A signal-arrest-release sequence mediates export and control of the phage P1 endolysin

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The Lyz endolysin of bacteriophage P1 was found to cause lysis of the host without a holin. Induction of a plasmid-cloned lyz resulted in lysis, and the lytic event could be triggered prematurely by treatments that dissipate the proton-motive force. Instead of requiring a holin, export was mediated by an N-terminal transmembrane domain (TMD) and required host sec function. Exported Lyz of identical SDS/PAGE mobility was found in both the membrane and periplasmic compartments, indicating that periplasmic Lyz was not generated by the proteolytic cleavage of the membrane-associated form. In gene fusion experiments, the Lyz TMD directed PhoA to both the membrane and periplasmic compartments, whereas the TMD of the integral membrane protein FtsI restricts Lyz to the membrane. Thus, the N-terminal domain of Lyz is both necessary and sufficient not only for export of this endolysin to the membrane but also for its release into the periplasm. The unusual N-terminal domain, rich in residues that are weakly hydrophobic, thus functions as a signal-arrest-release sequence, which first acts as a normal signal-arrest domain to direct the endolysin to the periplasm in membrane-tethered form and then allows it to be released as a soluble active enzyme in the periplasm. Examination of the protein sequences of related bacteriophage endolysins suggests that the presence of an N-terminal signal-arrest-release sequence is not unique to Lyz. These observations are discussed in relation to the role of holins in the control of host lysis by bacteriophage encoding a secretory endolysin.

Double-stranded DNA phage typically use a holin-endolysin system to achieve lysis of their bacterial hosts (1, 2). Holins are small proteins with no known enzymatic function. They are localized to the cytoplasmic membrane and function to control the timing of lysis. Endolysins are proteins with one of several muraletic activities responsible for the destruction of the peptidoglycan. For example, in the phage λ infection cycle, the holin, a product of the S gene, accumulates in the inner membrane throughout the period of late gene expression, whereas the active endolysin, R, accumulates in the cytoplasm without deleterious effects on the host (3). Suddenly, at a genetically determined time, the holin disrupts the inner membrane, allowing R to attack the peptidoglycan of the infected cell; lysis occurs within seconds. The λ paradigm, where the holin is required for the export of the endolysin from the cytoplasm to the periplasm, was long thought to be universal. However, Sao Jose et al. (4) reported that the endolysin (Lys-44) from oenococcal phage fOg44 carries a cleavable, N-terminal signal sequence that functions in both Escherichia coli and Oenococcus oeni. Thus, the catalytic domain of Lys-44 is exported by the sec translocon, and its signal sequence is proteolytically removed by leader peptidase. Many other phages of Gram-positive bacteria have similar N-terminal signals and are thus likely to be similarly exported. Despite the presence of a secretory endolysin, however, phage fOg44 appears to have a canonical holin gene. Moreover, in fOg44 infections, significant secretion of the endolysin, as monitored by the appearance of the processed product, occurred only before lysis is achieved, indicating that the sec-mediated export of the endolysin was not sufficient for lysis. These findings give rise to a number of questions about the role and mode of action of holins in phages where the endolysin is sec-exported. What is the role of the holin, if not to release the endolysin to the periplasm? How is lysis timing achieved if the endolysin is already secreted across the cytoplasmic membrane? Confounding the resolution of these issues is the limited understanding of the nature of the murein envelope in Gram-positive bacteria, where little is known about the chemical environment, and where, unexpectedly, high levels of a wide range cytosolic enzymes have been found (5).

An investigation of the lysis system of bacteriophage P1, one of the classic coliphages, warrants further study. P1 is unusual in that its endolysin gene, hyz, is not clustered with the holin and antiholin, as is found in all lambdoid and many other phage genomes (6). Moreover, unlike phage λ, which requires both its holin and endolysin to effect host lysis, P1 mutants deleted for the putative holin gene lyzA are plaque formers, although lysis is somewhat delayed when compared with the wild-type phage (7, 8). Here, we report the results of experiments to determine the mechanism of the apparent holin independence of P1 Lyz-mediated host lysis. These results are discussed in terms of a type of subcellular localization signal found in a number of endolysins from phages of Gram-negative bacteria, and how this localization is integral to the control of lysis.

