Isoprenoid Biosynthesis

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Moenomycin Biosynthesis: Structure and Mechanism of Action of the Prenyltransferase MoeN5

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Abstract: *The structure of MoeN5, a unique prenyltransferase involved in the biosynthesis of the antibiotic moenomycin, is reported. MoeN5 catalyzes the reaction of geranyl diphosphate (GPP) with the cis-farnesyl group in phosphoglycolipid* ***5*** *to form the (C_{25}) moenocinyl-sidechain-containing lipid* ***7****. GPP binds to an allylic site (S1) and aligns well with known S1 inhibitors. Alkyl glycosides, glycolipids, can bind to both S1 and a second site, S2. Long sidechains in S2 are "bent" and co-locate with the homoallylic substrate isopentenyl diphosphate in other prenyltransferases. These observations support a MoeN5 mechanism in which* ***5*** *binds to S2 with its C6–C11 group poised to attack C1 in GPP to form the moenocinyl sidechain, with the more distal regions of* ***5*** *aligning with the distal glucose in decyl maltoside. The results are of general interest because they provide the first structures of MoeN5 and a structural basis for its mechanism of action, results that will facilitate the design of new antibiotics.*

The moenomycins are a potentially important class of antibiotics that were discovered around 60 years ago,[1] and they function by inhibiting bacterial cell-wall biosynthesis.[2] Moenomycin A (MmA, **1**; Scheme 1) is one member of this class and is a more potent antibiotic in vitro than vancomycin. It has poor pharmacokinetics, but given the global increase in antibiotic resistance[3] there is renewed interest in the development of MmA analogues,[4] as well as the possibility of using MmA to treat infections of the gastrointestinal tract, such as those caused by *Helicobacter pylori*.[5] MmA biosynthesis is quite complex, with 17 enzymes being involved.[4a] The first committed step involves the condensation of 3-phosphoglycerate (**2**) with farnesyl diphosphate (**3**) to form 2-(*Z,E*)-farnesyl-3-phosphoglycerate (FPG, **4**) in a reaction catalyzed by MoeO5,[6] and in recent work, we reported the X-ray structure of MoeO5 and proposed a mechanism of action.[6b] Structure **4** then undergoes a series of glycosylations and other modifications to form a phosphoglycolipid, the FPG trisaccharide **5**, which then reacts with geranyl diphosphate (GPP, **6**) to produce the (C_{25}) moenocinyl trisaccharide **7**,[7] which after further transformations[4a] results in the formation of **1**. The **5**→**7** reaction is catalyzed by MoeN5[4a] and has been proposed[4a] to involve two carbocations. However, the structure of MoeN5 has not been reported and a Basic Local Alignment Search Tool (BLAST) search reveals no homologues with known structure. We thus sought to determine the structure of MoeN5 in the absence and presence of GPP, FPG, and a series of model glycolipids in order to clarify the mechanism of action of this very unusual "head-to-middle" prenyltransferase.

Recombinant MoeN5 from *Streptomyces ghanaensis* was first expressed with a 35-residue N-terminal tag, hereinafter denoted as MoeN5-NT, and its structure was solved by using single-wavelength anomalous diffraction (SAD) with a monoclinic C2 Se-Met data set (3.3 Å; Table S1 in the Supporting Information). The structure was highly α-helical, however, several regions were disordered and the resolution was low. We next sought to find better crystal forms by adding one of four protein fusion tags: thioredoxin from *Escherichia coli*; small ubiquitin-like modifier (SUMO) from *Saccharomyces cerevisiae*, and chromosomal protein 7d from the hyperthermophiles *Sulfolobus acidocaldarius* (Sac7d) and *Sulfolobus solfataricus* (Sso7d). The construct containing Sso7d linked via 5 residues to the MoeN5 C terminus, together with a 12-residue N-terminal His-tag, hereinafter referred to as MoeN5-CS, was successfully expressed, purified, and crystallized in two different orthorhombic space groups: C222 and I222. Both crystals yielded improved diffraction data sets: 2.29 Å for the C222 form, and 2.80 Å for the I222 form. The two structures were solved by molecular replacement, and the improved electron-density maps allowed full elucidation of the MoeN5 structure. Data acquisition and refinement details are given in Table S2, and full experimental details are given in the Supporting Information.

