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Transcription from efficient promoters can interfere with plasmid replication and diminish expression of plasmid specified genes

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The copy number of plasmids containing the ColE1 replicon is affected by changes in the transcriptional activity within the plasmid if these changes lead to transcriptional readthrough into the replication region towards the promoter priming DNA replication. Such readthrough e.g., from the *tet* region in pBR322 not only causes overproduction of a peptide known to affect the copy number negatively but also appears to interfere negatively with the replication of the plasmid itself. The proper placement of efficient transcriptional terminators prevents such interference and permits the stable integration of strong promoters. Due to this termination effect, up to 9-fold differences in plasmid copy number were observed, depending upon the particular growth conditions. The higher copy number is of course reflected by higher yields of plasmid-specified gene products indicating the relevance of the above effects for studies of gene expression.

Key words: promoter cloning/plasmid stability/plasmid replication/terminator cloning/gene expression

Introduction

The integration of strong promoters like those of coliphage T5 (Stueber *et al.*, 1978, Gabain and Bujard, 1979) into various cloning vehicles has been successful only since specialized plasmids became available which carried efficient transcriptional terminators downstream of the promoter integration site (Gentz *et al.*, 1981). However, even using these vehicles, some T5 promoters appeared to be unclonable, others were lost at high rates due to either rearrangements within the plasmid or, if no selection pressure was applied, the disappearance of the entire vehicle.

Here we describe experiments which show that at least two effects contribute to this instability: firstly, overproduction of a protein involved in the control of the plasmid copy number, the ROP (repressor of primer) protein (Twigg and Sherrat, 1980; Cesareni *et al.*, 1982) and secondly, transcriptional readthrough into the replication region itself.

Maximizing transcription in plasmids carrying ColE1-type replicons, therefore, may not only destabilize the vector system itself, but may also obscure the quantitation of expression signals because of the dependence of the copy number on transcriptional readthrough. The plasmid vector system used in the experiments presented here was developed for quantitative studies of transcriptional and translational signals and will be described in detail in a forthcoming publication.

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Results

The intracellular copy number of pBR322 at various stages of bacterial growth

Initial experiments suggested a strong dependence of the intracellular copy number of pBR322 on the growth conditions. The plasmid copy number was therefore determined at four different stages of bacterial growth (Figure 1): I, II, III

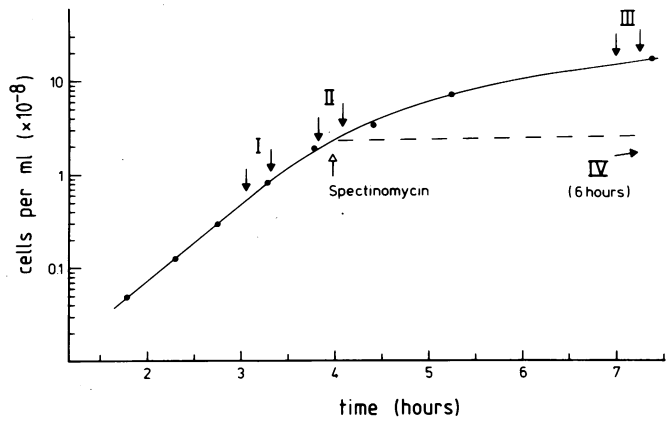

Fig. 1. Definition of cell stages I to IV within the bacterial growth curve. Cell densities were determined by monitoring A_{600} and by direct cell counts. For sample preparation, aliquots were withdrawn within the time periods indicated by arrows. It is obvious that cells in stage I and II already differ in their generation time which is 30 and 40 min, respectively.