Materials and Methods

Bacterial Strains, Growth Media, and Culture Conditions. All bacterial cultures were grown in standard LB medium, supplemented with various antibiotics when appropriate: 100 μg/ml for ampicillin, 10 μg/ml for chloramphenicol, 40 μg/ml for kanamycin, and 10 μg/ml for tetracycline. When indicated, isopropyl β-D-thiogalactoside, dinitrophenol (DNP), NaN3, or CHCl3 were added at final concentrations of 1 mM, 10 mM, 1 mM and 1%, respectively.

The E. coli strains MC4100 and XL1-Blue have been described (9). An azide-resistant mutant of XL1-Blue was selected by plating on LB media containing 1 mM NaN3. Some experiments used RY8653 (MC4100 pRho dshA:kan1 zih12::Tn10), kindly provided by T. J. Silhavy, Princeton University, Princeton; RY1531 (MC4100 secA)4, kindly provided by J. Beckwith (Harvard Medical School, Boston) (10); or TG1 [F' traD36 proAB lacZΔ(lacZ)M15 supF hsdD5 thiA Δ(lacZΔM15)F' Δ(lacZΔM15)lac Δ(lac − proAB)], a phenotypically PhoA− host (11). Standard conditions for the growth of cultures and the monitoring of lysis kinetics have been described (9, 12). When appropriate, the presence of active, cytosolic endolysin in nonlysing cultures was tested for by the addition of CHCl3.

Standard DNA Manipulation, PCR, and DNA Sequencing. Procedures for the isolation of plasmid DNA, DNA amplification by PCR, PCR product purification, DNA transformation, and DNA sequencing have been described (13–15). Oligonucleotides were

Abbreviations: DNP, dinitrophenol; pmf, proton-motive force; TMD, transmembrane domain; SAR, signal-arrest-release.

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obtained from Integrated DNA Technologies, Coralville, IA, and were used without further purification. Ligation reactions were performed by using the Rapid DNA ligation kit from Roche Molecular Biochemicals according to the manufacturer’s instructions. All other enzymes were purchased from Promega, with the exception of Pfu polymerase, which was from Stratagene. Automated fluorescent sequencing was performed at the Gene Technologies Laboratory in the Department of Biology at Texas A&M University.