The structure of MoeN5-CS (containing the Sso7d C-terminus tag; PDB ID: 5B02) is shown in Figure 1 A, and it can be seen that each molecule forms a dimer in the crystal. It is also clear from size-exclusion chromatography with multi-

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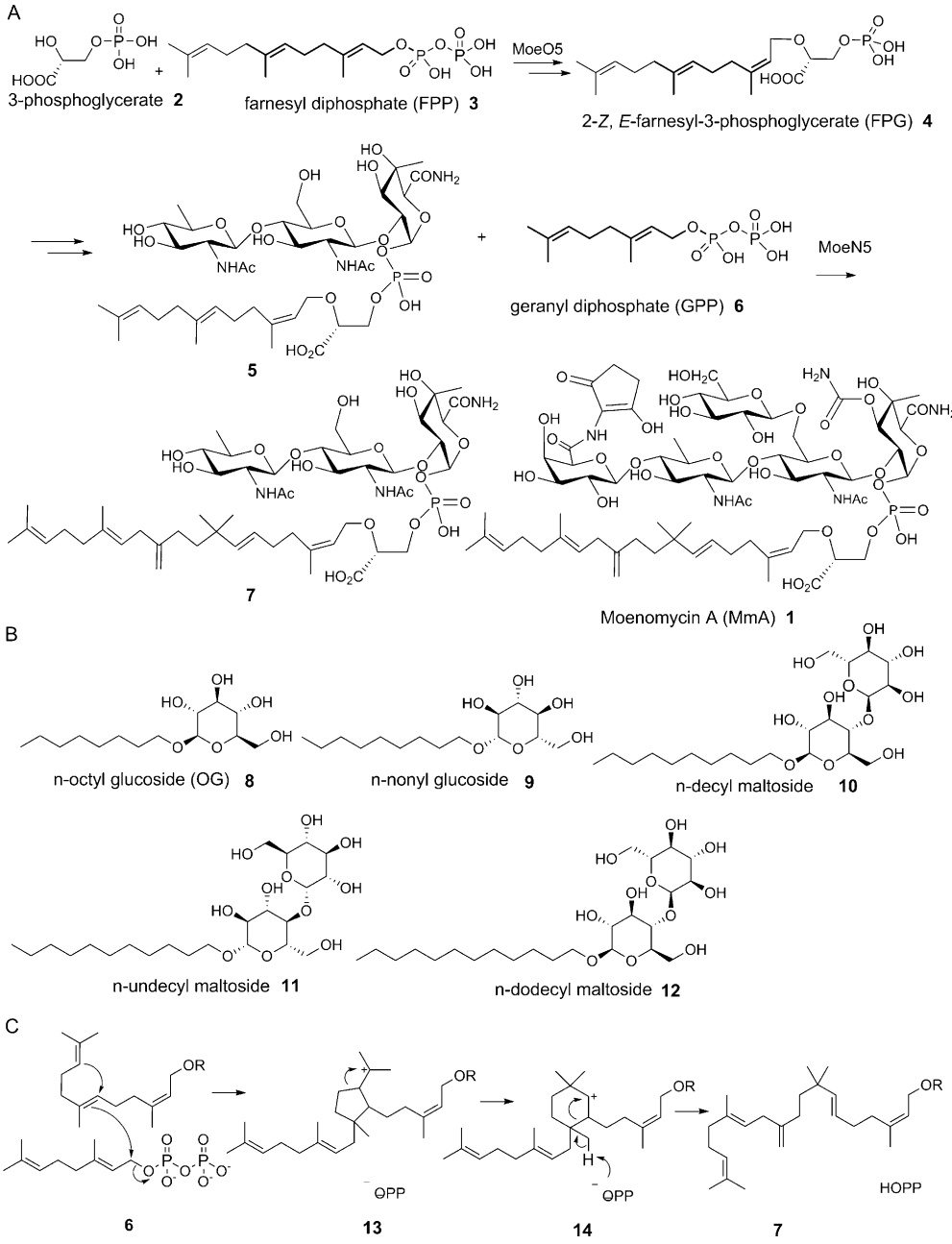

