Role of the colicin A lysis protein in the expression of the colicin A operon

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The involvement of the *cal* gene, which encodes the colicin A lysis protein, in the expression of the colicin A operon is demonstrated. Colicin A synthesis by *Escherichia coli* was studied at various temperatures in cells containing either the wild-type colicin A operon or the colicin A operon with the *cal* gene deleted. The amount of colicin A produced was lower in cells containing the colicin A operon devoid of the *cal* gene than in wild-type cells. In cells treated with the antibiotic globomycin, the synthesis of colicin A was blocked in null *cal* mutants at all temperatures. It was blocked only at low temperature in cells containing the wild-type colicin A operon, but not in cells subjected to heat shock or azide treatment. The *cal* gene product may be an activator of colicin A expression and of its own expression. An unidentified product, possibly a heat-shock protein, may also be involved and could complement the *cal* gene product in some situations.

Keywords: *Escherichia coli*, colicin, colicin lysis protein, globomycin, heat-shock protein

INTRODUCTION

The colicin A lysis protein (Cal) is a lipopeptide synthesized by cells of *Escherichia coli* carrying the plasmid pColA. Its structural gene, *cal*, is in the colicin A operon downstream from the colicin A structural gene, *caa*, both genes being under the control of an SOS promoter (Lloubès *et al.*, 1986). The two genes are expressed together after addition of mutagenic agents, but their products are addressed differently. Colicin A is a globular protein which accumulates in the cytoplasm before being exported into the medium with the help of Cal by an unknown mechanism. Cal is synthesized as a precursor that matures by acylation and processing in the inner membrane like all other lipoproteins (Braun & Wu, 1994).

The regulation of colicin operons is complex. They are under the control of SOS regulation, catabolite repression, stringent response, anaerobiosis (Pugsley, 1984; Eraso & Weinstock, 1992; Ferrer *et al.*, 1996) and growth phase (Eraso *et al.*, 1996). The expression of the colicin A operon is significantly delayed at low temperature. However, a heat shock restores normal synthesis, except in an *rpoH* mutant deficient in the RNA polymerase sigma factor σ^{32} , suggesting that one or more heat-shock proteins play a role in the expression of

the colicin A operon (Cavard, 1995). Similar results have been obtained for an operon containing only the *cal* gene, indicating that the synthesis of Cal is slowed down at low temperature. The insertion into the inner membrane of the precursor of Cal and its subsequent acylation and processing might be temperature-dependent like those of Braun's lipoprotein (Tian *et al.*, 1989). Consequently, the unmodified precursor form of Cal, pCal, may be involved in the delay of colicin A expression at low temperature (Cavard, 1995).

To investigate this possibility, in the study described here, cells induced for colicin A synthesis were treated with globomycin, a specific inhibitor of LspA, the lipoprotein signal peptidase that cleaves the acylated precursor form of lipoproteins into apolipoprotein and signal peptide (Inukai *et al.*, 1978; Hussain *et al.*, 1980). Globomycin provokes accumulation of the modified precursor form of Cal, pCal^m, and does not alter colicin A synthesis in cells induced at either 37 or 42 °C (Cavard *et al.*, 1989; Cavard, 1992). In induced cells grown at 30 °C in rich medium, globomycin significantly reduced the expression of the colicin A operon. In cells devoid of the *cal* gene, it blocked expression of the colicin A at all temperatures. This is the first time that such a phenotype caused by a null mutation of a gene encoding a colicin lysis protein has been described. The *cal* gene product

may be an activator of the expression of the colicin A operon, and an uncharacterized member of the heat shock regulon may also be involved.