**Plasmid Construction.** The various endolysins, endolysin chimeras, and genes encoding FtsI and PhoA were placed under the control of the lac promoter of pJF118 (16). The DNA inserts for these constructs were PCR-amplified from the following sources: for pJF1lyz, the P1 gene lyz was from P1vir FIIA; for pJFR, the λ R gene was from pST5 (13); for pJFR19, the P22 gene I9 was from P22 DNA; for pJFR31, the bacteriophage 21 R gene was from pBR121 (17); and for pJFSTs and pJFPH, the ftsI and phoA genes were from *E. coli* chromosomal DNA. To construct pZAdsbA, the dsbA gene was PCR-amplified from *E. coli* chromosomal DNA. The PCR product was digested with and cloned into unique *KpnI* and *XbaI* restriction sites in the chloramphenicol resistance plasmid pZAA-31, under control of the pLtetO-1 promoter (18). The plasmid pFtsIlyz, in which the transmembrane domain (TMD) of FtsI replaced the N-terminal hydrophobic domain of Lyz was constructed by first amplifying the DNA encoding the sequence FALLCGCIL-15 nucleotides of homology to positions LALAFLLG from FtsI. The upstream primer had, at its 5′-terminal hydrophobic domain of Lyz was constructed by first amplifying the DNA encoding the sequence FALLCGCIL-15 nucleotides of homology to positions LALAFLLG from FtsI. The downstream primer had, at its 5′-end, 15 nucleotides of homology to positions 3 to +12 of the gene lyz in pJF1lyz. The downstream primer had, at its 5′-end, 15 nucleotides of homology to positions +70 to +85 of lyz. The purified PCR product was then used to conduct a modified site-directed mutagenesis reaction by using the QuikChange kit from Stratagene with pJF1lyz as the template. The resultant PCR product was digested with *DpnI* and was transformed into XL1-Blue. The plasmids PR21-1lyz, in which the sequence encoding the N-terminal hydrophobic domain of the phage 21 endolysin R21 replaced that of Lyz, pLyzΦ19, in which sequence encoding the N-terminal hydrophobic domain of Lyz was inserted between the first two codons of gene I9 from phage P22; and pLyzΦPhoA, in which the signal sequence of PhoA was replaced with that of the N-terminal hydrophobic domain of Lyz, were constructed in a similar way. A cmyc-tagged allele of the R21 gene was generated by using primers encoding the epitope flanked either with 15 nucleotides of homology 5′ to the insertion site in R21 or 15 nucleotides 3′ to the insertion site. These primers were used for site-directed mutagenesis by using pJFR21 as described above to give pJFR′cmyc. The plasmid PCMycLyzΦ19, in which the cmyc epitope was inserted between the first two codons of the chimeric endolysin found in pLyzΦ19, was constructed in a similar way. All constructs were verified by DNA sequencing.

**Subcellular Fractionation and Alkaline Phosphatase Assay.** Cell pellets from 40-ml cultures were resuspended in 4 ml of FP buffer (0.1 M sodium phosphate/0.1 M KCl/5 mM EDTA/1 mM DTT/1 mM phenylmethylsulfonyl fluoride, pH 7.0) and were then disrupted by passage through a French pressure cell (Spectroline Instrument, Rochester, N.Y.) at 16,000 psi (1 psi = 6.89 kPa). The membrane and soluble fractions were separated by centrifugation at 100,000 × g for 60 min at 16°C. To isolate the periplasmic fraction, cell pellets from 25-ml cultures were resuspended in 500 μl of 25% sucrose/30 mM Tris-Cl, pH 8.0. Next, 10 μl of 0.25 M EDTA, 10 μl of lysozyme (20 mg/ml), and 500 μl of distilled water were added in sequence. After 5 min at room temperature, microscopic examination showed that ~95% of the cells had formed spheroplasts. The samples were centrifuged at 8,000 × g for 30 min to separate the released periplasm from the spheroplasts (membrane and cytosol).

PhoA activity assays was determined by using p-nitrophenyl phosphate as the substrate and a millimolar extinction coefficient of 18.3 for p-nitrophenol at 420 nm. One milliunit of activity is the amount of enzyme needed to form 1 μm of product per minute under standard assay conditions (19). TG1 cells carrying the empty vector, pJF, contained <5% of the activity detected in cells carrying pJF1lyzΦPhoA.

**SDS/PAGE and Western Blotting.** SDS/PAGE, Western blotting, and immunodetection were performed as described (13). Antibodies against the purified His6-tagged Lyz and λ R endolysins were prepared in chickens by Aves Labs, (Tigard, OR). Proteins tagged with the cmyc epitope were detected by using a mouse monoclonal antibody from Babco (Richmond, CA). For detection of PhoA and its derivatives, a rabbit polyclonal antibody from 5 Prime → 3 Prime was used. Horseradish peroxidase-conjugated secondary antibodies against chicken IgY, mouse IgG, and rabbit IgG were from Aves Labs, Pierce, and Pierce, respectively. Generally, primary antibodies were used at a 1:1,000 dilution, whereas secondary antibodies were used at a 1:3,000 dilution. Blots were developed by using the chromogenic substrate 4-chloro-1-naphthol (Sigma) or with the SuperSignal chemiluminescence kit (Pierce). Equivalent sample loadings were used whenever multiple fractions obtained from the same culture were analyzed.

