Structure to function of an α-glucan metabolic pathway that promotes *Listeria monocytogenes* pathogenesis

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7. The basis of ROK transcription induction.
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Supplementary Figure 1. Phylogenetic reconstruction of CAFE sequences. Seventeen CAFE homologs identified by protein BLAST were used to construct a phylogenetic tree. Values for nodes of 1000 random bootstrap replicate trees are shown. CAFE homologs are present in multiple phyla (Firmicutes and Actinobacteria), suggesting an ancient evolutionary origin of CAFE. The fact that so few organisms from these phyla possess CAFE homologs implies widespread gene loss over the course of evolutionary history.
Supplementary Figure 2. Representative operons with CA-associated genes. A comparative genomics analysis revealed that CAFE and branching enzyme genes are always associated with an intracellular GH31 protein and an ABC transporter and often with predicted transcription regulators and additional intracellular glycoside hydrolase family proteins. Gene clusters are colored by function: transcription regulators (pink), extracellular transglycosylases (green), ABC transporter components (yellow), and intracellular glycoside hydrolases (blue). (A) Representative genomes with a gene cluster that contains CAFE and branching enzyme alongside putative uptake/catabolism genes. (B) Representative genomes with two gene clusters – one with branching enzyme and CAFE and a second with putative uptake/catabolism functionality. (C) Representative genomes that lack branching enzyme and CAFE but possess a putative CA uptake/catabolism gene cluster.
Supplementary Figure 2. Representative operons with CA-associated genes.

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Supplementary Figure 3. CA binding to the ABC transporter substrate-binding protein Lmo0181. (A) ITC results generated by titrating CA into Lmo0181. This representative isotherm was fit by a one site binding model (N = 1.05 ± 0.005 sites, ΔH = -3023 ± 22.5 cal/mol, ΔS = 15.7 cal/mol/deg). (B) The CA binding site observed in the Lmo0181 crystal structure. Hydrogen bonds are represented as dashed lines. The Fo-Fc electron density map calculated with CA omitted from the model is contoured at 3σ. (C) The Lmo0181-CA complex (orange) superimposed with a representative ABC carbohydrate-binding protein (a S. glaucescens protein in complex with maltodextrin tetrasaccharide, PDB code 3K00). Like other linear substrates, the maltodextrin runs along the interdomain cleft. By contrast, the cyclic CA extends into a deeper C-terminal domain pocket in Lmo0181 (pink arrow).
Supplementary Figure 4. Structure-based inference of *Tp*Lmo0182 and Lmo0184 activities in CA catabolism. (A) Comparison of *Tp*Lmo0182 and Lmo2446. The enzymes have similar domain architectures and overall structures (RMSD = 1.0 Å). The nearly identical active sites between Lmo2446 and *Tp*Lmo0182 ([Fig. 2G](#)) suggested both bind CA and led us to hypothesize that they catalyze opposing reactions in CA anabolism and catabolism. (B) Lmo0184 is superimposed (RMSD = 0.6 Å) to the *Bacillus cereus* GH13 α-1,6-glycoside hydrolase homolog (PDB code 1UOK). The inset shows the glucose-bound Lmo0184 active site. The similar structure and conserved active site suggested a common enzymatic activity.
Supplementary Figure 4. Structure-based inference of TpLmo0182 and Lmo0184 activities in CA catabolism.

(A) Comparison of TpLmo0182 and Lmo2446. The enzymes have similar domain architectures and overall structures (RMSD = 1.0 Å). The nearly identical active sites between Lmo2446 and TpLmo0182 (Fig. 2G) suggested both bind CA and led us to hypothesize that they catalyze opposing reactions in CA anabolism and catabolism. (B) Lmo0184 is superimposed (RMSD = 0.6 Å) to the Bacillus cereus GH13 α-1,6-glycoside hydrolase homolog (PDB code 1UOK). The inset shows the glucose-bound Lmo0184 active site. The similar structure and conserved active site suggested a common enzymatic activity.

Supplementary Figure 5. Characterization of Lmo0178 operator- and inducer-binding.