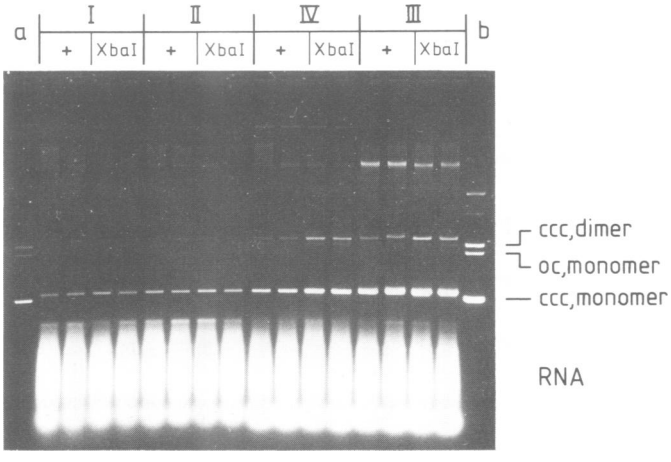

Fig. 2. Plasmid copy numbers of pBR322 and pBR322/XbaI at various stages of growth. Nucleic acid extracts were analyzed by agarose gel electrophoresis. Equal amounts of material as determined by A_{260} (namely 0.18 A_{260} units) were applied to each slot. The RNA which practically determines the absorption at 260 nm is seen in the lower part of the gel. The positions of the different forms of plasmid DNA are pointed out in the controls (**a**, **b**). I to IV indicate the probes from cell stages I to IV, '(+)' refers to pBR322 and 'XbaI' to pBR322/XbaI (Figure 3). The duplicate probes were independently prepared. It can be seen that the fastest growing cells (stage I) contain the lowest amount of plasmid DNA and that at all stages a non-functional coding sequence for ROP always leads to an increased plasmid copy number. More plasmid DNA per total A_{260} is obtained from cells of stage III as compared to cells of stage IV. The quantitative evaluation of this experiment is summarized in Table I and Figure 6.

(corresponding to 0.72 x 10^8, 2.2 x 10^8, and 1.7 x 10^9 cells/ml) and IV (2.2 x 10^8 cells/ml, amplified for 6 h after the addition of spectinomycin). Nucleic acids were extracted from the cells according to Birnboim and Doly (1979), and separated by agarose gel electrophoresis. The relevant parts of the resulting pattern were evaluated by densitometry. This procedure yields highly reproducible results as can be seen in Figures 2 and 4. Two independently prepared samples were always analysed along with concentration standards which allowed an estimate of the amount of DNA per band. Our data show (Table I) that the copy number of pBR322 increases ~4-fold between stage I and stage III and that the increasing copy number appears to correlate inversely with the growth rate of the culture (Figure 2).

It is interesting to note that amplification at stage II leads in stage IV to a 4-fold increase in the copy number which exceeds the copy number of a stage III cell by ~50%. However, the yield of plasmid DNA per ml of culture is more than three times higher in stage III as compared to stage IV cultures (Table I).

Integration of a XbaI linker into the PvuII site of pBR322 increases the copy number of the plasmid

We have integrated a synthetic octanucleotide carrying a cleavage site for the restriction endonuclease *Xba*I into the *Pvu*II site of pBR322 [position 2065 according to Sutcliffe (1979)] for two reasons: firstly, to disrupt the coding region of the ROP protein and, secondly to have a convenient site for either integrating a transcriptional termination signal or replacing the entire region coding for tetracycline resistance. The resulting plasmid pBR322/XbaI has an increased copy number per cell if compared to pBR322 (Figure 2, Table I). This increase is most significant in stage IV cells (after amplification) where a 2- to 3-fold difference in plasmid molecules per cell is observed.

pDS1 tolerates the integration of coliphage T5 promoter P_{207}

Using pLBU3-like vectors (Figure 3) developed for the cloning of efficient promoters (Gentz *et al.*, 1981) we observed, upon integration of some T5 promoters, either very low copy numbers per cell or various rearrangements within the vector or – if no selection pressure was maintained – loss of the entire plasmid. This suggested that either the overproduction of *tet*-proteins or interference with the plasmid's replication, or a combination of both, may have been responsible for the instability of these vectors.

We have, therefore, redesigned our vector system and pDS1 (Figure 3) is a representative of a new plasmid family which carries two indicator genes whose products are not only easily assayed but also tolerated in large amounts by the

Table I. Dependence of plasmid copy number and efficiency of CAT expression on specific vector properties and stages of cellular growth