Scheme 1. Structures of molecules of interest, and the MoeN5 mechanism. A) Selected steps in the biosynthesis of moenomycin A, showing the reaction catalyzed by MoeN5. B) Structures of the five β-*n*-alkyl glycosides investigated. C) The MoeN5 mechanism proposed by Walker et al..[4a]

angle laser scattering (SEC-MALS; Figure 1B) that MoeN5 without the fusion tag also forms a dimer in solution. Each MoeN5 domain contains 12 α-helices, designated A–L (Figure 1C). Dimer formation mainly involves helices E, F, and G, and together with the C-terminal regions of helices A and D, the dimer interface buries 1830 Å^2 of surface area on each monomer. The Sso7d fusion tag showed higher B-values than did MoeN5 and was visible as only one monomer in each MoeN5-CS dimer (Figures 1A,D), where it occupied one of two sites (Figure 1D). By using the PDBeFold server[8] we found that the MoeN5 structure has some similarity to a range of other prenylsynthases, including a (C_{10}) geranyl transferase from *Thermotoga maritima* (PDB ID: 2FTZ,[9] a 3.05 Cα root mean square deviation (rmsd) over 231 residues); a (C_{20}) geranylgeranyl diphosphate synthase from *Sinapis alba* (PDB ID: 2JLO,[10] 2.78 Å/214 residues); and the catalytic domain of a polyprenyl diphosphate synthase from *Shigella flexneri* (PDB ID: 2FOR,[11] 2.85 Å/221 residues). These (and many other) proteins catalyze well-known[12] "head-to-tail" *trans*-prenyl synthase reactions and contain two Asp-rich (DDXXD or DXXXD) domains. The first aspartate-rich domain (FARM) and the second aspartate-rich domain (SARM), both of which bind to Mg^{2+}, are involved in the ionization of allylic substrates. MoeN5 likewise contains two such Asp-rich domains, as illustrated in the stereoview in Figure 1E with orange (FARM; **DD**LM**D**) and yellow (SARM; **DD**LT**D**) spheres. The active site is expected to be located near these Asp-rich domains.

We next tried to determine the spatial locations of the two substrate binding sites, which, by analogy to work on other (head-to-head and head-to-tail) prenyl synthases,[12b,13] we shall call S1 (the allylic binding site to which GPP should bind) and S2 (to which the FPG-trisaccharide **5** should bind). As can be seen in Figure 2A, GPP binds to MoeN5-CS close to the Asp-rich domains, and the ligand electron density is well resolved. Similar results with GPP are found for all sites in the two structures (PDB IDs 5B00 and 5B03, Figures S1 and S2, respectively). There are actually slight differences in the site occupancies (in the unit cell) with all ligands investigated. Site occupancies (a total of 41) for all 7 systems are given in Table S3; ligand electron densities (there are 37 more and less well-defined densities) are given in Figures S1–7; and local ligand density fit (LLDF) values are also shown in Table S3.

