasmid copy number increases. The negative effect of the endogenous 2 μm circle plasmid on stability can be reduced by increasing plasmid copy number. For those plasmids carrying the entire 2 μm circle sequences, cellular copy levels are also influenced by plasmid incompatibility causing an increase in segregational instability(17). That is, the plasmid copy level in a [cir^o] strain is higher than that in a [cir$^+$] strain, presumably because 2 μm-based hybrid vectors share the same copy control system as the endogenous 2 μm plasmids. Hence, [cir^o] strains are the preferred hosts for vectors carrying the entire 2 μm circle sequences.

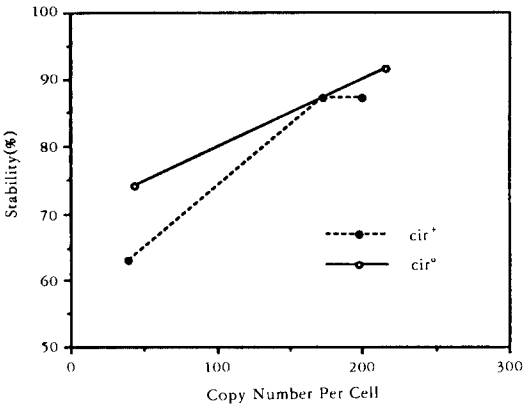


Fig. 3. Effect of 2 μm circle plasmid on stability (data are for ca. 10 generations). The data points are the stability of strains conferring the *REP*$^+$ function (except for strain S150-2B[cir^o]:pSI5 which cannot confer the *REP*$^+$ function).

Fig. 4 shows the plasmid stability of strains S150-2B[cir^o]:pSI4 and [cir$^+$]:pSI5 for the long term culture in order to confirm host-vector interactions. Strains S150-2B[cir$^+$]:pSI5 and [cir^o]:pSI4 provide *REP* functions and have very high copy levels and probability of plasmid transmission at cell division. Although there is no significant difference in probability of plasmid transmission ([cir$^+$]:pSI5, 0.988; [cir^o]:pSI4, 0.991), the stability of pSI4 in the [cir^o] strain is higher than that of pSI5 in the [cir$^+$] strain for the long term cultivation, as shown in Fig. 4. This means that even a small difference in probability of plasmid transmission results in a considerable difference in stability for the long term culture. Therefore, based on these results, it is possible, in principle, to design a desirable host-vector system in order to maintain the high stability and plasmid copy number. That is, a desirable host-vector system should confer the *REP* function for equipartitioning at cell division. When plasmids contain the entire 2 μm circle genome, a [cir^o] strain rather than a [cir$^+$] strain is a preferred host in order to avoid the negative effects of the endogenous 2 μm circle plasmid on probability of plasmid transmission, stability, and copy number.

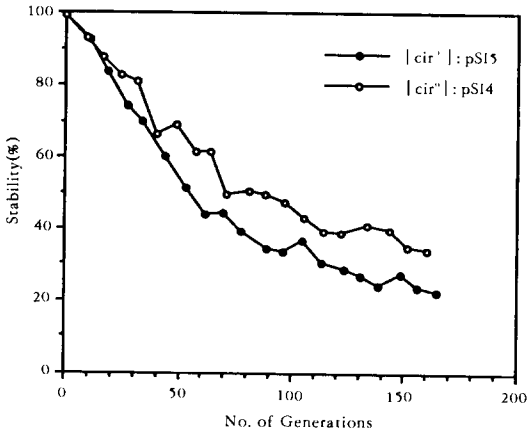


Fig. 4. Stability of strains S150-2B[cir$^+$]:pSI5 and S150-2B[cir^o]:pSI4.

Cloned Gene Expression

The effects of various genetic and environmental factors on cloned gene expression were studied in batch fermentation. Fig. 5 shows the stability of each strain in SD selective minimal medium. Strain S150-2B[cir^o]:pRY171 exhibits approximately 100% stability since this plasmid is integrated into the chromosome. The copy number of this plasmid is 1. Strain S150-2B[cir$^+$]:pRY131 and [cir$^+$]:LR1△20B have very high stability. In these strains, there is a *REP* system due to the presence of the endogenous 2 μm circle plasmids in cells, even though plasmids pRY131 and LR1△20B do not contain *trans*-acting *REP1* and *REP2* gene sequences. The copy number of plasmids pRY131 and LR1△20B is ca. 45. On the other hand, the stability of strain S150-2B[cir^o]:pRY131 is very low, even in the selective medium, due to the absence of the *REP* system. The average copy number of this plasmid is about 5.