Plasmid	Total nucleic acids[a] (A_{260} units/100 ml culture)				Yield of plasmid DNA[b] (10^{-12} mol/100 ml culture)				Copy number of plasmid per cell[c]				Molar ratio of CAT to TU protein[d]			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
pBR322	14.3 ±0.1	45.9 ±0.4	108 ±19	87.3 ±0.5	0.35 ±0.04	2.1 ±0.1	38.8 ±3.0	10.7 ±1.3	33 ± 5	67 ±5	141 ±11	269 ±43				
pBR322/XbaI	19.9 ±0.2	52.9 ±3.1	101 ± 2	88.4 ±1.9	0.58 ±0.10	3.8 ±0.1	57.2 ±4.8	26.4 ±0.4	56 ±10	113 ±5	223 ±19	662 ±19				
pDS1	17.6 ±0.5	49.3 ±3.0	110 ± 3	87.1 ±3.1	0.48 ±0.03	3.4 ±0.4	42.6 ±2.6	28.3 ±0.3	47 ± 1	84 ±6	149 ±11	569 ±13				
pDS1/P_{207}	18.6 ±2.0	54.2 ±1.5	110 ±15	88.0 ±8.3	0.22 ±0.01	1.3 ±0.1	46 ±4	3.2 ±0.2	23 ± 2	34 ±1	167 ±12	66 ± 3	0.17 ±0.01	0.15 ±0.00	0.63 ±0.06	0.40 ±0.05
pDS1/P_{207},$t_0$1$^+$	20.0 ±0.7	56.5 ±1.0	95 ± 1	87.4 ±9.1	0.55 ±0.04	3.1 ±0.2	51 ±1	24.4 ±2.6	56 ± 1	82 ±4	191 ± 5	553 ±49	<0.06	<0.06	<0.10	0.11 ±0.01
pDS1/P_{207},$t_0$1$^-$	24.3 ±0.0	48.8 ±2.5	108 ± 1	82.6 ±2.6	0.46 ±0.01	1.5 ±0.1	56.5 ±3.5	4.0 ±0.4	32 ± 0	44 ±2	200 ±11	88 ± 5	0.17 ±0.01	0.14 ±0.01	0.48 ±0.01	0.47 ±0.03
pDS1/P_{207},$t_0$2$^+$	23.9 ±1.1	47.0 ±5.3	111 ± 5	81.4 ±1.9	0.81 ±0.10	2.7 ±0.1	79.0 ±0.2	20.4 ±1.4	66 ± 2	79 ±1	284 ± 4	498 ±73	0.59 ±0.02	0.58 ±0.01	2.29 ±0.04	1.53 ±0.10
pDS1/P_{207},$t_0$2$^-$	24.4 ±0.8	49.9 ±5.2	108 ± 7	89.5 ±5.3	0.32 ±0.01	1.1 ±0.1	44.8 ±5.0	3.5 ±0.1	23 ± 1	29 ±2	151 ±21	67 ± 5	0.20 ±0.01	0.14 ±0.01	0.72 ±0.15	0.58 ±0.03

Summary of the results obtained from quantitative evaluation of experiments as shown in Figures 2, 4, and 5. The numbers given are mean values of two independent determinations with their maximal deviations.

[a]The total nucleic acids extracted from probes sampled at different stages of growth (I to IV). By relating the cell density of the original bacterial culture to the A_{260} of the extract the nucleic acid content per cell at various stages of growth can be calculated. It is interesting to compare the nucleic acid content of cells at stage II and IV: though host DNA and most of protein synthesis is blocked during amplification the continuing transcription significantly increases A_{260}.

[b]Plasmid yields per volume of culture. For plasmid isolation, stage III instead of stage IV cells should be used. This appears most important when transcription interferes with plasmid replication where up to 15-fold differences are observed between the two stages (pDS1/P_{207}). Best plasmid yields were obtained at stage III with pDS1/P_{207},$t_0$2$^+$: 0.8 nmol or 2.1 mg per liter of culture.

[c]The influence of cell stage and transcriptional activity on plasmid copy number per cell. According to these data plasmid copy number increases with generation time of the cell. Readthrough transcription has the most profound effect in stage IV, where up to 9-fold differences are found (e.g., pDS1 *versus* pDS1/P_{207}). The values were obtained by relating the amount of monomeric plasmid DNA obtained from analyses as described in Figure 4 and in Materials and methods to the number of cells used for the individual nucleic acid preparations.

[d]The amount of CAT produced under various conditions. To allow comparison, all values are standardised against the intracellular concentration of TU protein (mol. wt. 4.3 x 10^4, Arai *et al.*, 1980, 8.9 x 10^4 copies/cell, Pedersen *et al.*, 1978). Most efficient CAT production is observed under conditions which permit high intracellular plasmid copy number (stage III, no transcriptional readthrough, pDS1/P_{207},$t_0$2$^+$): 20 x 10^4 copies per cell (mol. wt. of CAT 2.57 x 10^4, Shaw *et al.*, 1979). The synthesis of CAT can be significantly increased by changing the translation efficiency of the mRNA (unpublished results). In comparing CAT production from pDS1/P_{207},$t_0$1$^+$ and pDS1/P_{207},$t_0$2$^+$ the efficiency of t_0 is estimated to be at least 95%. Plasmid-coded proteins are synthesized to a significant extent in the presence of spectinomycin (CAT production in stage II *versus* IV cells).

