Characterization of the Syringomycin Synthetase Gene Cluster

A LINK BETWEEN PROKARYOTIC AND EUKARYOTIC PEPTIDE SYNTHETASES*

(Received for publication, May 28, 1998, and in revised form, August 20, 1998)

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With this work we have completed the characterization of the syringomycin synthetase gene cluster. In particular, by sequencing additional 28.5 kilobase pairs we show that the nine modules involved in the binding of the nine amino acids of syringomycin are localized on SyrB and SyrE, with SyrE carrying eight modules. The recombinant SyrB and the first and second modules of SyrE (SyrE1 and SyrE2) have been expressed in Escherichia coli and purified. The biochemical data indicate that SyrB binds threonine, the putative precursor of the last amino acid of syringomycin, whereas SyrE1 and SyrE2 bind serine, the first and the second amino acids of syringomycin, respectively. On the basis of the sequence analysis and the biochemical data presented here, it appears that syringomycin synthetase is unique among peptide synthetases in that its genetic organization does not respect the “colinearity rule” according to which the order of the amino acid binding modules along the chromosome parallels the order of the amino acids on the peptide. This feature, together with the absence of a single transcription unit and the absence of epimerase-like domains make syringomycin synthetase more related to the eukaryotic peptide synthetases than to the bacterial counterparts.

Most phytopathogenic strains of Pseudomonas syringae pv. syringae secrete cyclic lipodepsipeptide toxins with phytotoxic activity and a wide spectrum of antimicrobial and antifungal properties. The cyclic lipodepsipeptide syringomycin is a key virulence determinant of P. syringae pv. syringae strain B301D and contributes to disease symptom development (1, 2) by disrupting ion transport and the electrical potential of host plasma membranes (3). Syringomycin is composed of a polar peptide head, having the sequence Ser-D-Ser-D-Dab-Dab-Arg-B301D and contributes to disease symptom development (1, 2) as a key virulence determinant of P. syringae.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank*™/EBI Data Bank with accession number(s) AF047828.

* This work was supported in part by a grant from the European Community, IV Framework Program (to G. G.), in part by Grant 97-35303-4460 from the National Research Initiative Competitive Grants Program of the U. S. Department of Agriculture, Science and Education Administration (to D. C. G.), partially by the contribution of the “Istituto Pasteur Fondazione Cenci Bolognetti,” Università di Roma “La Sapienza” (grant to I. G.), and by Ministero della Università e della Ricerca Scientifica e Tecnologica (MURST). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

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‡ The abbreviations used are: t-Ser, the d-isomer of Ser; Dab, 2,4-diaminobutyric acid; D-Dab, the D-isomer of Dab; Dhb, 2,3-dehydroaminobutyric acid; (3-OH)Asp, 3-hydroxyaspartic acid; (4-Cl)Thr, 4-chlorothreonine; bp, base pair(s); kb, kilobase(s); kbp, kilobase pair(s); PCR, polymerase chain reaction; GST, glutathione S-transferase; IPTG, isopropyl-b-D-thiogalactopyranoside; TE, thioesterase.

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¶ B. K. Scholz-Schroeder, I. Grgrurina, and D. Gross, unpublished observations.
the bacterial counterparts. Furthermore, the unusual architecture with which two amino acid binding modules are structured provides an additional example of how elegantly nature takes advantage of the organization of peptide synthetases in functionally independent domains to generate new enzymes.

MATERIALS AND METHODS

Media and Strains—Escherichia coli strains were grown at 37 °C in LB (Luria-Bertani) medium with the appropriate antibiotics. P. syringae pv. syringae B301D (5) was grown in SRR-C medium (10 g/liter manitol, 8 g/liter d-glucose, 0.2 g/liter MgSO4·7H2O, 0.02 g/liter FeCl2·4H2O, 0.1 g/liter CaCl2·2H2O, 0.5 g/liter NaOH2PO4·H2O, 0.8 g/liter KH2PO4) at 28 °C for 48 h.