Fig. 6 shows the effect of galactose concentration on cloned gene expression. At the mid-exponential phase, various concentrations of galactose were added to exponentially growing cultures containing an initial glucose concentraiton of 4g/ℓ. After 4-5 hours of induction during exponential growth, β-galactosidase specific activities were compared for the four strains at various galactose concentrations. Strains S150-2B[cir$^+$]:pRY131 and S150-2B [cir$^+$]:LR1△20B have very high β-galactosidase

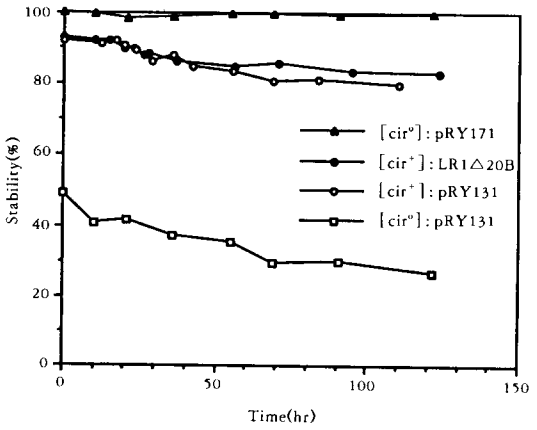


Fig. 5. Stability of each plasmid in batch culture. Labels for strains are :[cir^o]:pRY171, S150-2B[cir^o]:pRY171; [cir$^+$]:LR1△20B, S150-2B [cir$^+$]:LR1△20B; [cir$^+$]:pRY131, S150-2B [cir$^+$]:pRY131; and [cir^o]:pRY131, S150-2B [cir^o]:pRY131.

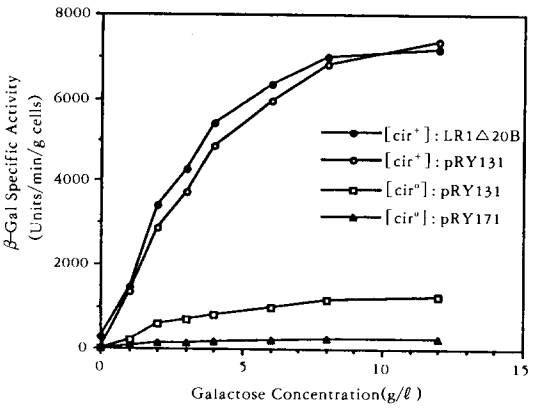


Fig. 6. Effect of galactose concentration on cloned gene expression. Labels for strains are :[cir$^+$]:LR1△20B, S150-2B[cir$^+$]:LR1△20B; [cir$^+$]:pRY131, S150-2B[cir$^+$]:pRY131; [cir^o]:pRY131, S150-2B[cir^o]:pRY131; and [cir^o]:pRY171, S150-2B[cir^o]:pRY171.

specific activity(ca. 7000 units/min/g cells) due to the high stability and copy number. Strain S150-2B [cir^o]:pRY131 has low β-galactosidase specific activity because the stability and copy number of the plasmid are low. Although strain S150-2B[cir^o]:pRY171 has a plasmid stability of approximately 100%, β-galactosidase specific activity is very low because

the plasmid copy number is only unity.

As shown in Fig. 6, β-galactosidase specific activity increases with galactose concentration. This means that the extent of induction increases with an inducer level since more galactose may be present to prevent gene *GAL80* repression by releasing a *GAL4*-encoded activator(19). The required inducer level increases with the plasmid copy number. Depending on the copy number, relatively less *GAL4* activator molecules may be required for the full induction of *lacZ* gene expression at low copy number. On the other hand, both strains, S150-2B[cir$^+$]:pRY131 and S150-2B [cir$^+$]:LR1△20B containing high copy level require more *GAL4* activator for the full induction of gene expression than strains S150-2B[cir^o]:pRY131 and S150-2B[cir^o]:pRY171 harboring a low plasmid copy number. Thus, gene dosage for expression is an important factor in determining the required inducer level and the extent of gene expression by induction, but intracellular production of *GAL4* may be an additional limiting factor for induced gene expression.