METHODS

Bacterial strains and plasmids. *Escherichia coli* K12 W3110 (Nal^R), *E. coli* W3110 *degP41* and *E. coli* 1320 *rpoH16 zbf50::Tn10* are from the author's laboratory collection (Cavard *et al.*, 1989, Cavard, 1995). The plasmid pColA9 contains the wild-type colicin A operon (Lloubès *et al.*, 1986). Plasmids pJMM1 and pColA9F1 contain the colicin A operon from which the *cal* gene has been deleted. pJMM1 is pColA with a Tn1 transposon located in the intergenic area between the *caa* and the *cal* genes; pColA9F1 is pColA9 from which a *StuI*-*EcoRV* fragment encoding the *cai* (colicin A immunity) and the *cal* genes has been deleted (Geli *et al.*, 1986). Plasmids containing a mutation within the *cal* gene have been constructed from pColA9 (Howard *et al.*, 1989). They include plasmids containing a single base substitution leading to an amino acid substitution in the mature form of Cal: pA2 (R7E), pG4 (G11E), pV1 (V14R); and plasmids with a stop codon in the *cal* gene: pS16 and pS18, which encode a truncated Cal containing the first 16 and the first 18 N-terminal amino acids, respectively.

Growth conditions. Strains were grown at 30 °C with shaking in LB medium or in M9 medium supplemented with thiamin (1 µg ml⁻¹), lactate (0.4%, v/v) and Casamino acids (0.01%, w/v). The OD₆₀₀ of the cultures was adjusted to 1 before induction with 300 ng mitomycin C ml⁻¹. At time zero of induction, the cultures were incubated at 30 °C, 37 °C or 42 °C. Globomycin, a gift of Dr M. Inukai (Sankyo Company, Tokyo, Japan), was either dissolved in methanol (10 mg ml⁻¹) or in dimethylsulfoxide (5 mg ml⁻¹), and added to a final concentration of 100 µg ml⁻¹ after 15–20 min induction. Sodium azide (either 2 or 3 mM as indicated), potassium cyanide (2 mM) and EDTA (3 mM) were added at time zero of induction.

Electrophoresis and immunoblotting. Routinely, 15 µl of culture was taken after 3 h induction at 42 °C, 3.5 h at 37 °C and 4 h at 30 °C, mixed with 10 µl of loading buffer, heated to 96 °C and loaded either on an 11% polyacrylamide-SDS gel for colicin A identification or on an 8–16% polyacrylamide-urea-SDS gel for Cal identification. Proteins in unstained gels were transferred onto a nitrocellulose filter (200 nm pore size; Scheicher & Schuell). To detect colicin A, the blots were incubated with either polyclonal or monoclonal antibodies. Serum from a rabbit inoculated with a hybrid protein, called NS protein, containing the N-terminal part of colicin A fused to the sequence of the mature Cal except for the first N-terminal cysteine residue (Howard & Lindsay, 1992) was used to detect Cal.

Radioactive labelling. Cells were grown in M9 medium and induced with mitomycin C. After 70 min induction at 37 °C or 130 min at 30 °C, [³⁵S]Protein Labeling Mix from NEN (1175 Ci mmol⁻¹) was added at 20 µCi ml⁻¹ (1 µCi = 37 kBq) and chased 1 min later with a mix of unlabelled methionine and cysteine (500 µg ml⁻¹). At various times thereafter, samples of the cultures were taken and immunoprecipitated with polyclonal antibodies against colicin A as described previously (Cavard, 1992).

RESULTS

The *cal* gene is required for colicin A synthesis in the presence of globomycin

The two gene products of the colicin A operon (colicin A and Cal) are synthesized immediately after induction by a mutagenic agent, except at 30 °C – at this temperature synthesis is delayed. To test the involvement of the precursor form of Cal in this delay, induced cells were treated with globomycin. Cells grown in rich medium at 30 °C were induced with mitomycin C, a mutagenic agent, and incubated at various temperatures in the presence or absence of globomycin. After induction, samples were analysed by SDS-PAGE (Fig. 1a). In W3110(pColA9) cells (containing the wild-type colicin A operon), large amounts of colicin A were produced at 37 °C and 42 °C in the presence or absence of globomycin. At 30 °C, colicin A accumulated in untreated cells, but not in the presence of globomycin.

The experiment was repeated with W3110 carrying either pColA9F1 (Fig. 1a) or pJMM1 (not shown), which both contain the colicin A operon with the *cal* gene deleted. In the absence of globomycin, colicin A production was somewhat reduced at all temperatures compared to that of cells carrying pColA9. But, strikingly, in the presence of globomycin, colicin A synthesis was inhibited at all temperatures. Thus the presence of the *cal* gene may be required for normal production of colicin A, but, more importantly, is essential for colicin A synthesis in the presence of globomycin.