DNA Preparation—P. syringae pv. syringae B301D chromosomal DNA was purified from 25 ml of overnight culture grown at 28 °C in SRR-C medium. The pellet was washed in 5 ml of physiological solution (50 mM NaCl), then resuspended in ET buffer (10 mM EDTA, 10 mM Tris-HCl, pH 8.0), and 10 mg of lysozyme was added and incubated without shaking at 37 °C for 15 min. After addition of the same volume of lysis solution (10% Sarkosyl, 250 μg/ml proteinase K in ET buffer) and further incubation at 37 °C for 15 min DNA was extracted twice with phenol/chloroform, precipitated with isopropanol, rinsed with 75% ethanol, and finally dissolved in 1 ml of water. Plasmid DNA was purified using the QIAquick spin or Plasmid MIDI kit (Qiagen, Hilden, Germany) and further precipitation using 2/3 volume of ethanolic alcohol. PCR Amplification and DNA Manipulations—The amplifications of the syr fragments were performed using AmpliTaqTM TdT DNA polymerase XL with proof-reading activity (Perkin-Elmer, Vaterstetten, Germany), 3.3 × 10 μl buffer and a specific set of primers. The reaction was performed in a final volume of 100 μl containing 10–20 ng of chromosomal DNA from P. syringae pv. syringae B301DR strain (5), 20–40 pmol of primers (equimolar), 800 μM deoxynucleotides (dNTPs), 1.5 mM magnesium acetate, 1 unit of rTth polymerase (Perkin-Elmer, Vaterstetten, Germany) following the supplier protocol. The PCR products were gel-purified using the JETPrime Gel Extraction Kit (Genomed, Bad Oeynhausen, Germany), digested with EcoRI and HindIII or SacI restriction enzymes (NEB, Schwalbach-Taunus, Germany) and cloned into pGEX-KG. Plasmids from three independent clones were sequenced to confirm the absence of any PCR errors. The ligation mixtures were used to transform E. coli TOP10. For the purification of the GST-fused domains, the recombinant clones were used to inoculate 500 ml of LB medium (Luria-Bertani) supplemented with 50 μg/ml of ampicillin. The plasmids were grown overnight and the culture was transferred to a polyvinylidene difluoride membrane. Sequencing, the protein was separated onto a 12.5% acrylamide gel and transferred to a polyvinylidene difluoride membrane.

RESULTS

Sequence Analysis of syrE, a 28.4-kbp Gene Located Downstream from syrC—For the sequencing of the chromosomal region downstream from syrC, a “chromosome walking” strategy was used (Fig. 1). For the first amplification step (reaction 1, Fig. 1), the forward primer was designed on the basis of the available sequence of the 3′ end of syrC whereas a degenerated oligonucleotide, matching the conserved “TT” box of the amino acid binding modules of peptide synthetases (Table I), was used as reverse primer, hypothesizing the existence downstream from syrC of such modules. Once amplified, the first fragment was cloned and sequenced, and its 3′ end sequence was used to design a second forward primer. The same reverse primer as for reaction 1 was used for the second amplification. Finally, a third amplification step was carried out using the chromosomal DNA. The sequencing was performed with appropriate restriction enzymes and self-
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FIG. 1. Cloning, sequencing, and biochemical characterization of the syringomycin biosynthesis genes. A. the organization along the chromosome of the syrB, syrC, and syrE genes is shown, as well as the PCR and inverse PCR clones used in this study. S. typhimurium strains carrying the different clones used in this study. B, gene fragments corresponding to the domains that were overexpressed and purified from E. coli to analyze their substrate specificity.

TABLE I

| Domain | Core(s) | Syringomycin synthetase | Peptide synthetases |
|--------|---------|-------------------------|---------------------|
| Adenylation | A1 | L/TS/XXL/[ND] | L/TS/YXEL |
| A2 | LKYG[Q/A]/ATY/V[L/P][A] | LKAYXAYLVLP[L/D] |
| A3 | A/YC/IV/I[L/T]STG/GX/PS/[KG] | LAYXXTYGST/IVTPKG |
| A4 | FD[XS] | FD[XS] |
| A5 | (NQ)XLYGS | NXYGTP |
| A6 | G/ED/XX/GE/GX/[V/L]AR/[L/G]YN | GELXIGXV/LP[AR]YL |
| A7 | Y/RR/[TS]GD | YRR/TCGD |
| A8 | (GA/R/NC/DXQ/X/R[G/L]RX/EXGE/E | GRXDXQV/KRIG/RXIELGEIE |
| A9 | L/PA/ER/YMP | LPGYMIVP |
| A10 | (NH/GR/KX/D | NGKVLIDR |

| Thiolation | T | D/XF/FL/XL/[GS]/HT/IS/[L]F/LA | DXFXXYLG/HD/[S/L] |
| Condensation | C1 | L | SXAQ/XR/[LM]/[WY]/XL |
| C2 | np | RHEXLRTXF |
| C3 | HXX/[SA]/D | MHHX/ISD/[WV]/S |
| C4 | Y | YX/D/[F]/AVW |
| C5 | G/X[N]/X/X/L/X/XR/[V]I | [IV]GXFVNT/[Q/L]/CAXR |
| C6 | (HN/[QXE]/[EX]/PS) | (HN/QU)[V]/PFE |
| C7 | (PQ/X/P[XXX] | RDXSNPL |

| Thioesterase | TE | GGSWSAGG | GR/[HY]/SIX |

* See Fig. 2.
* Marahiel et al. (8).
* np, not present.

