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Proteolysis and Modulation of the Activity of the Cell Division Inhibitor SulA in *Escherichia coli lon* Mutants†

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Intracellular accumulation of the inducible cell division inhibitor SulA is modulated by proteases that ensure its degradation, namely, the Lon protease and another ATP-dependent protease(s). Lon$^-$ cells are UV sensitive because SulA is stable. We asked whether these ATP-dependent proteases are more active when *lon* cells are grown at high temperature or in synthetic medium since these conditions decrease the UV sensitivity of *lon* cells. We found that these growth conditions have no direct effect on Lon-independent degradation of SulA. They may, instead, decrease the SulA-FtsZ interaction.

The inducible cell division inhibitor SulA (SfiA) is part of the SOS regulatory mechanism that tightly couples septum formation and chromosome replication in *Escherichia coli* (7, 8). The intracellular concentration of this potentially lethal protein is strictly controlled at both the transcriptional and posttranslational levels.

At the transcriptional level, expression of the *sulA* gene is negatively regulated by the cellular LexA repressor (7, 17) and is induced after any perturbation of DNA replication (7, 19). At the posttranslational level, the SulA protein has been shown to be actively degraded (18). Its striking instability is an essential part of the cell division control mechanism since it allows cell division to resume rapidly after the shutoff of SulA synthesis (14). In *lon* mutants defective in Lon protease (1, 2), SulA is stabilized (18) and even transient perturbations of DNA replication can lead to a lethal cell division arrest (6) as a result of induction of *sulA*.

The half-life of SulA in Lon$^-$ cells, however, remains relatively brief (30 min) (11, 18) compared with that of bulk cellular protein (several hours) (3). This suggests that Lon is not the only cellular protease that degrades SulA in vivo. Certain physiological conditions (i.e., medium composition, growth temperature) have been described which suppress the hypersensitivity of *lon* mutants to DNA-damaging agents (6, 20), suggesting that the accumulation of SulA may be lower in these conditions. Other Lon substrates have been described for which the rate of Lon-independent degradation is influenced by the growth conditions (4, 5). This raised the possibility that the activity of a potential Lon-independent protease(s) involved in SulA degradation is modulated by the growth conditions and thereby plays an important role in the regulation of cell division.

Lon-independent, energy-dependent, SulA degradation. To investigate the possibility that a Lon-independent protease(s) plays an important role in cell division regulation, we determined the half-life of SulA under different growth conditions by pulse-chase experiments. The uninduced level of SulA synthesis is unfortunately too low for the protein to be quantitatively visualized on polyacrylamide gels. Since it is induced by treatments that damage DNA (7), the SulA half-life has until now always been directly measured in UV-irradiated cells (11, 15, 18). This affects global cell physiology and could modify the process of SulA degradation. For this reason, we used a system in which the *sulA* gene was cloned under the *lac* promoter control on a pBR322-derived plasmid (pOHP293). In this construction, derived from pGC165 (9), 50 bases were deleted in vitro between the *lac* promoter and the beginning of the *sulA* gene; this deletion included the LexA binding site and improved the induced expression of SulA. Addition of isopropyl-β-D-thiogalactopyranoside to the growth medium of bacteria containing this plasmid induced a rapid block of cell septation and after a few minutes allowed easy detection of the 18-kDa ^{35}S-labeled SulA protein.

Pulse-chase experiments were done with this construct in cultures growing in M9 glycerol medium (16) supplemented with all of the amino acids except methionine. After brief (15 min) isopropyl-β-D-thiogalactopyranoside induction, [^{35}S] methionine was added at 10^{-4} mM for 2 min and then chased with 1 mM cold methionine for different times. Samples were taken during the experiments and immediately boiled in the presence of 2× Laemmli sample buffer (13) before being loaded on a 14% sodium dodecyl sulfate-polyacrylamide gel. The band on the gel corresponding to SulA was then cut out, and its radioactivity was quantified (18). The half-life was 3 min in Lon$^+$ cells and 30 min in Lon$^-$ isogenic cells (Fig. 1A). These values are in agreement with those previously measured in heavily UV-irradiated cells (11, 15, 18), and we verified directly that UV irradiation of the cells did not modify the rate of SulA degradation (data not shown).