Escherichia coli cell (unpublished observation). Promoters and terminators can be integrated at several unique cleavage sites and since the 63 amino acid ROP protein is non-functional, plasmid yields are comparable to those of pBR322/XbaI (Table I).

Using pDS1 it was possible to integrate a 211-bp fragment containing the strong T5 promoter P_{207} (Gentz *et al.*, 1981) into the *Eco*RI site (Figure 3) without the prior positioning of a downstream terminator. The stability of the resulting pDS1/P_{207} demonstrated that destruction of the *rop* gene and replacement of the *tet* region by the *cat* gene had made the vector less sensitive to high level transcription from P_{207}. However, when the copy number of pDS1/P_{207} was monitored it was only about one half to one third of pDS1 in stage I and II and even less than one eighth in stage IV (Figures 4 and 6, Table I). P_{207} was, therefore, tolerated but it still had a profound effect on the plasmid's replication.

Proper placement of an efficient terminator within pDS1/P_{207} reestablishes the copy number of pDS1

The pDS1/P_{207} vector contains two integration sites for terminators (Figure 3). Site 1 (*Hind*III) separates the two indicator genes and site 2 (*Xba*I) delineates the *dhfr/cat* transcriptional unit from the replication region. Both of these sites were used for integrating the efficient terminator t_0 of coliphage lambda (McKinney *et al.*, 1981). The copy number of both the resulting plasmids pDS1/P_{207},$t_0$1$^+$ and pDS1/P_{207},$t_0$2$^+$ was restored to the level of pDS1 (Figures 4 and 6, Table I). This shows that both integration sites are equally efficient for preventing transcriptional readthrough into the replication region. If, however, the terminator-carrying 103-bp fragment was inserted in the opposite orientation (pDS1/P_{207},$t_0$1$^-$ and pDS1/P_{207},$t_0$2$^-$), transcription was not interrupted and copy numbers were reduced to the same level as that of pDS1/P_{207} (Figures 4 and 6, Table I). These results clearly show that transcription from P_{207} directly interferes with the replication of the plasmid.

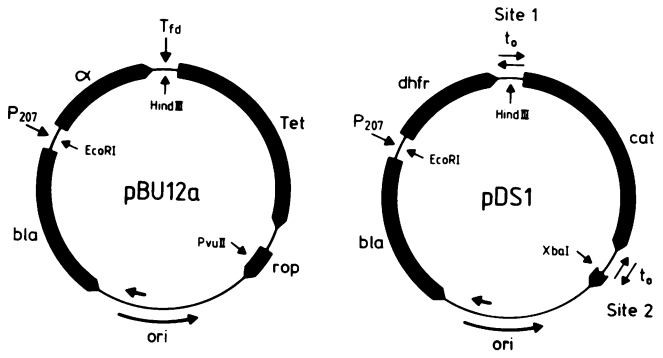

Fig. 3. Genetic and physical map of pBU12a and pDS1. Both plasmids contain two easily assayable functions which can be brought under the control of a single promoter (P) but which can also be separated by the insertion of a terminator (T). These functions are for the pBU series: β-galactosidase activity (*via* α-complementation) and tetracycline resistance *(tet)*; for the pDS series: trimethoprime (*via* dihydrofolate reductase, *dhfr*) and chloramphenicol (chloramphenicol acetyl transferase, *cat*) resistance. Both plasmids confer ampicillin resistance (*via* β-lactamase, *bla*) and carry the ColE1 replication region of pBR322 *(ori)*. A major difference of the two plasmid systems is the presence of the intact *rop* gene in pBU12a and a second site for the integration of a terminator (site 2) in pDS1. The direction of transcription of the coding sequences is indicated. Positions and directions of transcription for the primer RNA and RNA I are represented by arrows. The physical maps of both plasmids are not drawn to scale. Intergration of coliphage fd terminator into pBU12a results in pLBU3 which was used for the cloning of coliphage T5 promoters (Gentz *et al.*, 1981). Insertion of terminator t_0 from coliphage lambda at site 1 or 2 of pDS1 makes this plasmid an even better vehicle for promoter cloning. The following designations are used: pDS1/P_{207}, promoter P_{207} inserted into the *Eco*RI site; pDS1/P_{207},$t_0$1$^+$, pDS1/P_{207},$t_0$1$^-$, termintor t_0 inserted into site 1 of pDS1/P_{207} in functional and non-functional orientation, respectively; pDS1/P_{207},$t_0$2$^+$, pDS1/P_{207},$t_0$2$^-$, same as above but terminator inserted into site 2.