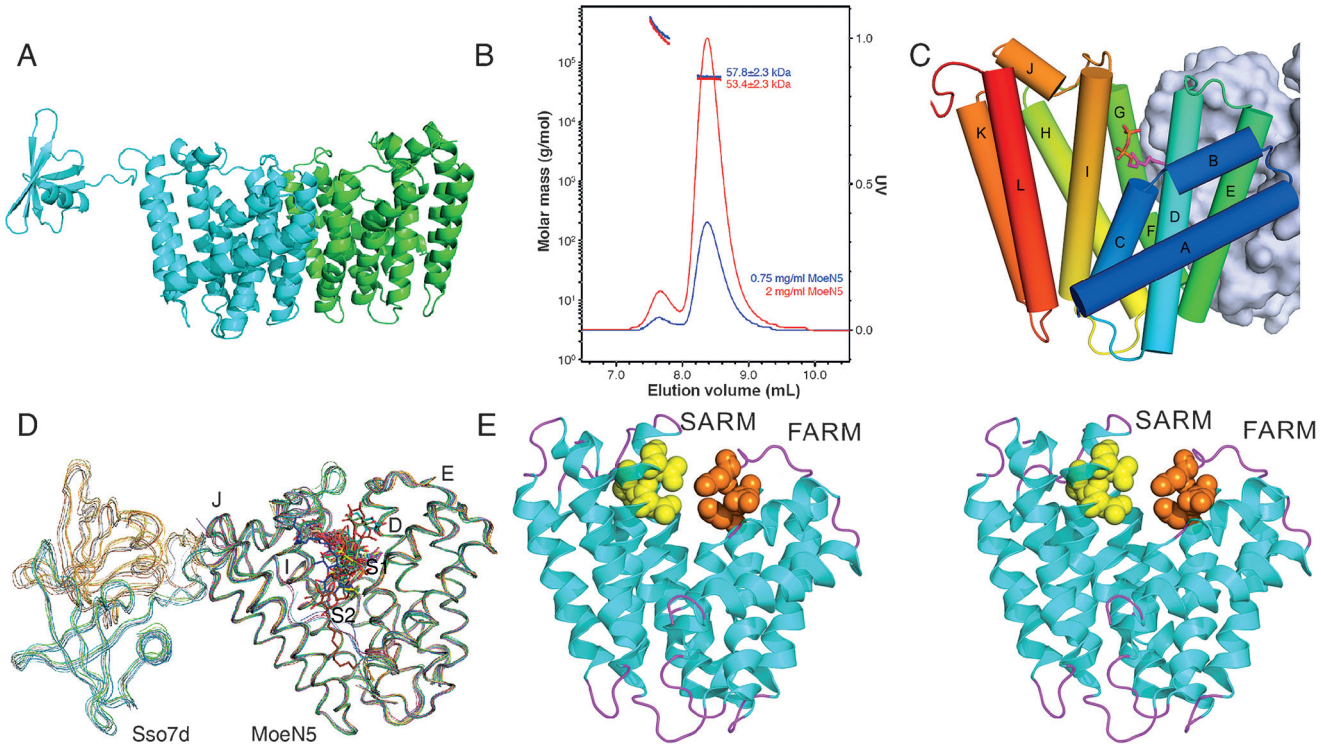

Figure 1. *Streptomyces ghanaesis* MoeN5 structures. A) The structure of the MoeN5-CS dimer (PDB ID: 5B02). The Sso7d tag is only ordered in one molecule in the dimer. B) SEC-MALS molecular-weight determination for MoeN5-NT, showing dimer (56 kD) formation in solution. Protein concentrations were 0.75 mg mL^{-1} and 2 mg mL^{-1}. C) The 12 α-helices (A–L) in MoeN5. D) Superposition of all MoeN5-CS structures, showing increased B-factors for the Ssod7 domains. E) Stereoview of MoeN5 (PBD ID: 5B00), showing the first (orange) and second (yellow) Asp-rich domains.

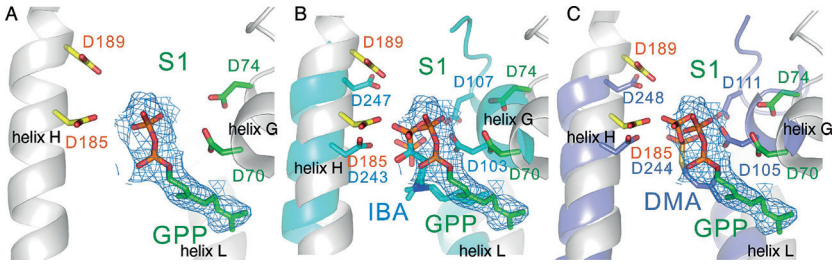

Figure 2. The S1 (GPP-binding) site in MoeN5. A) GPP electron density Fo-Fc omit maps are shown in a mesh representation (cyan, contoured at 1 sigma), together with several nearby amino acid residues. Maps contoured at 1 and 2.5 sigma are shown in Figure S2. B) Alignment of GPP in MoeN5 with the S1-site inhibitor ibandronate in human FPPS. C) Alignment of GPP in MoeN5 with the S1-site inhibitor S-thiolo-dimethylallyl diphosphate in *E. coli* FPPS.