**Results**

**The P1 Endolysin Causes Lysis of *E. coli* in the Absence of Holin Function.** The lyz gene encodes the P1 endolysin of 185 residues, which is homologous to the T4 gpe lysozyme. Unexpectedly, when lyz was cloned under an inducible promoter and expressed in logarithmically growing cells in the absence of a holin gene, overt lysis was observed beginning within 35 min (Fig. 1A). Inspection of the culture before lysis revealed that the cells began adopting a spherical morphology ~25 min after induction. Both of these observations suggest that Lyz can gain access to the periplasm and degrade the host peptidoglycan in the absence of its cognate holin. Inspection of the predicted Lyz sequence reveals a hydrophobic domain potentially capable of serving as a signal sequence (Fig. 2). The possibility that this sequence allows export of Lyz by using the sec system was tested by examining the effect of the SecA inhibitor, azide, on the holin-independent lysis observed after induction of the cloned lyz gene. Azide was found to inhibit Lyz-mediated lysis in a host carrying the wild-type secA locus, but not in an isogenic strain carrying an azide-resistant allele of secA. The addition of CHCl3 to permeabilize the membrane of the azide-treated culture resulted in its immediate lysis, which is consistent with the azide effect being at the level of membrane translation (Fig. 1A). Similar results were obtained by using a secA4 allele at the nonpermissive temperature. Again, lysis was blocked until CHCl3 was added to permeabilize the membrane (Fig. 1A).

Additional genetic evidence that Lyz is exported to the periplasm was obtained by using a dsbA host. There are seven cysteine residues in Lyz, six of which reside in its hydrophilic, catalytically active C-terminal domain and might form up to three disulfide bonds if this domain is externalized to the periplasm. These disulfides could be necessary for either the stability or activity of Lyz. As can be seen in Fig. 1B, Lyz-mediated lysis is not observed in a dsbA host but is recovered when dsbA gene function is provided from a compatible plasmid. Significantly, no Lyz could be detected by Western blot in dsbA cells, suggesting that in the absence of periplasmic DsbA activity all of the Lyz protein misfolds and is degraded. However, if dsbA cells are treated with 1 mM azide before induction, Lyz does accumulate and can be detected by Western blot (Fig. 6, which
Fig. 1. Induction of the Lyz endolysin results in holin-independent lysis. (A) Lyz-mediated lysis requires SecA. Azide-sensitive (●, ■, and ○) or resistant (□) XL1-Blue cells carrying pJFLyz were induced at time 0, and culture turbidity was followed as a function of time. To two of these cultures (● and ■), 1 mM sodium azide was added 10 min after induction, and to one of these cultures (○), CHCl₃ was added at 50 min. In another culture of MM52 secAΔ pJFLyz (○), the cells were shifted from 30°C to 42°C at 90 min before induction, and CHCl₃ was added at 85 min after induction. Cells retained at 30°C and then induced underwent lysis beginning at 50 min after induction (data not shown). (B) Lyz function requires DsbA activity. MC4100ΔsecAΔ pJFLyz (●), RY8653 ΔdsbA::kan pJFLyz pZ2-31 (■), and RY8653 ΔdsbA::kan pJFLyz pZA-dsbA (□) were induced at time 0 and were monitored for turbidity as a function of time. (C) Lyz-mediated lysis is triggered by energy poisons unless SecA-mediated secretion is inhibited. Cultures of XL1-Blue carrying pJFLyz were induced at time 0 and were monitored for turbidity as a function of time. To one culture (●), no further additions were made. To a second culture, 10 mM DNP was added 20 min after induction (□), and to a third, 1 mM sodium azide was added 10 min after induction, followed by 10 mM DNP at 70 min and CHCl₃ at 100 min (○). (PNAS 101 no. 17 6417 X. jatens, X. splendidus, and V. cholerae lysozyme sequences of the canonical lysozyme T4 gp26 (GI3676081). The putative TMD of the SAR signal is highlighted in gray, and basic and acidic residues are highlighted in red and teal, respectively. The residue after which there is a potential signal sequence cleavage site in the P1 Lyz sequence is in bold and is underlined. In addition to the endolysins from P1, T4, and P22, endolysins shown from functional bacteriophages include: Lyz of phage Mu (GI19633512), Lyz of Haemophilus influenzae phage HPI (GI1708889), Lyz of Erwinia amylovora phage phiE1H (GI11342495), gp45 of Pseudomonas aeruginosa phage 6KVM, R₁ of lambdoid phage 21 (GI126600), and gp19 of Salmonella typhimurium phage P534 (GI3676081). In the R₁ sequence, the Gly-16 residue altered to Cys in the R¹4Lyz fusion is underlined and is bold. Included in this list are putative endolysins from: a Yersinia pestis prophage (GI16122337), from a Fels-2-like prophage of uropathogenic E. coli (GI26246847), a prophage of Xylella fastidiosa (GI15837115), a prophage of Bordetella bronchiseptica (GI33602455), and the VT2 Sakai prophage of O157:H7 (GI5834216). Also included is the chromosomal endolysin NucD, encoded by a prophage remnant in Serratia marcescens, and the endolysin R from Qin, a cryptic prophage segment from E. coli K-12 (GI26249922), both of which have been demonstrated to have lytic function (32, 33). Only a representative set of the putative SAR endolysins is shown.)