Increased plasmid copy number yields more plasmid-specified gene products

When total protein of cells harbouring the various vectors were analysed in SDS-polyacrylamide gels the effect of plasmid copy number and transcriptional termination could be demonstrated by monitoring the production of chloramphenicol transacetylase (CAT). Figure 5 shows a portion of the protein pattern obtained at stages I to IV from cells containing the plasmids discussed above. All cells in which the number of plasmids is reduced owing to either the absence of a terminator (pDS1/P_{207}), or the insertion of t_0 in non-functional orientation (pDS1/P_{207},$t_0$1$^-$ and pDS1/P_{207},$t_0$2$^-$) produce the same amount of CAT. In contrast, if t_0 is inserted into site 1 in the functional orientation, little or no CAT can be seen despite the increased copy number of the plasmid. High yields of CAT are obtained when functional t_0 is positioned in site 2, thus allowing full transcription from P_{207} through the *cat* gene, but preventing a reduction of the

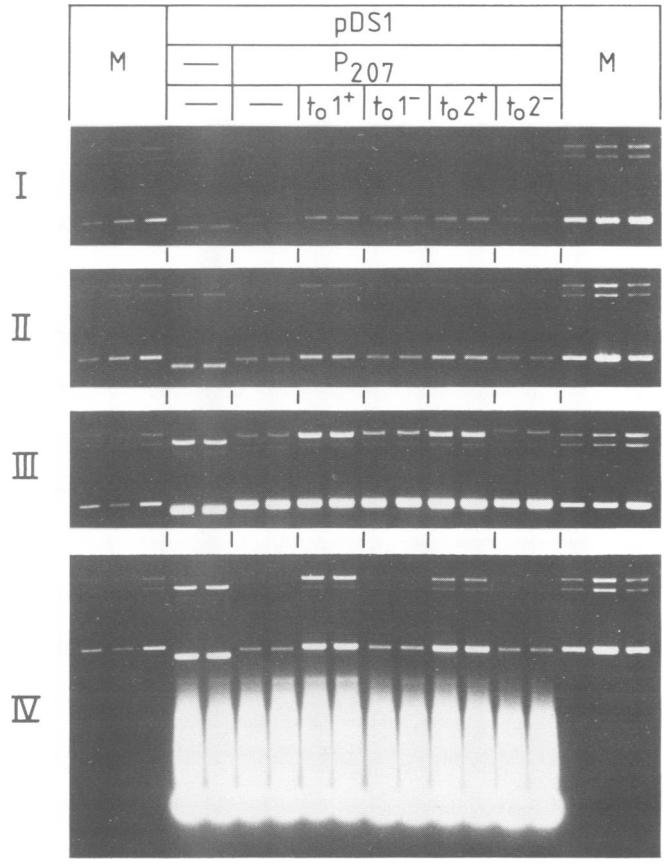

Fig. 4. Electrophoretic analysis of pDS1 derivatives at various cell stages. The roman numerals designate the preparations from cells of stages I to IV, M are concentration standards. The duplicate probes can be identified by the heading using the nomenclature given in Figure 3. The effects of promoter and terminator function on plasmid copy number are most clearly seen in IV. Equal amounts of A_{260} (namely 0.25 A_{260} units per sample) were applied to all four gels. The quantitation of these data is summarized in Table I.

plasmid copy number by reading through into the replication region.

Estimates of the total amount of CAT were carried out by quantitating the stained material in the 'CAT-band' in relation to the material representing the TU-protein. Assuming comparable molar extinction coefficients, the data summarized in the right hand column of Table I were obtained. Taking into account the change in copy number, it can be seen that t_0 in site 1 reduces production of CAT >10-fold. On the other hand, the two orientations of t_0 in site 2 show that in stage III the amount of CAT is reduced from 20 x 10^4 to 6 x 10^4 copies per cell owing to the drop in plasmid copy number. A surprisingly high yield of CAT is obtained in cells of stage IV containing pDS1/P_{207},$t_0$2$^+$ where 14 x 10^4 copies corresponding to ~8% of the total protein mass are found.