As can be seen in Figure 2B,C, there is good agreement between the position of GPP in MoeN5 and the positions of known S1-site inhibitors (and substrates) found in other *trans*-prenyl synthases. For example, both the bisphosphonate ibandronate bound to farnesyl diphosphate synthase (PDB ID: 2F94;[14] Cα rmsd 3.58 Å/236 residues) and *S*-thiolo-dimethylallyl diphosphate (DMA) bound to FPPS[15] (PDB ID: 1RQI; Cα rmsd 2.83 Å/218 residues) bind to the allylic site S1, as shown in the superposition with GPP in MoeN5 (PDB ID: 5B00; Figures 2B,C). This similarity helps confirm the location (and function) of the allylic GPP-binding site S1 in MoeN5. The locations of the conserved Asp residues are also shown in the Figure and are similar in both MoeN5 and the FPPSs.

We also anticipate a second, S2-like site in MoeN5 that would be analogous to that seen in other *trans*-prenyltransferases, although it would not formally be a "homoallylic" site. However, attempts to obtain structures with FPG (**4**), a model for **5** that might be expected to bind to an S2 site, as well as its mono and trimethyl esters and MmA, were not successful. We thus adopted an alternate strategy, which was to determine the binding sites of a series of glycolipid models, the alkyl glycosides (**8**–**12**; Scheme 1B). The idea was that it might be possible to map out the hydrophobic side-chain binding pocket (S2) to which the hydrophobic side chain of **5** should bind, and then suggest where the sugar residues (in **5**) would be located. Presumably, S2 should accommodate bent-sidechain species that would be close to C1 of GPP in S1 for catalysis, and in addition, the sugars might extend into the solvent.

We determined five structures (MoeN5-CS bound to ligands **8**–**12**), and as can be seen in Figures S3–S7, the electron densities in several cases are very well defined. Surprisingly, however, many of the glycosides bound to S1, and some bound to both S1 and a new site that we assign as S2. The two sites can be clearly seen in the electron-density map

of decyl maltoside (DM, **10**) bound to MoeN5-CS (PDB ID: 5B0K; Figure 3A and Figure 5B in the Supporting Information). This occupancy of both S1 and S2 sites is of interest since as can be seen in Figure 3A, the alkyl side chains have close contacts (as close as approximately 4 Å), as would be required for reaction of the S1- and S2-site substrates. What is even more interesting is the observation that the longer *n*-alkyl side chains of the ligands (**9**, **10**) that bind at S2 are bent at their C termini, as proposed[4a] for **5** during catalysis (see Scheme 1C). The bend is induced by a wall consisting of F149, L150, and I181 (Figure S8). The S2 site in MoeN5 overlaps with the isopentenyl diphosphate S2 site in FPPS (PDB ID: 1RQI,[15] Figure 3B). However, MoeN5 does not contain the highly conserved basic residue motifs (containing RR and K) that bind to diphosphates, since the phosphate in **5** is expected to be far removed from the typical location of the IPP phosphate binding site. When the ligands from all seven structures are superimposed (Figure 3C), it is clear that there are indeed two distinct ligand binding sites in MoeN5. The S1 domain corresponds to the allylic substrate, bisphosphonate inhibitor, and allylic *S*-thiolo-diphosphate inhibitor binding sites seen in, for example, FPPS, while S2 can house alkyl glycosides with a bent C terminus, and this bent C terminus is close to the position occupied by C1 of GPP. These structural results immediately suggest that the phosphoglycolipid **5** binds to S2 in a "bent" conformation with its C6,7 and C10,11 double bonds poised to attack the geranyl carbocation formed on diphosphate release from **6**.

The S1 and S2 site structures we have obtained support the structure-based model shown in Figure 4 for the interaction of the phosphoglycolipid **5** with GPP to form the moenocinyl phosphoglycolipid. Figure 4A shows a manual alignment of **5** (with the proposed "bent" C6–C11 side-chain geometry[7] shown in blue) with the alkyl side chain in **10** (pink) in S2, in which the first sugar in **5** is aligned with the distal glucose in the maltoside **10**. Also shown is GPP (yellow)