Various *cal* mutants from the laboratory collection were tested for production of colicin A. Colicin A production was similar to that in the wild-type in mutants with point mutations in the region of the *cal* gene encoding the mature form of Cal and in mutants with deletions of the C-terminal end of Cal. The synthesis of colicin A in induced W3110 cells carrying plasmid pA2 (R7E), or pV1 (V14R), or pS18 (which encodes a truncated Cal containing the first 18 N-terminal amino acids) (Fig. 1a), or pG4 (G11E), or pS16 (which encodes a truncated Cal containing the first 16 N-terminal amino acids) (not shown) was similar to that in cells carrying pColA9 although these plasmids encode inactive Cals. Thus the mature form of Cal seemed not to be responsible for the phenotype of null *cal* mutants described above, suggesting that the sequence signal of Cal might be involved.

The amount of colicin A produced was analysed by Western blotting using monoclonal antibodies against colicin A (Fig. 1b). This detection method provided evidence for colicin A production in all cells. In W3110(pColA9), high amounts of colicin A were detected in both the presence and absence of globomycin at 37 and 42 °C, whereas only small amounts were produced at 30 °C in the presence of globomycin. In W3110(pJMM1), the amounts of colicin A were significantly higher in the absence than in the presence of globomycin at each temperature, but were much reduced compared with W3110(pColA9). No colicin A fragments

Fig. 1. Effect of globomycin on the synthesis of colicin A at various temperatures. W3110 cells carrying pColA9, pColA9F1, pJMM1, pA2, pV1 or pS18 were induced with mitomycin C and incubated at the temperatures indicated. They were treated with globomycin (Globo) (+) or left untreated (–). After induction, samples were analysed by SDS-PAGE (a). The relevant parts of the gels stained with Coomassie blue are presented. The position of the colicin A is indicated by an arrowhead. Numbers on the right indicate the molecular masses in kDa of the standard proteins. (b) The blots were probed with the anti-colicin A monoclonal antibody 1C11 (only the reactive portion of the blots is shown).

were detected, indicating that proteolysis did not occur, particularly as the C-terminal part of colicin A is highly resistant to proteases (Martinez *et al.*, 1983) and would accumulate after proteolytic degradation.

Effect of heat shock and azide treatment on the synthesis of colicin A in the presence of globomycin

The delay in the synthesis of colicin A observed at 30 °C in the absence of globomycin is suppressed by either heat shock or treatment with azide or EDTA (Cavard, 1995). The effects of these treatments were therefore tested in the presence of globomycin (Fig. 2a). EDTA did not modify the production of colicin A in the presence of globomycin, at any temperature. In contrast, a heat

shock of 10 min at 42 °C restored the production of colicin A in the presence of globomycin by induced W3110(pColA9) cells incubated at 30 °C. Large amounts of colicin A were produced in the presence of globomycin when the heat shock was at the start of induction, and smaller amounts later during induction the heat shock was carried out. No such effect was obtained in an *rpoH* mutant deficient in σ^{32} , the sigma factor of the RNA polymerase that transcribes two-thirds of the heat-shock genes. No change of colicin A production was observed after a heat shock of 10 or 20 min in W3110(pJMM1) cells. Thus both the *cal* gene product and one or more heat-shock protein(s) were required for the production of colicin A in the presence of globomycin.

Fig. 2. Synthesis of colicin A in the presence of globomycin after heat shock and azide treatment. (a) Experiment with cells grown in LB. W3110 (wild-type, wt) cells carrying either pColA9 or pJMM1, and 1320 *rpoH* cells carrying pColA9 were induced and incubated at 30 °C with no addition (none) or with azide (3 mM), or after a heat shock at either time zero of induction (HS 0) or 30 min after induction (HS 30), or with EDTA. They were then treated with globomycin (+) or left untreated (-). At the end of induction, samples were analysed by SDS-PAGE. (b) The same experiment for W3110 in M9 medium. The relevant parts of the gels stained with Coomassie blue are presented. The position of the colicin A is indicated by an arrowhead. Numbers on the left indicate the molecular masses in kDa of the standard proteins.