Discussion

The replication of ColE1-like replicons is regulated by transcriptional events within the replication region itself (Itoh and Tomizawa, 1980; Lacatena and Cesareni, 1981; Tomizawa and Itoh, 1981; Wong *et al.*, 1982) as well as by at least one plasmid-coded protein, the 63 amino acid ROP protein (Twigg and Sherrat, 1980; Cesareni *et al.*, 1982) which appears to be involved in copy number control. Consequently, upsetting transcription around the origin of replication or overproducing ROP may not only affect propagation or maintenance of such plasmids but also – due to changes of the copy number – obscure the efficiency of expression of plasmid-coded proteins. Integration of the strong promoter P_{207} of coliphage T5 into pLBu3 (Gentz *et al.* 1981, Figure 3) does in fact distinctly lower the copy number of the resulting plasmid pGBu207. This copy number is raised again by the insertion of an octanucleotide (*Xba*I linker) into the coding sequence of ROP (data not shown). In an analogous experiment (Figures 2 and 3, Table I) the copy number of pBR322 is raised 1.6- to 2.5-fold; the magnitude of the effect depends on the cell stage as does the basic copy number. The latter increases 4-fold from early log phase (stage I) to early stationary phase (stage III) and is highest in stage IV (after amplification, Table I).

From these data we conclude: (i) destruction of the *rop* gene raises the copy number of pBR322 significantly (see also Twigg and Sherrat, 1980) and prevents overproduction of that protein by readthrough transcription as it most likely occurs in pGBu207; and (ii) intracellular concentrations of plasmid increase with increasing generation time and are highest after amplification, best yields per unit volume of culture, however, are obtained in early stationary phase (e.g.,

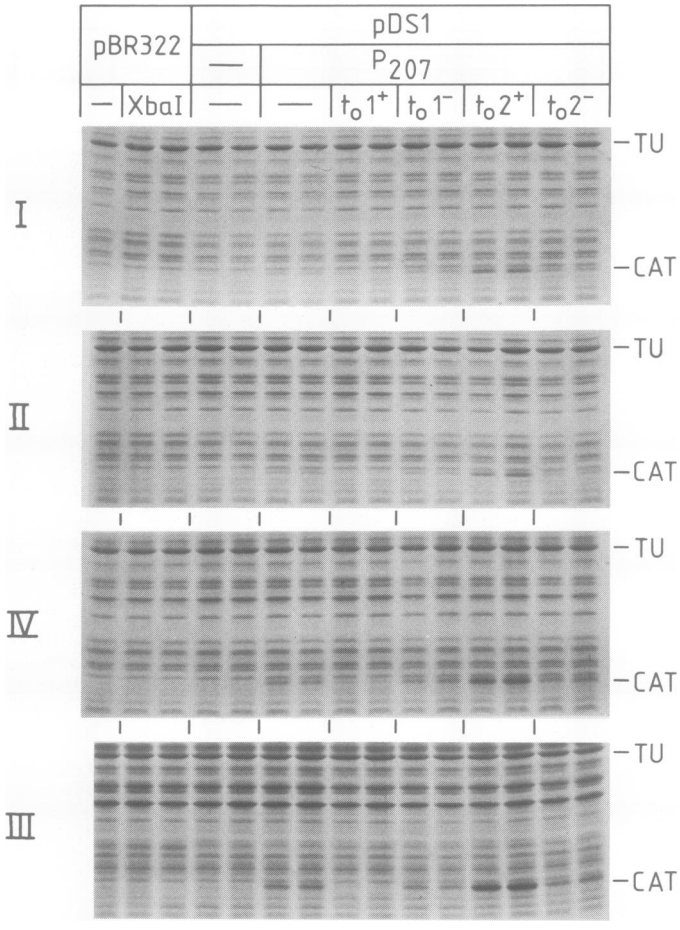

Fig. 5. Production of CAT protein in relation to plasmid copy number. The nomenclature of the four SDS-polyacrylamide gels is identical to that in Figure 4 except that probes of pBR322 and pBR322/XbaI containing cells are included. Equal amounts of the various cultures (0.6 x 10^8 cells of stage I, 0.7 x 10^8 cells of stages II through IV) were prepared and applied to the gels. The positions of TU and CAT protein are indicated. The results of the quantitative evaluation are summarized in Table I.