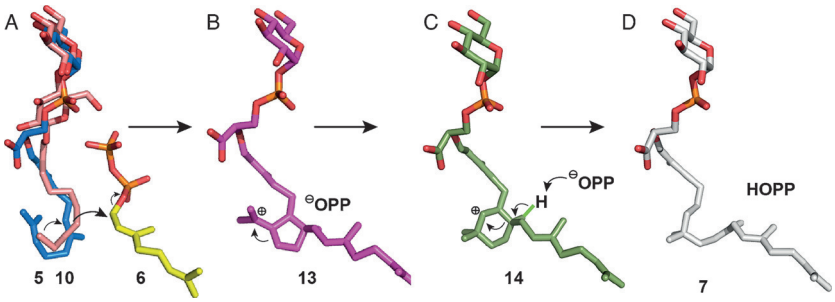

Figure 4. Structural models for the MoeN5 mechanism. A) The phosphoglycolipid **5** (blue, shown for simplicity with a single sugar) binds to S2 with its bent (C6–11) sidechain occupying the same region as does **10** (pink); the proximal sugar in **5** overlaps with the distal glucose on the maltoside. Other sugars will be in solvent. Also shown is GPP (**6**, yellow). B) Diphosphate release from GPP **6** and attack by C6,7/C10,11 in **5** yields the cyclopentyl carbocation **13**. C) **13** rearranges to the cyclohexyl carbocation **14**. D) Removal of a methyl proton in **14** by PPi yields the product **7**.

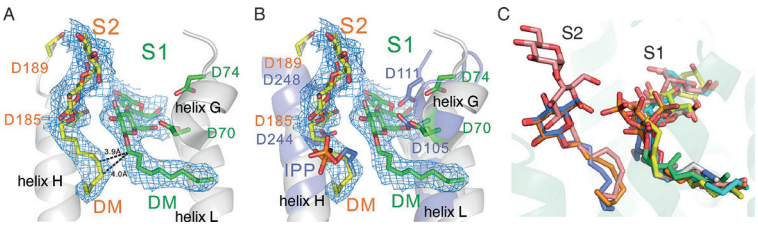

Figure 3. Ligands bind to both the S1 and S2 sites in MoeN5. A) β-*n*-decyl maltoside (DM, **10**) binds to both S1 and S2. Electron density Fo-Fc omit map (cyan, contoured at 1 sigma) are given together with several nearby amino acid residues. The chains approach to within around 4 Å. For additional density results, see Figure S5B. B) Alignment of DM (**10**) in MoeN5 with the canonical *trans*-prenyltransferase S2-site ligand IPP in human FPPS. C) Superposition of all six ligands investigated (GPP and the 5 glycosides), bound to MoeN5. The ligands shown are those with the most complete electron densities in Figures S1–S7.

in S1. Upon diphosphate ionization, C6,7[7] attacks C1 of GPP and the 5- and 6-membered-ring carbocations form (**13** and **14**; Figures 4B,C). The diphosphate group released from **6** is then poised to remove the methyl proton in **14** (Scheme 1C, Figure 4C) to form the product **7** in a manner reminiscent of the general-base role of the diphosphate group (in dimethylallyl diphosphate) in isoprene synthase.[16] We find no serious clashes with any protein residues, which supports this structural model and, consequently, the mechanism of action first proposed by Arigoni et al.[7]

In summary, we report the first X-ray structures of *S. ghanaensis* MoeN5, a prenyltransferase involved in biosynthesis of the antibiotic moenomycin A. Structure determination was made possible by the use of chromosomal protein 7d from the hyperthermophile *Sulfolobus solfataricus* as a fusion tag, an approach that may be applicable to other systems. The MoeN5 substrate GPP binds to the allylic site S1 seen in other prenyltransferases. Amphiphilic glycosides can bind to both S1 and S2, with **9** and **10** binding to S2 in a "bent" C-terminus conformation. This conformation serves as a template for binding of the second MoeN5 substrate (**5**), which enables attack by C6,7 in **5** at the C1 of GPP to form **7**. The first glycoside group in **5** would then be located in the same region as the distal glucose in **10**, with additional sugars (in **5** and **7**) extending into the solvent. Overall, the results are of broad general interest since they can be expected to facilitate the future development of MmA analogues as new anti-infective drug leads. In addition, the use of small fusion tags such as Sso7d can be expected to facilitate the structure determination of other proteins.

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