Accession nos. are for the GenBank database.

Subcellular Localization of Lyz: The Signal-Arrest-Release (SAR) Sequence. When cells expressing the lyz gene were disrupted and fractionated, the Lyz protein was found in both soluble and membrane fractions (Fig. 3). Analysis of the Lyz sequence by algorithms demonstrated that Lyz is externalized by the sec system, requires DsbA to catalyze the formation of stabilizing disulfide bonds in the periplasm, and can be activated to induce lysis by collapse of the pmf.

is published as supporting information on the PNAS web site). This finding suggests that normally there is no cytoplasmic pool of Lyz.

In comparison with the saltatory and rapid lysis seen in a P1-infected culture (20), the lysis observed in cells expressing P1 gene lyz alone is gradual. However, the addition of DNP to cultures 20 min after the induction of Lyz expression dramatically accelerates lysis (Fig. 1C). Cyanide has a similar effect (data not shown), suggesting that the activity of the exported Lyz remains largely cryptic until the proton-motive force (pmf) across the cytoplasmic membrane is dissipated. Significantly, the addition of DNP to azide-inhibited cultures did not result in lysis, even though the cells contained sufficient, active Lyz to cause lysis after the addition of CHCl₃ (Fig. 1C). We conclude that Lyz is externalized by the sec system, requires DsbA to catalyze the formation of stabilizing disulfide bonds in the periplasm, and can be activated to induce lysis by collapse of the pmf.