Because the *lon* strain (OHP7) in which we measured the SulA half-life carries a large deletion of the *lon* gene (*Δlon-510*) (15), it seems unlikely that the remaining degradation is due to residual activity of the Lon protease. It suggests, on the contrary, the existence of Lon-independent proteolysis of SulA. To test whether the Lon-independent SulA degradation is mediated by an ATP-dependent protease, we measured the half-life of SulA in *lon* cells after energy deprivation (effected by addition of 1 mM KCN to the culture medium). The presence of KCN almost completely blocked SulA degradation (Fig. 1B), suggesting that, like the

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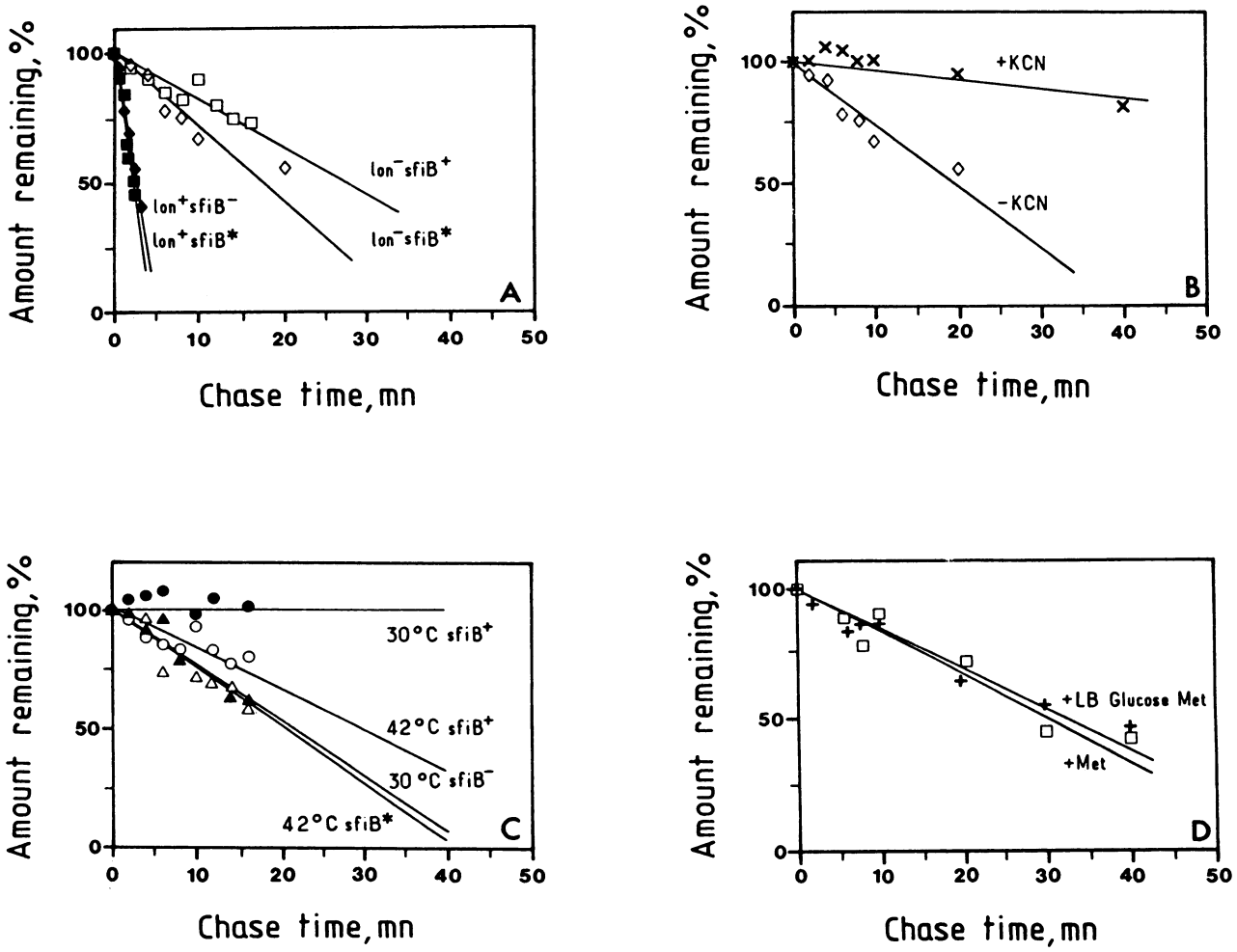