ligated. After self-ligation, amplification products were generated with synthetic oligonucleotides (see “Materials and Methods”) and cloned for sequence analysis.

Using this strategy, the 28.4-kbp region downstream from the syrC gene was fully sequenced (GenBank™ accession number AF047828). The sequence analysis revealed the presence of a single ORF, named syrE, which is translated in the same direction as syrB and syrC. Although the absence of a start codon preceded by a canonical ribosome binding site prevents the easy localization of the beginning of the protein, based on the sequence homology with other peptide synthetases modules, we predict that SyrE starts with the ATG located 134 bp downstream from the syrC stop codon. The correctness of the start codon localization is further supported by the fact that 12 of the 16 upstream nucleotides (AAGAGTTGCTTGTCATG) are conserved in the same region preceding the ATG of the syrB gene (ACACAGGATTGTCATG). Therefore, SyrE is predicted to be a 9454-amino acid-long protein, with a calculated molecular mass of 1,038,663 Da. Similar to what was found for syrB, syrC, and syrP (5, 7), no typical E. coli σ70-like promoter is present upstream from syrE. A ρ-independent terminator-like structure, with a calculated free energy of stability of ~6.5 kcal/mol, was observed 53 bp downstream of the syrE stop codon.

As illustrated in Fig. 1, the sequence analysis of SyrE revealed that the protein is organized in eight highly homologous modules having the typical organization of the amino acid binding domains of all peptide synthetases so far characterized (8). In fact, the eight modules have in common the three major domains known as condensation, adenylating, and thiolation domains (8) (Fig. 2). In particular, the common boxes identified within all amino acid binding modules (8) are highly conserved (Table I). Interestingly, the overall identity among the SyrE modules is unusually high. In particular, if only the adenylating domains are taken into account, the identities range from 45% (between module E5 and E8) to 90% (between modules E1 and E2 and between E3 and E4).

Another unexpected feature of SyrE is the absence of any racemase-like domain. Indeed, considering that two of the nine amino acids of syringomycin are in the D configuration (the second and the third amino acid (4)) one would expect that, as it is the case for all bacterial peptide synthetases so far char-
acterized (16–18), these two amino acid binding modules are followed by racemase units.

Unique is the organization of the SyrE8 module. In addition to the condensation, adenylation, and thiolation domains, the module carries a thioesterase domain (TE), a motif constantly fused to the last amino acid binding domains of all bacterial peptide synthetases (8, 16–20). However, the architecture of the SyrE8 module is such that the TE motif is kept apart from the thiolation domain by an intervening sequence that includes a condensation domain and a second thiolation domain (Fig. 2). Looking at the architecture from another perspective, one can say that between the thiolation domain of SyrE8 and the TE motif a ninth module is present in which the adenylation domain is missing.

Interestingly, when the SyrE modules are compared with the other syringomycin synthetase subunit, SyrB1, a module having both the adenylation and thiolation domains but not the condensation domain (Fig. 2), the overall homology is in the 37% range, remarkably lower than the homology found among the SyrE modules.

Amino Acid Specificity of SyrB, SyrE1, and SyrE2—As mentioned above, the high homology between SyrE1 and SyrE2 and between SyrE3 and SyrE4 strongly suggests that the homologous domains recognize the same amino acid, because in syringomycin the first and the second amino acid are serine and the third and forth amino acid 2,4-diaminobutyric acid (Dab), one would predict that SyrE1 and SyrE2 recognize Ser and SyrE3 and SyrE4 Dab. This organization would be in contrast with the colinearity rule of peptide synthetases according to which the order with which the amino acid binding modules are aligned along the chromosome parallels the sequence of the peptide. In fact, the genetic organization of syringomycin synthetase is such that the syrE1 module is preceded by the syrB module, and therefore one would expect that SyrB and SyrE1 recognize serine and SyrE2 and SyrE3 Dab.

