

Sortases make pili from three ingredients

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Many bacterial pathogens have evolved hair-like extensions that project from their surfaces and enable adhesion to host tissues, formation of biofilms, motility, and even transfer of genetic material (1). Gram-positive bacteria use sortases, membrane-anchored transpeptidases, to cleave precursor proteins at cognate recognition motifs within C-terminal sorting signals and assemble pili in their cell wall envelope (2, 3). The transient product of this reaction is an acyl enzyme that, depending on the sortase involved, is relieved by a specific nucleophile (4). Currently studied sortases accept only one nucleophile to complete their transpeptidation reaction (5–8). For example, sortase A of *Staphylococcus aureus* forms an acyl enzyme with surface proteins bearing LPXTG motif sorting signals, which are subsequently relieved by the nucleophilic attack of the amino group of lipid II, the precursor of peptidoglycan synthesis in bacteria (9, 10). In this issue of PNAS, Mandlik *et al.* (11) provide new insight into sortases and their complicated recipes for pilus assembly.

Starting with simple recipes, some sortases make pili from two ingredients (12, 13). *Bacillus cereus* sortase D cleaves the sorting signal of pilin precursors and accepts the side chain ϵ -amino group of lysine (K) within the YPKN motif of the major pilin protein as its nucleophile (14). Products of this reaction are covalently polymerized pilin subunits, glued together by an amide bond between the C-terminal carboxyl group of one subunit and the side-chain amino group of the YPKN motif of another (14). Conservation of sortase D, as well as the YPKN motif and cell wall sorting signal of major pilin proteins in many different species, suggests that this pilus assembly reaction is universal in Gram-positive bacteria (14, 15). A simple recipe for pili therefore requires only one chef, sortase D, and two ingredients, BcpA and BcpB (12). Sortase D acyl intermediates are relieved by the nucleophilic attack of the BcpA YPKN motif, thereby generating a pilus with BcpB at the tip joined to a BcpA homopolymer (14). Pilus assembly is terminated when sortase A cuts BcpA and accepts its nucleophile, lipid II. Because BcpA can still be joined to the pilus via its YPKN motif, incorporation of lipid II at the C-terminal end causes pili to be immobilized in the cell wall envelope.