Sodium azide also restored the synthesis of colicin A by W3110(pColA9) cells at 30 °C in the presence of globomycin to an amount similar to that observed in the absence of globomycin. It did not restore the synthesis of colicin A by induced W3110(pJMM1) cells treated with globomycin, except at 42 °C, at which temperature it provoked a slight increase in the amount of colicin A produced (not shown). As azide is an energy poison, the amount of colicin A produced was smaller in azide-treated than in untreated cells. Another energy poison, potassium cyanide, had the same effects as azide (not shown).

The production of colicin A by induced cells grown in minimal medium was studied (Fig. 2b). W3110(pColA9) produced colicin A at 30, 37 and 42 °C and there was no evidence of a delay in synthesis at 30 °C. The amount of colicin A was higher in globomycin-treated than in untreated cells. However, W3110(pJMM1) did not

produce colicin A in the presence of globomycin at any of the three temperatures, except small amounts at high temperature. Thus growth in minimal medium and treatment with energy poisons had similar effects.

Effect of globomycin on the rate of colicin A synthesis

The effect of globomycin might have been due to a delay in colicin A synthesis after induction. Colicin A production was therefore followed throughout the induction by SDS-PAGE. In W3110(pColA9), colicin A was detected immediately after induction and gradually accumulated with time at 37 °C (Fig. 3) and 42 °C (not shown), in both the presence and absence of globomycin. At 30 °C, in the absence of globomycin, colicin A was detected from the onset of induction: the amount remained unchanged for 2 h and increased thereafter. This late increase did not occur in the presence of globomycin. In W3110(pJMM1), the amount of colicin A increased regularly with time of induction at 37 °C in the absence of globomycin. This increase was delayed at 30 °C. In the presence of globomycin, no colicin A was detected at any temperature.

The rate of colicin A production was analysed in induced cells either treated with azide or subjected to heat shock. The amount of colicin A in W3110(pColA9) cultures increased with time in the presence or absence of globomycin. In W3110(pJMM1), it increased in the absence but not in the presence of globomycin, regardless of temperature (Fig. 3).

The rate of colicin A production in cells grown in minimal medium was determined by pulse-chase labelling. The cells were labelled for 1 min with [³⁵S]methionine/cysteine mix and chased with non-radioactive amino acids. At various times after the chase, colicin A was immunoprecipitated with a specific antiserum (Fig. 4). Colicin A was detected from the first minute of the chase and its concentration was stable throughout: it was significantly higher in W3110(pColA9) than in W3110(pJMM1). For W3110(pColA9), it was similar in the presence and absence of globomycin, whereas in W3110(pJMM1) cultures it was significantly lower in the presence than in the absence of globomycin.

Effect of globomycin on the synthesis of the *cal* gene product

As globomycin blocked the synthesis of colicin A at 30 °C in cells carrying pColA9, it should also block that of Cal. Cal synthesis in the experiments described above was analysed by immunoblotting, as Cal cannot be visualized by Coomassie blue staining. The antiserum used reveals three forms of Cal: the precursor form pCal, the modified precursor form pCal^m, and the mature form Cal. In the presence of globomycin, pCal^m is cleaved at two sites near its C-terminal end by the DegP/HtrA protease, giving two shortened forms of pCal^m which accumulate (Cavard *et al.*, 1989). As they are not recognized by the polyclonal antibodies used, the

Fig. 3. Rate of synthesis of colicin A in the presence of globomycin. W3110 cells carrying either pColA9 or pJMM1 were induced and incubated at either 37 °C or 30 °C, with or without azide (3 mM), or after heat shock at time zero of induction (HS) as indicated. They were then treated with globomycin (+) or left untreated (-). At various times of induction as indicated in h, samples were taken and analysed by SDS-PAGE. The relevant parts of the gels stained with Coomassie blue are presented. The position of the colicin A is indicated by an arrowhead. Numbers on the left indicate the molecular masses in kDa of the standard proteins.