In the attempt to shed light on the domain organization and specificity in syringomycin, we isolated the syrB, syrE1, and syrE2 genes using the PCR approach described under “Materials and Methods.” Three fragments were obtained, one of 2,847 bp encoding the putative SyrB protein of 949 amino acid (105 kDa), the syrE1 domain of 3,678 bp encoding a protein of 1226 amino acids (135 kDa), and the syrE2 domain of 3,243 bp encoding a protein of 1081 amino acids (119 kDa). These DNA fragments were inserted into plasmid pGEX in such a way that the modules could be expressed in E. coli fused to the C-terminal end of the GST protein. Although we used the ULTma™ DNA polymerase with proof-reading activity to perform the PCR reactions, three independent clones for each construct were randomly selected and sequenced to rule out the possibility of PCR-generated errors. In all cases the sequences were identical. However, the sequence analysis of the plasmids carrying the syrB gene revealed a difference from the published syrB sequence (5) in that the G originally found at position 2207 was absent, leading to a change in the reading frame and to a stop codon at position 2211. Interestingly, since an ATG codon is present at position 2229, which is preceded by a putative ribosome binding site sequence (AGGAA), one could predict that a second protein can be generated from syrB, translated with the same reading frame as the preceding one.

In conclusion, syrB encodes two putative proteins, SyrB1 of 613 amino acids (67 kDa) carrying the typical adenylation and thiolation domains (Figs. 1 and 2) and SyrB2 of 331 amino acids (36 kDa), with a still unknown function.

When the total proteins from the clones carrying the three genes were analyzed by SDS-polyacrylamide gel electrophoresis, it appeared that the fusions proteins were expressed (Fig. 3), mostly as insoluble material. However, when the cells were grown at 26 °C and the expression of the fusion proteins was induced with a low concentration of IPTG (100 μM) a sufficient amount of proteins partitioned in the soluble fraction thus allowing their partial purification using a glutathione affinity column (Fig. 3). The three fusions, GST-SyrB1, GST-SyrE1, and GST-SyrE2, migrated according to the expected molecular size on SDS-polyacrylamide gel (96, 164, and 148 kDa, respectively, Fig. 3). After purification, minor contaminants were still present that could represent host proteins retained on the affinity column (as is the case for the 30-kDa protein species found in all protein preparations) or the degradation products of the fusion proteins. Furthermore, as expected, the clone carrying the syrB gene also produced a 36-kDa protein not retained on the affinity column (Fig. 3A, lane 3). To confirm that the 36-kDa protein species corresponded to SyrB2, the band was cut out from a polyvinylidene difluoride membrane and subjected to N-terminal sequencing. The sequence of the first seven amino acids was MSKKFAL, corresponding to the N-terminal sequence of SyrB2.

The purified SyrB1 and SyrE1 proteins were also subjected to thrombin digestion (in both fusions a thrombin recognition...
site is located at the junction between the GST protein and the amino acid binding domain). As shown in Fig. 3A, lane 4, and 3B, lane 5, the digestions gave two bands, one with a molecular mass of 29 kDa corresponding to the GST moiety whereas the other with a molecular mass of 67 kDa and 135 kDa corresponded to SyrB1 and SyrE1, respectively.

The fusion proteins were then utilized to determine the amino acid specificity of SyrB1, SyrE1, and SyrE2. To this purpose, the partially purified proteins were incubated with each of the amino acids present in syringomycin, including threonine as both the L isomer and 4-chloro-substituted derivative and the 3-hydroxy derivative of the aspartic acid. In the case of SyrB1 (Fig. 4A), threonine was the only amino acid able to promote pyrophosphate exchange from PPi to ATP, whereas SyrE1 and SyrE2 turned out to be specific for serine. Similar results were obtained when the thrombin-cleaved domains from GST-SyrB1 and GST-SyrE1 were used in the assay (Fig. 4B). These data indicate that SyrE1, SyrE2, and SyrB1 recognize the first, the second, and the last amino acids of syringomycin, respectively.

**DISCUSSION**

With the present work we have completed the characterization, started by Gross and co-workers (1, 5), of the genetic region which in P. syringae is responsible for the synthesis of the lipopeptide syringomycin. From the previously published work as well as from the data reported here, it appears that syringomycin synthetase has a number of features both at the genetic and structural level that make it unique among all bacterial peptide synthetases so far characterized.