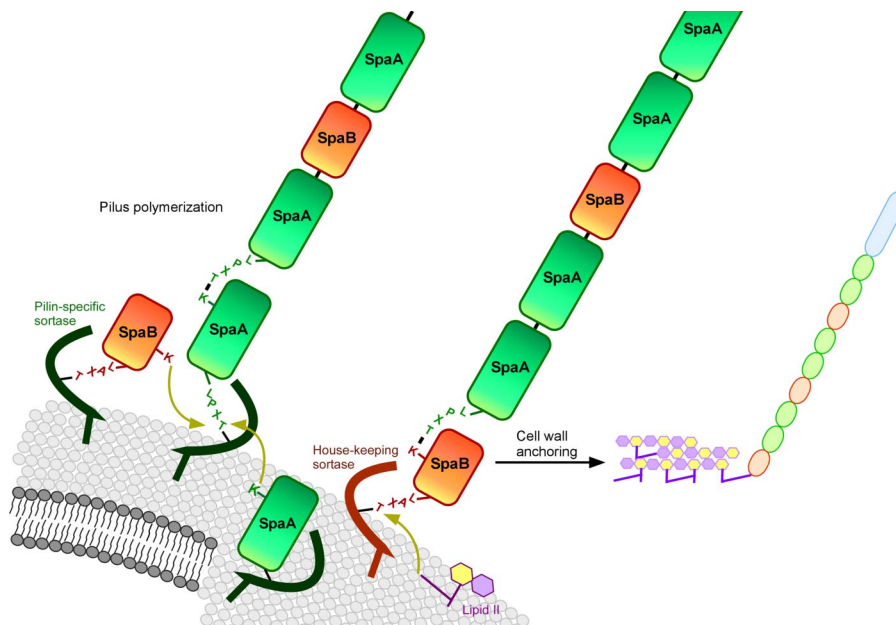


Fig. 1. Pilus assembly in *Corynebacterium diphtheriae*. Pilin-specific sortase cleaves the sorting signal of SpaA and SpaB at the threonine residue (T) of the LPXTG motif, generating an acyl-enzyme intermediate. Nucleophilic attack by the side chain of lysine (K) in the YPKN motif of SpaA forms an amide bond between SpaA or SpaB and provides for pilus polymerization. SpaB is cleaved by the housekeeping sortase. Nucleophilic attack by lysine 139 (K139) of SpaB connects the minor subunit to the pilus. Nucleophilic attack of lipid II at the acyl intermediate of the housekeeping sortase with SpaB transfers the pilus to the cell wall envelope.

In chemical terms, anchoring of pili to the cell wall envelope occurs by a mechanism where another chef, sortase A, competes with sortase D for the same ingredient (BcpA substrate).

Pili made from three subunits thus far have presented an enigma to the pilus sortase hypothesis (3). The pilin-specific sortase of *Corynebacterium diphtheriae* (sortase A) is responsible for assembly of pili that harbor SpaC at the tip joined to a heteropolymer made of SpaA, the major pilin subunit with YPKN motif, and SpaB (3) (Fig. 1). Immune electron microscopy studies suggest an occasional incorporation of SpaB, a minor subunit that harbors a C-terminal sorting signal but no YPKN motif (3). Mandlik *et al.* (11) report that deletion of *spaB* does not abrogate pilus assembly; instead, the SpaC-SpaA_n polymer is released into the extracellular medium, a phenotype similar to that of *srtF* mutants (the *C. diphtheriae* homolog of sortase A) (16). What is the nucleophilic attribute that makes SpaB an ingredient of pili? Mandlik *et al.* use a molecular genetic approach and identify the smallest domain that allows for incorporation of

SpaB into SpaA pili. Substitution experiments identify lysine 139 (K139) as absolutely required and, similar to the YPKN motif (3), suggest that the side-chain amino group of this residue functions as the SpaB nucleophile (11). This discovery provides fresh and important views on pilus assembly in Gram-positive bacteria, because most sortase chefs use three ingredients to make pili and these pili are used for vaccine development (1, 17). If one assumed that *C. diphtheriae* sortase A cuts three subunits (SpaA, SpaB, and SpaC), the resulting acyl intermediates could be relieved by nucleophilic attack of two different substrates, SpaA or SpaB, which would be a true novelty of sortase chemistry. This recipe could get even more complicated because some pili involve two pilin-specific sortases for assembly, leaving open the possibility of

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multiple substrate specificities (18, 19). Whatever models turn out to be true, currently available data suggest that some pilin-specific sortases must accept more than one nucleophile to complete their transpeptidation reaction (11). Another attribute of recipes with three ingredients is that two chefs (sortases A and F) may compete for the minor pilin, whereas the major subunit is apparently only cut by sortase A (16). Similar mechanisms may apply for pili of *Streptococcus agalactiae* (group B strepto-

cocci), where deletion of the minor subunit also results in the release of pili (20). Unlike bacilli, corynebacteria, or group B streptococci, pili of *Streptococcus pneumoniae* were found localized to the cell wall in a mutant that lacked the housekeeping sortase A, the enzyme that accepts lipid II nucleophiles (21). If so, should we expect that pilin-specific sortases of *S. pneumoniae* accept two nucleophiles from different chemical groups, pilin precursor (a protein) and lipid II (a glycolipid)?

In sum, recipes for pili with three ingredients seem complicated and require sortases that cleave multiple substrates and accept multiple nucleophiles. What's needed now are biochemical approaches that recapitulate these assembly reactions *in vitro* and reveal the molecular details of substrate recognition and sortase catalysis. Looking deep into chefs' recipes will not only provide insight into Gram-positive pili, but should also generate menus of astonishing variety that can be used in imaginative ways for biological investigation (22).

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