Fig. 4. Stability of the colicin A synthesized in the presence of globomycin. W3110 cells carrying either pColA9 (a) or pJMM1 (b) were grown at 30 °C in M9 medium, induced and incubated at either 37 °C or 30 °C. They were treated with globomycin (+) or left untreated (-). They were labelled with [³⁵S]Protein Labeling Mix for 1 min and chased with non-radioactive amino acids. At various times after the chase, as indicated in min, samples were taken, immunoprecipitated with anti-colicin A antibodies and analysed by SDS-PAGE. The volume of the loaded samples of W3110(pJMM1) was three times that of the W3110(pColA9) samples. The relevant parts of the fluorograms are presented.

analysis was carried out on both isogenic wild-type and *degP* cells (deficient in the DegP protease) (Fig. 5).

In induced cells of W3110(pColA9), the three forms of Cal were detected at each temperature in the absence of globomycin, in lower amounts at low than at high temperature. Cal was the major form. In the presence of globomycin, pCal^m did not accumulate in W3110 (pColA9), but did in W3110 *degP* cells at either 37 or

42 °C. At 30 °C, pCal^m was present in low amount. It was often not detected. Thus both genes of the colicin A operon were expressed weakly at 30 °C in the presence of globomycin.

In cells treated with sodium azide, the amount of each form of Cal was similar at each temperature in the absence of globomycin. Especially, the level of pCal was identical at every temperature, while it was lower at 30 °C than at 37 and 42 °C in the absence of azide as already reported (Cavard, 1995). In the presence of globomycin, pCal^m was detected in *degP* cells at each temperature. The wild-type cells contained a small amount of pCal^m at 30 °C. This was not due to inhibition of the DegP protease by azide since DegP was active in the presence of this agent as evidenced at 37 and 42 °C by the absence of pCal^m, but rather to a defect at 30 °C of this enzyme, which is a heat-shock protein (Lipinska *et al.*, 1988; Strauch *et al.*, 1989).

The amounts of the various forms of Cal found at 30 °C increased after a heat shock of 10 min at 42 °C. The levels of pCal and Cal in cells submitted to a heat shock at zero time of induction in the absence of globomycin were similar to those in cells incubated at 42 °C. In the presence of globomycin, the amount of pCal^m in *degP* cells after a heat shock was similar to that at 42 °C. Less of each of the Cal intermediates was detected when the heat shock was performed 30 min after induction (not shown). Thus, the relative proportions of the *cal* gene product and colicin A were constant, confirming that they are co-expressed.

The *cal* gene product does not modify the sensitivity to globomycin

Some of the above experiments might suggest that the *cal* gene product protected cells against killing by globomycin. Globomycin provokes the accumulation of the acylated prolipoproteins by inhibiting their processing into apolipoproteins and signal sequences. This accumulation is toxic, as some minor lipoproteins are

Fig. 5. Effect of globomycin on the synthesis of Cal in various growth conditions. W3110 (wild-type, wt) and W3110 *degP* cells carrying pColA9 were induced and incubated at various temperatures as indicated without (none) or with azide (3 mM), or after a heat shock at time zero of induction (HS), before being treated with globomycin (+) or left untreated (–). At the end of induction, samples were analysed by urea-SDS-PAGE. The Western blot of the bottom of the gel is presented. The various forms of Cal are indicated: ○, precursor form; ●, modified precursor form; ◄, mature form.

essential for the viability of the cells (Gan *et al.*, 1993). Early killing of the cells would prevent colicin induction from proceeding. Therefore the sensitivity to globomycin of induced and control cells devoid or not of the *cal* gene was determined as a function of time at various temperatures by measuring the colony-forming ability. The percentage survival was determined from the ratio of colony-forming units of globomycin-treated to untreated samples at a given time. It decreased with time and was influenced by temperature, as killing was obtained faster at 42 °C than at 30 °C (Fig. 6a). It was unchanged by induction with mitomycin C in either W3110 or W3110(pJMM1) cells (not shown).

The *cal* gene product is lethal for the producing cells and, consequently, the colony-forming ability decreases rapidly after induction of W3110(pColA9) with mitomycin C (Cavard *et al.*, 1989). It did not protect cells against globomycin. Survival of induced W3110 (pColA9) cells treated by globomycin was slightly weaker than that of untreated ones (not shown).