The first unusual feature is the organization of the syringomycin synthetase structural genes. Four genes had been previously identified to be involved in the synthesis of syringomycin, including threonine as both the L isomer and 4-chloro-substituted derivative and the 3-hydroxy derivative of the aspartic acid. In the case of SyrB1 (Fig. 4A), threonine was the only amino acid able to promote pyrophosphate exchange from PPi to ATP, whereas SyrE1 and SyrE2 turned out to be specific for serine. Similar results were obtained when the thrombin-cleaved domains from GST-SyrB1 and GST-SyrE1 were used in the assay (Fig. 4B). These data indicate that SyrE1, SyrE2, and SyrB1 recognize the first, the second, and the last amino acids of syringomycin, respectively.

**Fig. 3.** SDS-polyacrylamide gel electrophoresis of the GST/SyrB, GST/SyrE1, and GST/SyrE2 fusions expressed in E. coli. A, SyrB1 module. Lanes: 1, whole cell extract before induction with IPTG; 2, whole cell extract after induction for 5 h at 26 °C with IPTG (100 μM); 3, partially purified GST/SyrB1 fusion; 4, purified product cleaved by thrombin. B, SyrE1 and SyrE2 modules. Lanes: 1, whole cell extract of GST/SyrE2 after induction for 5 h at 26 °C with IPTG (100 μM); 2, soluble fraction of the cell extract of GST/SyrE2; 3, partially purified GST/SyrE2 fusion; 4, partially purified GST/SyrE1 fusion; 5, purified GST/SyrE1 product cleaved with thrombin. M, apparent molecular mass marker. The black arrows show the band corresponding to the purified GST fusions before and after digestion with thrombin. The white arrow shows the product of the syrB2 gene.

**Fig. 4.** Substrate specificities of SyrB, SyrE1, and SyrE2 modules. A, the ATP/PPi exchange activity of the partially purified GST fusion proteins were measured in the presence of the amino acids contained in syringomycin. The highest activities were set to 100% (85,000 cpm for the SyrB modules and ~21,000 cpm for the SyrE1 and SyrE2 modules; this represents the average of at least 5 experiments with 2 independent clones for each domain). The SyrB1, SyrE1, and SyrE2 domains were able to activate L-threonine and L-serine, respectively. B, ATP/PPi exchange activity of the SyrB1 and SyrE1 fusion proteins before and after cleavage with thrombin. The results are similar in the presence or absence of the GST moiety.
SyrB subunit, which activates the last amino acid of syringomycin, has to interact specifically with the C-terminal end of SyrE in order to build up a functional enzyme.

Secondly, our biochemical data indicating that SyrB1 binds threonine, the putative precursor of the amino acid found at the ninth position in syringomycin, and SyrE1 binds the first amino acid serine, clearly show that the colinearity rule, according to which the order of the amino acid binding modules along the chromosome parallels the order of the amino acids in the peptide, does not hold for the syringomycin synthetase system. These experimental data fit nicely the observation that in SyrB1 the condensation domain typically found at the N terminus of the first domains of lipopeptide synthetases is missing whereas such a domain is fused to SyrE1. This is the first example of such an organization among all peptide synthetases studied so far. Interestingly, very recently we have demonstrated that in surfactin synthetase the coordinated transcription of the enzyme subunits can be altered and the amino acid binding modules can be dissociated without substantially affecting the synthesis of surfactin at both the qualitative and quantitative level (19). These data, together with the experimental evidence showing that the purified surfactin synthetase subunits can reassociate in vitro to give a functional enzyme (22), led us to the conclusion that protein-protein interactions rather than coordinated transcription and translation guide the correct assembling of peptide synthetases. In this context, syringomycin synthetase is a natural example of what we have demonstrated by gene manipulations.

Thirdly, in all prokaryotic peptide synthetases the thioesterase moiety, which appears to be involved in the release of the mature peptide chain from the enzyme, is fused to the C-terminal end of the last amino acid binding module (8). In the case of syringomycin synthetase, the TE domain is found fused to the C-terminus of SyrE8 and not, as one would predict, at the end of SyrB1. Apart from its unusual feature, this structural organization poses the question on how can a premature peptide of eight amino acids not be released before the completion of syringomycin synthesis. Most likely, the answer to this question is found in the atypical architecture of SyrE8. Differently from the other synthetases in which the TE domain is directly fused at the C-terminal end of the last amino acid binding module, in SyrE8 an intervening sequence keeps TE apart from the thiolation motif and most likely prevents the thioesterase from releasing the 8-amino acid peptide. Even more interesting is the fact that the intervening sequence is constituted by an amino acid binding module in which the adenylation domain is missing. Considering that in SyrB1 the adenylation domain is not preceded by the condensation domain, one could envisage a situation in which a fully functional module is reconstituted by the proper interaction of SyrB1, which provides the amino acid specificity domain, with the intervening sequence of SyrE8, which provides the condensation and the thiolation domain (Fig. 5).