To determine the optimum galactose concentration, various galactose concentrations were added at different times, i.e., at the mid-exponential phase and at the start of cultivation, while the whole cultivation time including induction time was approximately the same in both cases (ca. 26-27 hours). Fig. 7 shows the results from these two strategies with strain S150-2B[cir$^+$]:LR1△20B. Within a range of low galactose levels, both strategies exhibit a linear correlation between galactose concentration and β-galactosidase activity. However, beyond 4.0g/ℓ of galactose concentration, the rate of increasing β-galactosidase activity decreases when galactose is added at the mid-exponential phase, whereas the linear correlation is maintained up to 8.0g/ℓ of galactose concentration when galactose is added at the start of cultivation. It seems that there is a glucose repression from the start of cultivation. Thus, more galactose is needed to overcome this glucose repression. At 8.0g/ℓ of galactose concentration, β-galactosidase specific activities in both strategies are approximately the same. These results suggest that 8.0g/ℓ of galactose concentration may be sufficient to induce β-ga-

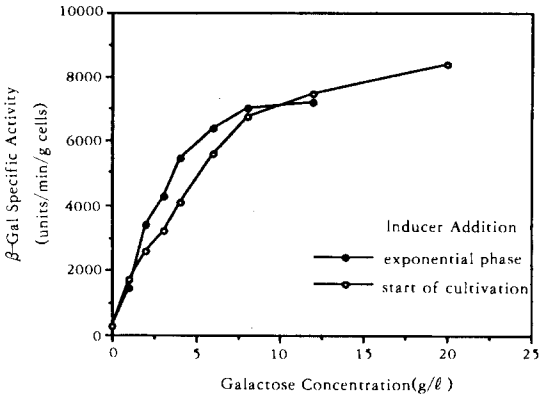
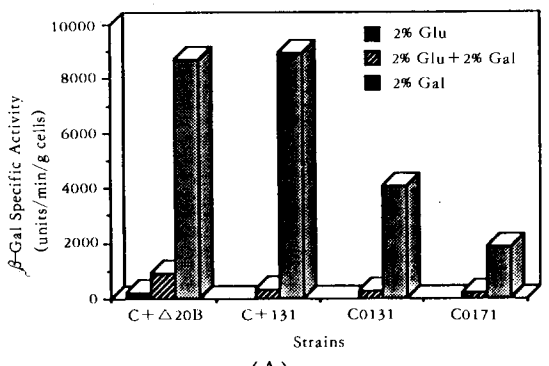


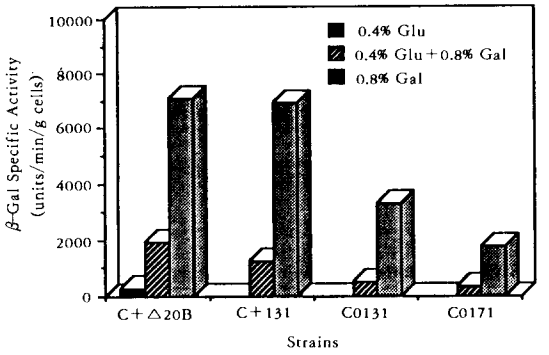
Fig. 7. Effect of galactose concentration on cloned gene expression under variable induction timing with strain S150-2B[cir$^+$]:LR1△20B.

lactosidase synthesis. In addition, this strain S150-2B [cir$^+$]:LR1△20B can produce β-galactosidase without galactose induction, even though in small amounts. This is due to the deletions between UAS_G and *GAL1* TATA box, which reduce the glucose repression(10).

Yeast cells containing a *GAL1-lacZ* fusion plasmid synthesize β-galactosidase only in the presence of galactose, and this synthesis is inhibited by glucose (8). Fig. 8 shows β-galactosidase specific activity at various combinations of glucose and galactose concentration. β-galactosidase specific activity was measured at ca. 50 hours of culturing time to study the effect of glucose repression on cloned gene expression at the late-exponential grwoth phase. In Fig. 8a, the medium contains 2% glucose, 2% galactose, or both, and in Fig. 8b, the medium contains 0.4% glucose, 0.8% galactose, or both. When there was no galactose in the medium, only strain S150-2B[cir$^+$]:LR1△20B could produce β-galactosidase, even though in a small amount. If the medium included galactose only, β-galactosidase activity was very high in each strain. For all strains, β-galactosidase activities were somewhat higher in 2 % galactose than in 0.8% galactose. On the other hand, when the medium contained both glucose and galactose(galactose was added at the start of the cultivation), cloned gene expression was severely repressed in all strains tested, i.e., even in the case of strain S150-2B[cir$^+$]:LR1 △20B. In the case