(A) Two overlapping quasi-palindromic sequences identified using the RegPredict software.1 Positions relative to the reported lmo0178-lmo0184 operon transcription start site (59 base pairs upstream of lmo0178 start codon) are shown.2 The 4 base pair offset between operators is consistent with a previously characterized ROK-DNA interaction.3 (B) Results from a representative EMSA conducted with oligonucleotides that span different portions of the putative operator. The range of each oligonucleotide is shown in the first panel. In lane 1, a single larger shift is observed for the two palindrome-encompassing 29 base pair oligonucleotide. Lanes 6 and 7 correspond to isolated Site 1 and Site 2 sequences, respectively. The smaller shift in lane 6 implies a lower stoichiometry of the Lmo0178-oligonucleotide complex. This is consistent with the larger band representing a 4:1 complex and truncation of the lower affinity binding site resulting in a 2:1 complex. This interpretation suggests that Lmo0178 binds Site 2 with higher affinity than Site 1. The lack of a 2:1 band with the two palindrome-encompassing 29 base pair oligonucleotide, despite the presence of excess DNA, suggests cooperative Lmo0178 binding to the lower affinity site. (C) ITC results generated by titrating isomaltose into Lmo0178. This representative isotherm was fit by a two site binding model (K1 = 4.0 x 10^5 ± 6.2 x 10^4 M^{-1}, ΔH1 = -9,516 ± 180 cal/mol, ΔS1 = -6.3 cal/mol/deg, K2 = 2.7 x 10^4 ± 3.6 x 10^2 M^{-1}, ΔH2 = -3,899 ± 314 cal/mol, ΔS2 = 7.2 cal/mol/deg).
Supplementary Figure 6. Conformational change in the three Lmo0178 states. (A) The extent of HTH domain disorder differs between the three Lmo0178 states. Regions that are disordered in the unliganded structure are colored red and cyan on the operator-bound Lmo0178 dimer. In the inducer-bound state the entire HTH is disordered. (B) The relative asymmetry within the homodimer differs across Lmo0178 states. For each state, the C-terminal effector subdomains of the two promoters in the dimer are superimposed. Subtle differences are evident in the N-terminal effector subdomain in the unliganded state and extend to the HTH in the operator-bound structure. By contrast, the N-terminal subdomain adopts the same position in the inducer-bound state. (C) The effector binding domain openness and dimerization mode changes across the Lmo0178 states. Operator- and inducer-bound Lmo0178 C-terminal (C1) effector subdomains are superimposed to the unliganded structure (gray). Arrows denote the differences in N-terminal effector subdomain position (N1, green/red arrows) and dimerization mode (C2, purple arrows) relative to the unliganded state. HTH domains have been omitted for clarity.

Supplementary Figure 7. The basis of ROK transcription induction. (A) Operator- and inducer-bound Lmo0178 dimers are shown. To determine the approximate position of disordered HTHs in the inducer-bound state, the N-terminal effector subdomains from the operator-bound complex (gray, only HTH domains shown) were superimposed. In the operator-bound complex HTHs dimerize through the α1-helix. In the inducer-bound state, closure of the effector domains causes the HTHs to move ~25 Å apart, to a position incompatible with dimerization. (B) Superposition of operator- and inducer-bound Lmo0178 complexes. F385 from one protomer (pink) makes van der Waals interactions with the isomaltose (ISM, yellow) bound to the other protomer (brown) in the dimer. In the operator complex, structural reorganization of the dimer interface causes F385 to withdraw from the inducer binding site (arrow). As such, inducer binding is poised to disfavor the operator-bound dimerization mode. (C) Schematic representation of conformational changes associated with ROK transcriptional induction. Arrows indicate conformational changes that occur as the repressor transitions between specified states. Transitions in effector domain openness (black arrows) and dimerization mode (red arrows) likely act synergistically to influence DNA binding.
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Supplementary Figure 8. Structural insight into the evolution of ROK repression.

Previous studies have shown that ROK kinases and repressors share a common dimerization mode. A comparison of the inducer-bound Lmo0178 monomer to an AMP-PNP and glucose-bound ROK glucokinase (PDB code 3VGL) demonstrates that they also share a similar sugar-binding mode. One glucose in the isomaltose disaccharide adopts the same relative position as the kinase-bound glucose. The other glucose in isomaltose occupies the same space as the disordered gamma-phosphate of AMP-PNP. Thus, the kinase’s sugar-binding site is conserved in the repressor, with distinctions in the would-be nucleotide-binding site accommodating the larger disaccharide. Furthermore, the effector domain closure caused by inducer binding to Lmo0178 (Supplementary Figure 6C) resembles that caused by substrate binding to kinase. As the organization of the kinase and repressor dimers is nearly identical and the kinase substrate and repressor inducer bind in the same site and result in similar domain closures, the evolution of ROK repressors appears to have resulted by fusing an HTH domain to a functioning ROK kinase and then leveraging the preexisting dimerization mode and substrate-induced conformational changes to yield an allosterically regulated DNA-binding protein.
Supplementary Figure 8. Structural insight into the evolution of ROK repression. Previous studies have shown that ROK kinases and repressors share a common dimerization mode. A comparison of the inducer-bound Lmo0178 monomer to an AMP-PNP and glucose-bound ROK glucokinase (PDB code 3VGL) demonstrates that they also share a similar sugar-binding mode. One glucose in the isomaltose disaccharide adopts the same relative position as the kinase-bound glucose. The other glucose in isomaltose occupies the same space as the disordered gamma-phosphate of AMP-PNP. Thus, the kinase’s sugar-binding site is conserved in the repressor, with distinctions in the would-be nucleotide-binding site accommodating the larger disaccharide. Furthermore, the effector domain closure caused by inducer binding to Lmo0178 (Supplementary Figure 6C) resembles that caused by substrate binding to kinase. As the organization of the kinase and repressor dimers is nearly identical and the kinase substrate and repressor inducer bind in the same site and result in similar domain closures, the evolution of ROK repressors appears to have resulted by fusing an HTH domain to a functioning ROK kinase and then leveraging the preexisting dimerization mode and substrate-induced conformational changes to yield an allosterically regulated DNA-binding protein.