Finally, because the second and third amino acids of syringomycin are in the D configuration (D-serine and D-aminobutyric acid (4)), comparisons to other bacterial peptide synthetases predict the presence of an epimerization domain fused to the C terminus of both SyrE2 and SyrE3. However, such domains are missing in the SyrE subunit. Therefore, the mechanism by which D-amino acids are incorporated into syringomycin could involve the direct binding of the D-isomers to the adenylation domains, as postulated for the eukaryotic cyclosporin and Helminthosporium carbonum toxin synthetases (23, 24).

In the attempt to shed light on the mechanism of D-amino acid incorporation, we have also cloned and expressed the SyrE2 domain to determine its amino acid specificity. From our data it appears that SyrE2 recognizes l-serine, in line with the sequence analysis showing a 90% identity between the adenylation modules of SyrE1 and SyrE2. This raises the interesting question on how and when the change of configuration takes place during syringomycin biosynthesis. A possible mechanism could envisage that racemization occurs at the peptidyl or at the aminoacyl stage, as has been proposed for the incorporation of D-valine in both GrsA and TycA (25, 26), provided that an external racemase takes part in the synthetase complex. Considering that various amino acid racemases and epimerases with broad substrate specificity have been characterized from several Pseudomonas and Aeromonas strains (27, 28), it is likely that some of them might play a role in syringomycin biosynthesis. The mechanism of racemization in syringomycin synthetase could therefore differ from the one proposed for the D-amino acid incorporation in cyclosporin A and Helminthosporium carbonum toxin, where the direct binding of D-amino acids has been postulated. However, it has to be pointed out that there is no experimental evidence showing that purified, recombinant domains of eukaryotic peptide synthetases bind the D-isomers. Therefore, the existence of these two different pathways of D-amino acid incorporation still awaits experimental demonstration.

As far as substrate specificity is concerned, the observation that SyrB1 appears to bind threonine and not its chloro derivative is interesting, suggesting that, as is the case for serine

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3 GenBank\textsuperscript{TM} sequence data base, http://www.ncbi.nlm.nih.gov (submission 7/98).
The length of SyrE, the absence of racemase motifs, and the independent transcription of the coding genes make syringomycin synthetase more similar to the eukaryotic enzymes than to the prokaryotic counterparts. For instance, the 9454-amino acid long SyrE resembles the cyclosporin synthetase constituted by a single giant protein of 15,282 amino acids containing 11 modules (23), and as is the case for syringomycin synthetase, no racemase-like domain can be identified in cyclosporin synthetase (23).

How can an enzyme with eukaryotic features be present in a prokaryotic organism? One possible explanation is direct gene transfer from an eukaryotic organism to P. syringae. In line with this hypothesis is (i) the relative poor homology between SyrB and SyrE as compared with the homologies existing among SyrE modules (50% identities among SyrE domains versus the lesser 37% identity between SyrB and any of the SyrE modules) and (ii) the GC content of syrB. While it is similar to the average GC content of other P. syringae pv. syringae genes (59.5% (5)), the GC content of syrE is 62.5%. The horizontal transfer of a peptide synthetase gene from prokaryotes to eukaryotes has already been postulated in the case of the ACV (β-(L-α-aminoadipyl)-L-cysteinyld-valine) synthetase (30, 31). Our data would suggest that in peptide synthetases such gene exchange could also go in the other direction.

If SyrE has been acquired from an eukaryotic organism, why has its structure not changed during evolution to become more similar to the prokaryotic enzymes? Among the possible explanations an attractive one is that the gene could be transcribed and translated in the infected plant cells to exacerbate its pathogenic functions. It should not be difficult to test this hypothesis experimentally. Of relevance is the fact that the syringomycin synthetase genes are activated in response to specific plant signal molecules (7, 32), thus making syringomycin synthetase the only known thiopentolate system for toxin synthesis regulated by external host factors.

Acknowledgments—We thank Giorgio Corsi for the artwork and Antonietta Maiorino for her expert secretarial assistance.
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J. Biol. Chem. 1998, 273:32857-32863.
doi: 10.1074/jbc.273.49.32857

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