FIG. 1. Stability of SulA as measured by pulse-chase experiments under different conditions (see text) and with different isogenic strains. (A) Measurement of SulA half-life at 37°C in *lon*$^+$ *sfiB*$^+$ cells (OHP59; ■), *lon sfiB*$^+$ cells (OHP60; □), *lon*$^+$ *sfiB** cells (OHP17; ◆), and *lon sfiB** cells (OHP7; ◇). (B) Effect of energy deprivation. Measurement of SulA half-life at 37°C in *lon* cells (OHP7) in the absence (◇) or presence (×) of 1 mM KCN. (C) Effect of temperature. Measurement of SulA half-life in *lon sfiB*$^+$ cells (OHP60) at 30°C (●) and 42°C (○) and in *lon sfiB** cells (OHP7) at 30°C (◇) and 42°C (◆). (D) Effect of nutritional shift-up. Measurement of SulA half-life at 37°C in *lon* cells (OHP60) in minimal medium (□) and after shift to rich (LB plus glucose) medium (+).

Lon protease, the protease(s) mediating this reaction also requires ATP for its activity.

Temperature effect on Lon-independent degradation of SulA. The UV sensitivity of *lon* mutants has been shown to be due to the stabilization of SulA (18). Several authors have previously observed that some aspects of the Lon$^-$ phenotype are suppressed when the bacterial mutants are grown in synthetic medium at 42°C (4–6). We show here (Fig. 2) that under these conditions the UV sensitivity of a *lon* null mutant (*Δlon-510*) can likewise be completely suppressed. We know from previous experiments that this is not due to a defect in SulA inducibility (7). Since other Lon substrates have been reported to be more rapidly degraded in *lon* cells at 42 than at 30°C (4, 5), we speculated that the thermoconditional UV resistance of the *lon* mutant could be due to the stimulation, at 42°C, of a Lon-independent mechanism of SulA degradation.

The results (Fig. 1C) show that the SulA protein is indeed more rapidly degraded in minimal medium at 42°C than it is at 30°C. However, the half-life measured at 42°C (30 min) in *Δlon-510* cells is much longer than that previously measured in *lon*$^+$ cells (3 min) and in fact is similar to that observed in the *lon* mutant in conditions that do not suppress its UV sensitivity (cf. Fig. 1A). Thus, the complete suppression of the UV-sensitive phenotype at 42°C could not be explained by the increased rate of SulA degradation.

An alternative explanation for the conditional UV resistance is that perhaps SulA activity as a cell division inhibitor is decreased at 42°C compared with its activity at 30°C. For example, it could interact less tightly with its target, the FtsZ (also called SfiB or SulB) protein, at high temperature than it does at low temperature. It is known that SulA is partially protected against degradation when it interacts with FtsZ (11). The more active degradation of SulA observed at 42°C could therefore reflect a weaker interaction between SulA and FtsZ, which would allow greater access of SulA to the Lon-independent proteolytic system. To test this hypothesis, we have compared the half-life of SulA at 30°C with that at 42°C in an *sfiB** mutant in which the SulA-FtsZ interaction is reduced or abolished (9, 11). The results (Fig. 1C) show that in this mutant the SulA half-life is the same at 30 as it is