(A)



(B)

Fig. 8. Glucose repression on gene expression: (A) 2 % glucose (B) 0.4% glucose. Labels for strains are: C+△20B, S150-2B[cir$^+$]:LR1△20B; C+ 131, S150-2B[cir$^+$]:pRY131; C0131, S150-2B [cir^o]:pRY 131; and C0171, S150-2B[cir^o]: pRY171.

of this medium, glucose was completely depleted when β-galactosidase specific activity was measured. On the contrary, galactose concentration was not reduced severely, indicating that glucose was the primary carbon source. This means that the initial glucose concentration could severely inhibit the cloned gene expression until glucose was depleted. However, in a medium with 0.4% glucose and 0.8% galactose, the glucose repression was reduced considerably for all strains by a factor of 2 to 5, indicating production of a fairly high amount of β-galactosidase in the presence of glucose, as shown in Fig. 8b. Therefore, glucose repression can be reduced by applying low glucose concentration even though

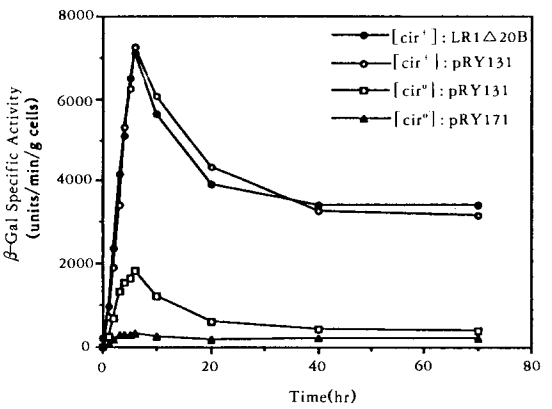


Fig. 9. Gene expression profiles(t=0:galactose addition(8g/ℓ) at the mid-exponential growth phase). Labels for strains are :[cir$^+$]:LR1△ 20B, S150-2B[cir$^+$]:LR1△20B; [cir$^+$]:pRY 131, S150-2B[cir$^+$]:pRY131; [cir^o]:pRY131, S150-2B[cir^o]:pRY131; and[cir^o]:pRY171, S 150-2B[cir^o]:pRY171.

the medium has a low galactose concentration. These results suggest that the relative inducer level(ratio of galactose/glucose concentration) affects the extent of gene expression and glucose repression. Also, generally, the beneficial effect of higher galactose concentrations is weaker than the repressive effect of higher glucose concentrations.

Batch Induction Behavior

Batch induction experiments were performed to study the relationship between induction behavior and plasmid stability and copy number. The medium initially contained 4.0g/ℓ glucose. 8.0g/ℓ of galactose was added either at the mid-exponential phase (Fig. 9) or at the start(Fig. 10) of the batch cultivation to induce gene expression.

If Fig. 9, β-galactosidase specific activity increased linearly with time up to 6 hours. In strains S150-2B [cir$^+$]:LR1△20B and [cir$^+$]:pRY131 which exhibit high stability and copy number level, the initial induction rates were much faster by a factor of 4 to 25 than in strains with low stability and low copy level, i. e., [cir^o]:pRY131 and [cir^o]:pRY171, respectively.

Thus, similar to the induction behaviors as a function of galactose concentration, plasmid stability

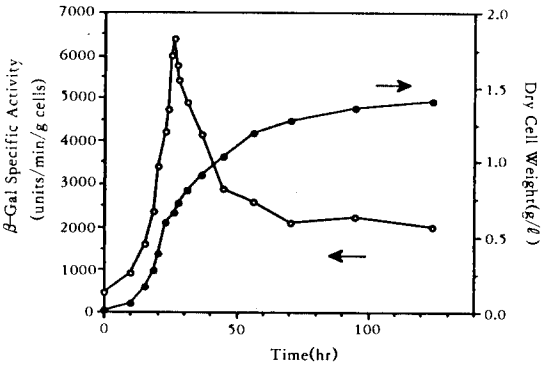


Fig. 10. Batch culture induction behavior with strain S150-2B[cir$^+$]:LR1△20B (t=0: galactose addition(8g/ℓ) at the start of the cultivation).

and copy number are very important determinants of the extent of gene expression. At 6 hours, the levels of β-galactosidase specific activity reached the maximum for all strains, indicating that about 6 hours were required for the full induction of β-galactosidase synthesis. After the peak point, β-galactosidase activity decreased with the fermentation time. The level of β-galactosidase activity at the stationary phase was reduced to less than one-half the peak level. Although it is not known why, the reasons may presumably be deactivation of β-galactosidase, decrease in efficiency of gene expression, or cell deactivation.