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The SAR Domain of Lyz Is Necessary and Sufficient for Localization to Two Cell Compartments. To test whether the SAR domain of Lyz is sufficient to localize a protein to both the cytoplasmic membrane and the periplasm, this sequence was either used to replace the normal, cleavable signal sequence of the periplasmic enzyme, PhoA, or fused to the N terminus of the soluble, cytoplasmic domain, the endolysin R21 from bacteriophage 21 (21). The FtsI-Lyz chimera was recovered exclusively in the membrane fraction (Fig. 4B). We were unable to localize the reciprocal Lyz-FtsI fusion protein, presumably because its instability prevented detection by Western blotting. Finally, we examined the distribution of another endolysin with a potential SAR sequence, the endolysin R21 from bacteriophage 21 (Fig. 2). When synthesized in E. coli, R21, like Lyz, causes lysis in the absence of its cognate holin (data not shown). As with Lyz, R21 was found in both soluble and membrane-bound forms (Fig. 4B). When the putative SAR domain of R21 was used to replace the SAR sequence of Lyz, the chimera R21-Lyz, while enzymatically inactive, was localized similarly to wild-type R21 (Fig. 2). The lack of activity of R21-Lyz was surprising, considering the similarity of the sequences of these two endolysins (Fig. 2). The most obvious difference between the R21 and Lyz SAR sequences was the lack of a cysteine residue in the former. When Gly-13 in R21-Lyz (corresponding to Gly-16 in the R21 sequence; see Fig. 2) was changed to a Cys residue, the chimera became lytically active (data not shown). Thus, the SAR domains of Lyz and R21 are essentially interchangeable, except for the requirement of a cysteine residue in the former, a finding that will be considered elsewhere (M.X., A. Areland, D.K.S., S. Swanson, J. Sacchettini, and R.Y., unpublished work).

Discussion

A Subcellular Localization Signal: The SAR Sequence. The results presented here demonstrate that the phage P1 endolysin, Lyz, is secreted by the sec translocon of the host, unlike the well studied endolysins of other phages of Gram-negative bacteria, all of which have been shown to accumulate in the cytosol (1, 2). The N-terminal secretory signal of Lyz is not removed during export, suggesting that it constitutes a signal-arrest domain, which, on
completion of translocon function, tethers the exported protein to the membrane. Surprisingly, however, a significant portion of the Lyz is found in the periplasm. This finding is independent of the level of expression of lyz, because identical results were found with lyz mounted on a low copy number plasmid (Fig. 7) and also when lyz, with its cognate translation signals, was used to replace the SR genes of phage λ (see Supporting Materials and Methods and Fig. 8, which are published as supporting information on the PNAS web site). Because the sec translocon will initially localize an uncleaved, signal-arrest domain to the membrane, the presence of a significant fraction of the protein in the periplasm indicates that some of the initially membrane-bound protein was subsequently released into the periplasm. Consequently, the N-terminal domain of the P1 endolysin represents another type of subcellular localization signal, the SAR sequence. The unusual feature of the SAR sequence is that it endows a protein with the ability to convert from a membrane-integrated state to a freely soluble state, without proteolytic cleavage. The SAR sequence from Lyz was found to confer the two-compartment disposition on fusions with the P22 lysozyme, gp19 and with PhoA (Fig. 4A). Thus, the Lyz SAR sequence is both necessary and sufficient for the sec-mediated export to a membrane-tethered state and subsequent release to the periplasm.

The endolysin gene R21 of lambdoid phage 21 also encodes a T4 gpe homolog with a functional SAR sequence. The SAR sequences of Lyz and R21 do not share significant sequence similarity but do share the characteristic of having 40–60% of the residues as Gly or Ala, which contribute very little to the similarity but do share the characteristic of having 40–60% of protein from the membrane in certain signal-sequence mutants. When the protein is released from the membrane, it will be of aT 4g p

Interestingly, a precedent exists for the release of a tethered protein from the membrane in certain signal-sequence mutants of lamB. A double mutation near the cleavage site (A23D and A25Y) abolishes leader peptidase cleavage, leaving the LamB tethered to the membrane by its uncleaved signal sequence, where it is rapidly degraded. However, a suppressor R6L allows LamB to reach the outer membrane and fold into functional porin and λ receptor, with the mutant signal sequence intact (25). Inspection of the mutant sequences suggests that the suppressor may be creating a SAR sequence from the LamB signal sequence (Fig. 9, which is published as supporting information on the PNAS web site). The triple mutant has a signal-arrest domain of 19 uncharged residues because the four-carbon aliphatic segment of the Lys side chain allows its α carbon to be buried a full helical turn within the bilayer (26). This domain resembles the SAR sequences in having 10 of 19 residues either negligibly hydrophobic (Ala or Gly) or overtly hydrophilic (Lys, Ser, Thr, and Gln), with only a single basic side chain to serve as a membrane anchor. Duguay and Silhavy (25) speculated that this mutant protein might be assisted in exiting the membrane by periplasmic folding factors, a notion that could also apply to the endolysins described here.