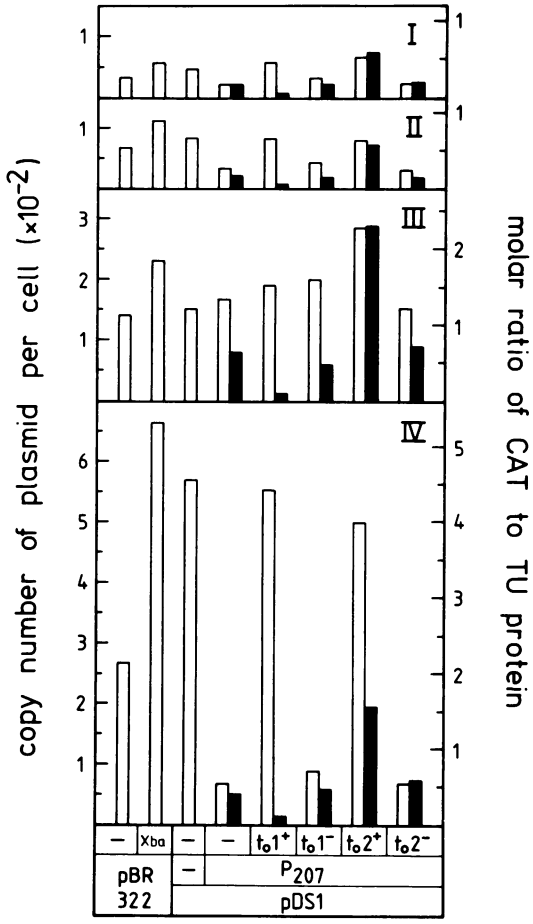

Fig. 6. CAT production and plasmid copy number at different cell stages. The various probes are defined as in Figure 5. The white and black bars indicate plasmid copy numbers per cell and molar ratios of CAT *versus* TU protein, respectively. Best yields of the protein are obtained at stage III (pDS1/P_{207},$t_0$2$^+$). It can be easily seen that in stage III the differences in copy number seen in stage I and II are much less pronounced, however, this is not reflected in the synthesis of CAT.

with pDS1/P_{207},$t_0$2$^+$ up to 800 pmol or 2.1 mg per liter of culture, Table I).

For analysing whether transcriptional readthrough into the ColE1 replication region directly interferes with the replication of the plasmid, we used pDS1 (Figure 3), a representative of a recently developed vector system (Stueber *et al.*, in preparation) which allows the selective cloning and *in vivo* quantitation of transcriptional as well as translational signals. In pDS1 the ROP protein is not functional and transcription into the replication region from any integrated promoter can be prevented by the insertion of terminators at two different sites (Figure 3). Figure 4 and Table I show that integration of promoter P_{207} into pDS1 (resulting in pDS1/P_{207}) lowers the copy number 2- to 3-fold again depending on the phase of cellular growth. The original amount of plasmid per cell is, however, reestablished if the efficient terminator t_0 of phage lambda (McKinney and Rosenberg, 1981) is integrated in functional orientation at site 1 or 2, demonstrating the interference of transcriptional readthrough from P_{207} with the plasmid's replication. It is interesting to note that the most dramatic interference is observed at stage IV (up to 8-fold differences in copy number) whereas in stationary phase (stage III) the prevention of transcriptional readthrough has a rather small effect. We assume that this reflects the difference in transcriptional activity at the two cell stages. Finally, as seen in Figure 4, replication of the dimeric form of the plasmids appears considerably more disturbed than the monomeric form. Our results show that: (i) in pDS1/P_{207} transcriptional readthrough into the origin of replication towards the promoter priming DNA replication (Itoh and Tomizawa, 1981) diminishes the plasmid copy number ~2-fold; and (ii) inactivation of the *rop* gene raises the copy number in pBR322 by a factor of ~2. This suggests that in a hypothetical plasmid carrying P_{207}, as well as the intact *rop*-coding sequence, an at least 4-fold reduction of the copy number would be expected. Since, however, in such a plasmid the enhanced transcription would most likely also cause an overproduction of ROP, the copy number of the plasmid may be reduced below the limit necessary for its stable propagation. The finding that pGBu207 is not viable upon removal of the phage fd terminator (Gentz *et al.*, 1981) whereas pDS1/P_{207} is stably maintained, though at a reduced copy number, strongly supports this hypothesis.