Supplementary Figure 9. Phenotypes of CA pathway mutants. (A) Mice were infected orally by gastric gavage with the indicated Lm strain. Bacterial burdens in livers and spleens at 48 hours post-infection are shown. Each symbol in the box plot represents one animal and a dotted line indicates the limit of detection. Asterisks indicate statistical significance of $P \leq 0.05$ by two-tailed Wilcoxon Rank-Sum Test compared to wildtype. Error bars represent ±1 SEM. (B) Intracellular growth of indicated Lm strains in Caco-2 cells. Monolayers grown on glass coverslips were infected with bacteria at an MOI of 100:1. Gentamicin was added one hour post-infection and coverslips were removed at the indicated time points. Caco-2 cells were lysed and the amount of intracellular bacteria was determined. Representative averages from measurements performed in triplicate are graphed with ±1 SEM error bars. (C) Mice were infected intravenously by tail vein injection with the indicated Lm strain. Bacterial burdens in livers and spleens at 72 hours post-infection are shown. Error bars represent ±1 SEM. (D) Growth of indicated Lm strains in ½ Luria broth (LB) or ½ LB supplemented with 1% maltodextrin (MD). Error bars represent ±1 SEM. In ½ LB the CA pathway mutants were indistinguishable from wildtype and are thus omitted for clarity. Note that the addition of maltodextrin increases the stationary phase cell density. Consistent with a second pathway rendering the CA pathway non-essential for maltodextrin utilization, CA pathway mutants grow more slowly but ultimately reach the wildtype stationary phase cell density.
Supplementary Figure 10. Gels.
## Supplementary Table 1. General Data Collection and Refinement Statistics\(^a\)

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\(^a\)Highest resolution shell in parenthesis.

\(^b\)Definition of R\(_{work}\), R\(_{free}\): \(R = \sum_{hkl} |F_{obs}| - |F_{calc}| / \sum_{hkl} |F_{obs}|\), where \(hkl\) are the reflection indices used in refinement for R\(_{work}\), and 5% not used in refinement for R\(_{free}\). F\(_{obs}\) and F\(_{calc}\) are structure factors deduced from measured intensities or calculated from the model, respectively.
### Supplementary Table 2. Lmo0178 Data Collection and Refinement Statistics

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*a* Highest resolution shell in parenthesis.

*b* Definition of $R_{work}$, $R_{free}$: $R = \sum_{hkl} |F_{obse} - |F_{calc}| / \sum_{hkl} |F_{obs}|$, where $hkl$ are the reflection indices used in refinement for $R_{work}$, and 5% not used in refinement for $R_{free}$. $F_{obs}$ and $F_{calc}$ are structure factors deduced from measured intensities or calculated from the model, respectively.
**Supplementary Table 3.** Summary of Δlmo0182/Δlmo2446 phenotypes in ½ LB media supplemented with α-glucan

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<td>&gt;100</td>
<td>-</td>
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<tr>
<td>Glycogen</td>
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<tr>
<td>Dextrin</td>
<td>5</td>
<td>20</td>
<td>-</td>
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<tr>
<td>Maltodextrin</td>
<td>4-7</td>
<td>14-25</td>
<td>+</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>13-17</td>
<td>6-8</td>
<td>+</td>
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<tr>
<td>Maltodextrin</td>
<td>16.5-19.5</td>
<td>5-6</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>50</td>
<td>2</td>
<td>+</td>
</tr>
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</table>

“+” signifies phenotype
Supplementary References