Host Lysis by Bacteriophage P1. Phage P1 is one of the most intensively studied phages, which was established as a major experimental system the same year as phage λ (27). P1, like other classical phages, was subjected to thorough amber mutant screening, by which all essential genes were identified. Two essential genes with primary lysis phenotypes were found: lyz, which encodes the endolysin of P1 and has a lysis-negative null phenotype; and hdb, encoding the antiholin, which has a null phenotype of early lysis, such that no plaque-forming units are generated (20). Strikingly absent was a lysis defect that could be attributed to a holin. The results presented here provide an rationale for this long-term mystery. We have shown conclusively that lysis can be mediated by the endolysin, Lyz, which is capable of attacking the host murein without requiring a holin to disrupt the membrane. Instead, Lyz is exported by the host sec machinery. Thus, the hdb amber mutant was never isolated because it does not have a lysis-negative phenotype detectable in simple plate tests.

To our knowledge, this report is the first of its kind of a sec-exported endolysin in phages of Gram-negative bacteria and serves to both confirm and generalize the results reported by Santos and colleagues (4), who have shown that endolysins encoded by phages of Gram-positive hosts can have secretory signal sequences. In the well studied cases of phage A and T4, where the endolysins absolutely require holin function for escape from the cytoplasm, it has been demonstrated that the timing of lysis, and thus the yield of virions for the infection, is an exquisitely sensitive function of the primary structure of the holins (28–31). Thus, the finding of the endolysins with cleavable signal sequences was a surprise and posed a challenge to the presumed central regulatory role of holins. Nevertheless, the available physiological data and also genomic analysis suggest that even in phages with secretory endolysins, in both Gram-negative and Gram-positive systems, a holin is present.

Role of the Holin in the Regulation of the SAR Endolysin. With the properties of the SAR endolysins in mind, it is proposed that there are two different modes by which holins can control lysis timing; both can be viewed as activation of the endolysin (Fig. 5). At the programmed time, the canonical holins of λ and T4 disrupt the membrane and allow escape of the cytoplasmically located, active endolysin to the periplasm, after which degradation of the murein and lysis follow within seconds (Fig. 5 A and B). With the SAR endolysins, we suggest that the endolysin is first localized to the periplasm in its membrane tethered form, where it is either inactive or its activity is cryptic (i.e., restrained from access to the peptidoglycan). Triggering of the holin at the programmed lysis time will facilitate the instantaneous and quantitative release of the SAR endolysin from the membrane. This result might simply be due to the collapse of the pmf that occurs after holin triggering. Indeed, energy poisons were found to trigger Lyz-mediated lysis unless Lyz secretion is prevented by the inhibition of SecA (Fig. 1C). The observation that loss of the pmf alone is sufficient to release Lyz from the membrane might explain why much of this endolysin is found in the soluble/periplasmic fractions when cells are subjected to subcellular fractionation well before...
Fig. 5. Model for triggering of lysis with SAR endolysins. (A) A SAR endolysin is initially tethered in an inactive form to the energized membrane, in which the holin protein accumulates without affecting the pmf. (B) At the programmed lysis time, the holin triggers, disrupting the membrane sufficiently to abolish the pmf, and perhaps also to assist the liberation of the endolysin from the membrane, which results in activation of the endolysin. In contrast, with the canonical lysis systems of T4, λ, and T7, the endolysin accumulates in its active form in the cytosol (C) and, when the holin triggers, is released to the cell wall by membrane disruption sufficient to allow passage of large proteins (D).

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