Survival of globomycin treatment was measured in cells treated with azide or submitted to heat shock (Fig. 6a). Both azide treatment and heat shock significantly reduced globomycin killing of induced and control W3110 cells carrying either no plasmid or pJMM1. Azide protected the cells against globomycin killing more efficiently at low than at high temperature.

The optical density of cultures treated at 37 °C with globomycin decreases slowly with time (Hussain *et al.*, 1980). Globomycin seemed to make the cells leaky, as various cellular proteins were found in the spent medium of treated cells, as checked by analysis of the pellets and the supernatants of centrifuged cells on SDS-PAGE (Fig. 6b). This leakiness decreased when the temperature increased: more proteins were found in the supernatants of cells incubated at 30 °C than in those incubated at 37 °C, and very few at 42 °C. It was similar in uninduced and induced cells carrying either no plasmid or pColA9

or pJMM1. Colicin A, which is a cytoplasmic protein, did not leak from induced cells, suggesting that only the periplasmic proteins were leaking. Treatment with azide significantly decreased the rate of the leakiness provoked by globomycin, but heat shock did not (not shown). This effect of azide on the leakiness provoked by globomycin was obtained on both control and induced cells of W3110 carrying either pColA9 or pJMM1. It cannot thus explain the difference in colicin A synthesis observed in these cells in the presence of globomycin.

Traces of colicin A released by induced cells carrying pJMM1 and pColA9F1 might be sufficient to kill the cells and prevent colicin A induction from proceeding, as neither plasmid contains the immunity gene whose product protects the cell from external colicin A (Geli *et al.*, 1986). However, they grew as well as uninduced cells during the first hours of induction as assessed both by optical density measurements and by colony-forming ability, indicating that there was no killing. Furthermore, the production of colicin A was unchanged when the induction was carried out in the presence of either trypsin, which destroys colicin free in the medium, or vitamin B₁₂, which protects the cells against colicin A killing by blocking its receptor BtuB (James *et al.*, 1996) (not shown). Thus, in the absence of a *cal* gene, colicin A was never released, whatever the presence of globomycin.

DISCUSSION

This work demonstrates that the *cal* gene product plays a positive role in the synthesis of colicin A by *E. coli* producing cells. This role was clearly evidenced by treatment with the antibiotic globomycin during induction of the colicin A operon. In the presence of globomycin, colicin A was synthesized in cells carrying the wild-type operon, except at low temperature. At 30 °C, it was synthesized in small amounts in the presence of globomycin and in large amounts in its

Fig. 6. Effects of globomycin on the survival and on the leakiness of the cells at various temperatures. (a) W3110 cells were treated with globomycin at various temperatures, without azide (LB) or with 2 mM sodium azide (LB+azide) or after being submitted to a heat shock (HS). At various times of incubation, samples were diluted in LB and plated on LB agar. Percentage survival was determined from the ratio of the number of colonies from globomycin-treated samples to the number from untreated samples taken at a given time. (b) W3110(pColA9) cells were treated with globomycin after being induced with mitomycin C (MG) or uninduced (CG) and incubated at various temperatures as indicated, without (top) or with sodium azide 2 mM (bottom). At the end of induction,

absence. In cells devoid of the *cal* gene, only traces of colicin A were synthesized in the presence of globomycin whatever the temperature. Thus a novel phenotype of null *cal* gene mutants of *E. coli* is described, revealed by treatment with globomycin.

In the absence of globomycin, the amount of colicin A produced was lower in cells with the *cal* gene deleted than in wild-type cells. This may be due to a positive regulation of colicin A synthesis by the *cal* gene. Such a regulation by a colicin lysis gene has been previously proposed by Lawrence & James (1984), who showed that insertion of transposons into the *lys* gene encoding the colicin E8 lysis protein reduces the production of colicin E8. However, the expression of the colicin A operon provokes a shut-off of chromosomal protein expression (Cavard *et al.*, 1989). Such a shut-off is observed after induction of every operon of the group A colicins which contains a lysis gene, but not after induction of operons of the group B colicins, which are naturally devoid of a lysis gene (reviewed by Pugsley, 1984; van der Wal *et al.*, 1995). The shut-off seems therefore to be due to the expression of the colicin lysis gene. It must favour colicin expression, as the entire cell machinery of transcription and translation may be devoted to it. Thus the reduced synthesis of colicin A observed in cells devoid of the *cal* gene is presumably caused by the absence of the turn-off of chromosomal protein expression provoked by Cal.