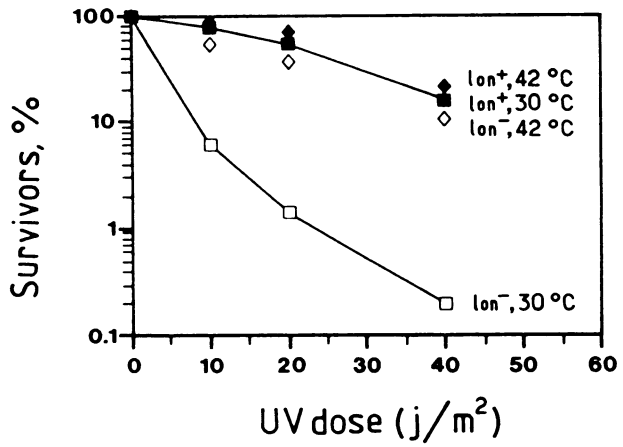

FIG. 2. UV sensitivity of *lon*$^+$ cells (OHP59) at 30°C (■) and 42°C (◆), and *lon* cells (OHP60) at 30°C (□) and 42°C (◇). Cells were grown in glycerol minimal medium at 30 or 42°C, centrifuged, resuspended in 10 mM $MgSO_4$, and irradiated; portions were withdrawn at different times, and viable bacteria were assayed on minimal plates at 30 or 42°C.

at 42°C and is roughly equivalent to that in *sfiB*$^+$ strains at 42°C.

This result strongly suggests that the Lon-independent degradation system of SulA is not stimulated at high temperature. It further suggests that the temperature-dependent UV resistance of the *Δlon-510* mutants reflects a difference in the accessibility of SulA to the Lon-independent protease(s), probably because of the decreased interaction of SulA with FtsZ at 42°C.

Effect of nutritional shift-up on Lon-independent degradation of SulA. *lon* bacteria are known to be hypersensitive to a nutritional shift-up. They overelongate after a shift from minimal glycerol to LB glucose medium, returning to the normal bacterial length after 2 h (21). We have previously shown that this effect is SulA dependent (8) but does not involve *sulA* transcriptional induction (7, 10). Since the Lon$^-$ phenotype is medium dependent (6), we speculated that SulA, like other unstable proteins (12, 22), may be degraded at different rates depending on the medium composition. Indeed, if the half-life of SulA is longer in rich medium than in minimal medium, the nutritional shift-up would lead to an accumulation of SulA and therefore delay cell septation.

To test this hypothesis, we compared the half-life of SulA in glycerol minimal medium with that after a shift-up in LB broth (16) supplemented with 0.4% glucose and 1 mM methionine. In this experiment, LB with glucose and methionine was added in place of the cold methionine chase; the rest of the experiment was as previously described. This did not reveal any significant difference in SulA decay (Fig. 1D), suggesting that the overelongation observed in these conditions after a nutritional shift-up is not due to a modification in the rate of SulA degradation. This result also suggests that the Lon-independent proteolytic activity responsible for SulA instability does not differ in rich and minimal medium.

Several hypotheses could explain the delay in cell division observed after the shift-up in *lon* mutants. The shift-up could stimulate SulA synthesis at a posttranscriptional level. This may not have been detected by our previous experiments in which SulA expression was monitored by means of a *sulA*::*lac* operon fusion. An alternative explanation is that the relative intracellular concentration of SulA versus that of FtsZ may vary during the shift-up, leading to a transient excess of SulA over FtsZ that would delay cell septation.

This study confirms that there is a Lon-independent degradation of SulA and shows that this mechanism, like Lon, requires energy. However, the Lon-independent degradation of SulA does not seem to play an important role in cell division nor in the SulA activity under different physiological conditions tested here. Nevertheless, this proteolytic activity may play a fundamental role in adjusting the concentration of other physiological substrates. The fact that it consumes energy actually reinforces this latter possibility.