In Fig. 10, β-galactosidase synthesis in strain [cir$^+$]: LR1 △ 20B was induced by addition of galactose (8.0g/ℓ) from the start of batch cultivation. Here, the strategy of galactose addition time is different from the previous experiments. β-galactosidase specific activity increased rapidly up to 26 hours to the peak level of about 6400 units. This means that the required time for the full induction was longer than that of the previous set of experiments(6 hours). The reason for this seems to be glucose repression occurring from the start until glucose concentration was reduced to a certain level at which the induction of β-galactosidase synthesis and be activated by galactose.

요 약

효모균에서의 유전자 재조합에 의한 단백질 생산에 미치는 유전학적, 환경적인 요인의 영향을 연구하였다. Plasmid 안정도와 개수는 *REP*$^+$ 체계에서 대단히 높은 반면, *rep*$^-$ 체계에서는 매우 낮았다. 2μm circle plasmid genome을 포함하는 plasmid의 경우에 있어서, [cir^o]형 세포에서의 plasmid 안정도와 개수가 [cir$^+$]형 세포에서보다 높기때문에 [cir$^+$]형 세포가 더 선호되는 세포였다. 유전자 발현은 plasmid 개수와 안정도에 좌우되었다. 촉진제의 양이 유전자 발현에 매우 중요한 역할을 했다. 유전자 발현의 촉진에 필요한 galactose의 농도는 0.8%이면 충분했다. 높은 안정도와 개수를 갖는 plasmid의 경우 촉진속도는 매우 빨랐다. Galactose가 배양의 시작부분부터 첨가될 때가 mid-exponential phase에 첨가될 때보다 유전자 발현의 극대점에 이르는 시간이 길었다. 상대적 촉진제의 양이 증가함에 따라 glucose억제현상은 감소되었다.

REFERENCES

1. C. Gerbaud and M. Guerineau(1980), *Curr. Genet.*, **1**, 219.
2. B. Futcher(1989), *Genetic Engineering-Principles and Methods*, (J. K. Setlow, ed). Vol. **11**, 33, Plenum Press, New York.
3. F.C. Volkert and J.R. Broach(1987), *Biological Research on Industrial Yeasts*, (G.G. Stewart, I. Russell, R.D. Klein, and R.R. Hiebsch, eds), Vol. **2**, 145, CRC Press, Boca Raton, Florida.
4. M. Jayaram, A. Sutton, and J.R. Broach(1985), *Mol. Cell. Biol.*, **5**, 2466.
5. Y. Kikuchi(1983), *Cell*, **35**, 487.
6. D.J. Mead, D.C. J. Gardner, and S.G. Oliver (1986), *Biotechnol. Lett.*, **8**, 391.
7. N.A. Da Silva and J.E. Bailey(1989), *J. Biotechnol.*, **10**, 253.
8. R.R. Yocum, S. Hanley, R. West Jr., and M. Ptashne(1984), *Mol. Cell. Biol.*, **4**, 1985.
9. J.R. Broach(1983), *Methods Enzymol.*, **101**, 307.
10. R.W. West Jr., R.R. Yocum, and M. Ptashne (1984), *Mol. Cell. Biol.*, **4**, 2467.
11. F. Sherman, G. R. Fink, and J.B. Hicks(1986),

Laboratory Course Manual for Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
12. H. Ito, Y. Fukuda, K. Murata, and A. Kimura (1983), *J. Bacteriol.*, **153**, 163.
13. J. H. Miller(1972), *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
14. A. B. Futcher(1988), *Yeast*, **4**, 27.
15. B. E. Veit and W. L. Fangman(1988), *Mol. Cell. Biol.*, **8**, 4949.
16. F. C. Volkert, D. W. Wilson, and J. R. Broach (1989), *Microbiol. Rev.*, **53**, 299.
17. A. B. Rose and J. R. Broach(1990), *Methods Enzymol.*, **185**, 234.
18. T. Som, K. A. Armstrong, F. C. Volkert, and J. R. Broach(1988), *Cell*, 52, 27.
19. J. Ma and M. Ptashne(1987), *Cell*, **50**, 137.