In the presence of globomycin, the *cal* gene seems to play a positive role in colicin A expression. The precursor forms of the *cal* gene product might be particularly required for the regulation of colicin A synthesis. Analysis of some *cal* mutants indicated that the N-terminal part of the *cal* gene product, that is the signal sequence, was involved in that regulation. The modified precursor form of Cal, pCal^m, like the acylated precursor form of every lipoprotein (Inukai *et al.*, 1978), accumulates in the inner membrane in the presence of globomycin. It is then cleaved by DegP, a periplasmic protease. pCal^m and its fragments are presumably on the periplasmic face of the cytoplasmic membrane and thus unlikely to be involved in the expression of the colicin A operon. The involvement of the unmodified precursor form of Cal, pCal, formed in the presence and absence of globomycin, is more likely.

The synthesis of colicin A seemed to start immediately after induction in both the presence and absence of globomycin. At 30 °C, in cells carrying the wild-type colicin A operon, the amount of colicin A was low during the first few hours and only increased thereafter in the absence of globomycin. Thus, at this temperature,

samples were centrifuged, and pellets (P) and supernatants (S) were analysed on SDS-gels. The gels stained with Coomassie blue are presented. The position of the colicin A is indicated by an arrowhead. Numbers on the left indicate the molecular masses in kDa of the standard proteins.

synthesis was either arrested or slowed rather than delayed as suggested previously (Cavard, 1995). After a lag, the synthesis either started again or speeded up. This lag would be so long that killing would have occurred in cells treated with globomycin, preventing colicin synthesis. At 37 and 42 °C, the lag would be either null or so short in wild-type cells that a large amount of colicin A could be synthesized before killing by globomycin. In contrast, the lag would not end in cells devoid of a *cal* gene. The signal needed to speed up synthesis would thus involve the *cal* gene product together with a gene product appearing at normal and high temperature.

Some unrelated agents suppressed the arrest or the slow-down of colicin A synthesis at 30 °C in cells carrying the *cal* gene, in both the presence and absence of globomycin. They did not have similar effects on cells devoid of the *cal* gene, indicating that they act either on or through the *cal* gene product. All of them – azide, heat shock and growth in synthetic medium – slowed down the rate of cell growth and reduced the sensitivity of the cells to globomycin. Azide is an inhibitor of the SecA translocase and, consequently, of membrane insertion (Oliver *et al.*, 1990). It may favour the insertion of pCal into the membrane, which does not require SecA (Cavard, 1992). On the other hand, azide may speed up the timing of the colicin lysis proteins as it does on the ‘lysis clock’ of the phage lysis proteins (Young, 1992; Bläsi & Young, 1996). The Rz lysis gene of bacteriophage λ has been recently shown to encode a small lipoprotein (Kedzierska *et al.*, 1996) similar to colicin lysis proteins. Thus the unknown mode of action of azide on phage and colicin lysis proteins might be exerted on lipoproteins. Heat shock provoked the same effect as azide, except in an *rpoH* strain, suggesting that one or more proteins regulated by σ^{32} was/were required for colicin A synthesis. There was a weak production of colicin A in the presence of globomycin at 42 °C by cells with no *cal* gene, indicating that one or more heat-shock protein(s) may complement the *cal* gene product.

Cal, the colicin A lysis protein, thus appears to exert a positive effect on its own synthesis as it is co-expressed with colicin A. Various phage lysis proteins are regulated at the transcriptional level by complex mechanisms involving themselves (Young, 1992). The self-regulatory role of the *cal* gene product may be common to all colicin lysis proteins, as their genes are similarly localized in the various colicin operons and their structures are highly homologous (van der Wal *et al.*, 1995). Recently, an activator required for SOS induction of a colicin-like bacteriocin has been described. It is regulated by the SOS response and its sequence is similar to those of phage lysis proteins (Ferrer *et al.*, 1996).

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