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LITERATURE CITED

1. **Charette, M. F., G. W. Henderson, and A. Markovitz.** 1981. ATP hydrolysis-dependent protease activity of the *lon* (*capR*) protein of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **78**:4728–4732.
2. **Chung, C. H., and A. L. Goldberg.** 1981. The product of the *lon* (*capR*) gene in *Escherichia coli* is the ATP dependent protease, protease La. Proc. Natl. Acad. Sci. USA **78**:4931–4935.
3. **Goldberg, A. L., and A. C. St. John.** 1976. Intracellular degradation in mammalian and bacterial cells. Annu. Rev. Biochem. **45**:747–803.
4. **Gottesman, S., and D. Zipser.** 1978. Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. **133**:844–851.
5. **Grossman, A. D., R. R. Burgess, W. Walter, and C. A. Gross.** 1983. Mutations in the *lon* gene of *E. coli* K12 phenotypically suppress a mutation in the sigma subunit of RNA polymerase. Cell **32**:151–159.
6. **Howard-Flanders, P., E. Simson, and L. Theriot.** 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K12. Genetics **49**:237–246.
7. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *Escherichia coli*. Nature (London) **290**:797–799.
8. **Huisman, O., R. D'Ari, and J. George.** 1980. Inducible *sfi* dependent division inhibition in *Escherichia coli*. Mol. Gen. Genet. **177**:629–636.
9. **Huisman, O., R. D'Ari, and S. Gottesman.** 1985. Cell division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. Proc. Natl. Acad. Sci. USA **81**:4490–4494.
10. **Huisman, O., M. Jacques, R. D'Ari, and L. Caro.** 1983. The role of the *sfiA* dependent cell division regulation system in *Escherichia coli*. J. Bacteriol. **153**:1072–1074.
11. **Jones, C., and I. B. Holland.** 1985. Role of the SulB(FtsZ) protein in division inhibition during the SOS response in *Escherichia coli*: FtsZ stabilizes the inhibitor SulA in maxicells. Proc. Natl. Acad. Sci. USA **82**:6045–6049.
12. **Kowit, J. D., and A. L. Goldberg.** 1977. Intermediate steps in the degradation of a specific abnormal protein in *Escherichia coli*. J. Biol. Chem. **252**:8350–8357.
13. **Laemmli, U. K.** 1970. Cleavage of the structural proteins during assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Maguin, E., J. Lutkenhaus, and R. D'Ari.** 1986. Reversibility of SOS-associated division inhibition in *Escherichia coli*. J. Bacteriol. **166**:733–738.
15. **Maurizi, M. R., P. Trisler, and S. Gottesman.** 1985. Insertional mutagenesis of the *lon* gene in *Escherichia coli*:*lon* is dispensable. J. Bacteriol. **164**:1124–1135.
16. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Mizusawa, S., D. Court, and S. Gottesman.** 1983. Transcription of the *sulA* gene and repression by LexA. J. Mol. Biol. **171**:337–343.
18. **Mizusawa, S., and S. Gottesman.** 1983. Protein degradation in

Escherichia coli: the *lon* gene controls the stability of SulA protein. Proc. Natl. Acad. Sci. USA **80**:358–362.

19. **Quillardet, P., O. Huisman, R. D'Ari, and M. Hofnung.** 1982. SOS chromotest, a direct assay of induction of an SOS function in *Escherichia coli* K12 to measure genotoxicity. Proc. Natl. Acad. Sci. USA **79**:5971–5975.
20. **Walker, J. R., and A. P. Pardee.** 1967. Conditional mutations involving septum formation in *Escherichia coli*. J. Bacteriol. **93**:107–114.
21. **Walker, J. R., and J. A. Smith.** 1970. Cell division of the *Escherichia coli lon* mutant. Mol. Gen. Genet. **108**:249–257.
22. **Zipser, D., and P. Bhavsar.** 1976. Missense mutations in the *lacZ* gene that result in degradation of β-galactosidase structural protein. J. Bacteriol. **127**:1538–1542.