Efficient transcription and translation are prerequisites for high level expression of cloned genes. Since, however, strong promoters can drastically influence the copy number of cloning vehicles, as described above, we have monitored the effect of gene dosage (i.e., copy number) on the production of CAT protein in varous pDS1 plasmids at different stages of cell growth. As seen in Figures 5, and 6 and Table I, for stage I and II there is a rather good linear relationship between plasmid copy number and synthesis of CAT protein if different plasmids are compared within one cell stage: all plasmids with reduced copy number (pDS1/P_{207}, pDS1/P_{207},$t_0$1$^-$, pDS1/P_{207},$t_0$2$^-$) produce about the same amount of CAT protein whereas cells harbouring pDS1/P_{207},$t_0$2$^+$ having a 3-fold higher copy number contain correspondingly more. This linearity is not observed in stage III where high copy numbers yield large amounts of protein only in the case of pDS1/P_{207},$t_0$2$^+$. An explanation might be that in stationary phase the high copy number, e.g., of pDS1/P_{207},$t_0$1$^-$, is reached only after the rates of transcription and translation have already dropped.

The synthesis of CAT protein per plasmid copy at various stages of growth was calculated using the intracellular concentration of TU protein, which under our conditions is 89 000 copies per cell (Figure 5, Table I) as a standard. The results show that, at all stages of growth, the gene dosage directly affects the yield of CAT protein. Thus, having t_0 in functional orientation at position 2 increases CAT synthesis 3- to 4-fold. Maximal yields are reached in stage III where 20 x 10^4 copies of CAT per cell are produced.

It is interesting to note that, despite the presence of spectinomycin, rather good yields of CAT protein can be obtained (e.g., 14 x 10^4 copies per cell or 8% of the total protein mass for pDS1/P_{207},$t_0$2$^+$), and it seems that the gene dosage efficiently specifies the programme of the residual protein synthesis. Pulse labelling proteins under these conditions might, therefore, be a simple way to identify plasmid-coded gene products.

Materials and methods

Bacterial growth

Overnight cultures of plasmid harbouring *E. coli* (C 600 r$^-$ Δlac M15) in Luria broth were diluted 1:1000 into fresh medium containing 100 μg/ml ampicillin. The growth rates of the cultures were determined by monitoring A_{600} and by direct cell counts. All cultures exhibited closely comparable growth rates. For DNA and protein analysis samples were withdrawn at various times and cell densities were determined by A_{600} measurements and by direct cell counts before the bacteria were sedimented. Typical cell densities at various stages of growth were as follows: stage I, A_{600} = 0.25 ≙ 0.72 x 10^8 cells/ml; stage II, A_{600} = 0.65 ≙ 2.2 x 10^8 cells/ml; stage III, A_{600} = 3.5 ≙ 1.7 x 10^9 cells/ml; stage IV, same as stage II (after addition of spectinomycin to 300 μg/ml final concentration and further incubation at 37°C for 6 h).

Analysis of plasmid DNA

To determine the intracellular plasmid content, 20 ml (stage I) or 10 ml (stage II to IV) of bacterial cultures were centrifuged at 8000 r.p.m. for 6 min and the resulting sediment was treated as described by Birnboim and Doly (1979). All determinations were carried out in duplicate. Whenever necessary, samples were adjusted to identical A_{260} before they were applied to 0.7% agarose gels along with DNA concentration standards. Electrophoresis was carried out for 4 h at 3.4 V/cm and room temperature (Hayward and Smith, 1972). The gels were stained in aqueous ethidium bromide solution (1 μg/ml), illuminated with u.v. light, and photographed by a series of increasing exposure times on Ilford Pan F film. The amounts of monomeric plasmid DNA were quantitated with respect to the concentration standards by microdensitometry with a Joyce-Loebl 3CS.

Analysis of proteins

Aliquots of the various bacterial cultures (0.6 x 10^8 cells of stage I, 0.7 x 10^8 cells of stage II through IV) were centrifuged for 1 min at 10 000 r.p.m. and the resulting pellets were resuspended in sample buffer (3% SDS, 5% sucrose). After heating to 95°C for 5 min the probes were applied to 12% (30:0.8) polyacrylamide slab gels and electrophoresis was carried out according to Laemmli (1970). After 3 h at 12 V/cm and room temperature the gels were stained (Coomassie blue R-250) and destained. Photographs of the protein patterns on Ilford Pan F film were quantitatively evaluated by microdensitometry using the TU protein as an internal standard.

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