

10. E. S. Goodrich, *Studies on the Structure and Development of Vertebrates* (Macmillan, London, 1930).
11. J.-L. Li, Y. Wang, Y.-q. Wang, C.-k. Li, *Chin. Sci. Bull.* **45**, 2545 (2000).
12. X.-L. Wang et al., *Vert. Palasiat.* **36**, 81 (1998).
13. F. A. Jr. Jenkins, C. R. Schaff, *J. Vertebr. Paleontol.* **8**, 1 (1988).
14. Z. Kielan-Jaworowska, D. Dashzeveg, *Acta Palaeontol. Pol.* **43**, 413 (1998); H.-J. Kuhn, *Abh. Senckenb. Naturforsch. Ges.* **28**, 1 (1971).
15. Q. Ji, Z.-x. Luo, S.-a. Ji, *Nature* **398**, 326 (1999).
16. G. W. Rougier, J. R. Wible, M. J. Novacek, *Am. Mus. Novit.* **3187**, 1 (1966).
17. Y.-m. Hu, Y.-q. Wang, Z.-x. Luo, C.-k. Li, *Nature* **390**, 137 (1997).
18. Z.-x. Luo, A. W. Crompton, A.-L. Sun, *Science* **292**, 1535 (2001).
19. K. A. Kermack, F. Mussett, H. W. Rigney, *Zool. J. Linn. Soc.* **53**, 87 (1973).
20. H.-J. Kuhn, *Abh. Senckenb. Naturforsch. Ges.* **28**, 1 (1971).
21. U. Zeller, in *Morphogenesis of the Mammalian Skull*, H.-J. Kuhn, U. Zeller, Eds. (Verlag Paul Parey, Hamburg and Berlin, 1987), pp. 17–50.
22. C. T. Clark, K. K. Smith, *J. Morphol.* **215**, 119 (1993).
23. H.-J. Kuhn, in (21), pp. 1–12.
24. M. R. Sánchez-Villagra, K. K. Smith, *J. Mamm. Evol.* **4**, 119 (1997).
25. P. P. Gambaryan, Z. Kielan-Jaworowska, *Acta Palaeontol. Pol.* **40**, 45 (1995).
26. R. Owen, *Monograph of the Fossil Mammalia of the Mesozoic Formations* (Paleontographical Society, London, 1871).
27. G. G. Simpson, *A Catalogue of the Mesozoic Mammalia in the Geological Department of the British Museum* (Clowes & Sons, London and Beccles, 1928).
28. A. W. Crompton, Z.-x. Luo, in (8), pp. 30–44.
29. A. W. Crompton, A.-L. Sun, *Zool. J. Linn. Soc.* **85**, 99 (1985).
30. K. A. Kermack, F. Mussett, H. W. Rigney, *Zool. J. Linn. Soc.* **71**, 1 (1981).
31. Z.-x. Luo, A. W. Crompton, *J. Vertebr. Paleontol.* **14**, 341 (1994).
32. A. W. Crompton, *Proc. Zool. Soc. London* **140**, 697 (1963).
33. H. R. Barghusen, J. A. Hopson, *Science N.Y.* **168**, 573 (1970).
34. Supplementary material is available at www.sciencemag.org/cgi/content/full/294/5541/357/DC1.
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Genetic Basis for Activity Differences Between Vancomycin and Glycolipid Derivatives of Vancomycin

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Small molecules that affect specific protein functions can be valuable tools for dissecting complex cellular processes. Peptidoglycan synthesis and degradation is a process in bacteria that involves multiple enzymes under strict temporal and spatial regulation. We used a set of small molecules that inhibit the transglycosylation step of peptidoglycan synthesis to discover genes that help to regulate this process. We identified a gene responsible for the susceptibility of *Escherichia coli* cells to killing by glycolipid derivatives of vancomycin, thus establishing a genetic basis for activity differences between these compounds and vancomycin.

Vancomycin (Fig. 1A) is the drug of last resort for treating resistant Gram-positive bacterial infections, and the emergence of vancomycin resistance presents a serious threat to public health. Vancomycin inhibits the maturation of the peptidoglycan layer surrounding bacterial cells by binding to D-Ala-D-Ala, a dipeptide found in peptidoglycan precursors (Fig. 1B) (1). Resistance to vancomycin arises when microorganisms acquire genes that lead to the substitution of D-Ala-D-Ala by D-Ala-D-Lac (2), which vancomycin does not bind. Remarkably, vancomycin derivatives with a hydrophobic substituent on the carbohydrate moiety are active against

vancomycin-resistant strains (3) even though they contain the same peptide binding pocket as vancomycin. The mechanism of action of these derivatives may be fundamentally different from that of vancomycin (4). Unlike vancomycin, they retain activity against both vancomycin-sensitive and vancomycin-resistant strains even when the peptide binding pocket is damaged (5). In vitro, they block a different step of peptidoglycan synthesis than does vancomycin (5). In addition, they kill bacteria very rapidly, whereas vancomycin only stops growth (6).

Because vancomycin and its derivatives affect cells differently (i.e., produce different phenotypes), it might be possible, using a chemical genetics approach, to identify genes involved in the cellular response to these compounds. The synthesis of peptidoglycan from its disaccharide precursor involves numerous enzymes with overlapping functions that are subject to tight temporal and spatial

regulation (7). Most of the major enzymes in peptidoglycan synthesis—the transglycosylases and transpeptidases—have been identified, but how these enzymes are regulated remains poorly understood. In developing an experimental approach to probe the cellular response to glycolipid derivatives of vancomycin, we focused on the following facts: Vancomycin blocks the transpeptidation step of peptidoglycan synthesis and kills cells slowly; glycolipid derivatives of vancomycin block the transglycosylation step of peptidoglycan synthesis (Fig. 1B) and provoke a rapid lethal response in cells (Fig. 1C). Moenomycin, another transglycosylase inhibitor, also induces a rapid lethal response in cells (8). Thus, inhibiting the transglycosylation step of peptidoglycan synthesis may activate a pathway that triggers rapid cell death. If so, it should be possible to identify components of this pathway by selecting for mutants that are resistant to small molecules that inhibit transglycosylation.

We initiated a search for mutants resistant to three different transglycosylase inhibitors: chlorobiphenyl vancomycin, desleucyl chlorobiphenyl vancomycin, and moenomycin (Fig. 1A). Moenomycin binds directly to key bacterial transglycosylases (9). Chlorobiphenyl vancomycin inhibits transglycosylation by binding to the D-Ala-D-Ala terminus of the peptidoglycan precursor lipid II and also by binding to components of the transglycosylation complex (5). Desleucyl chlorobiphenyl vancomycin cannot bind D-Ala-D-Ala and is proposed to inhibit transglycosylation primarily by the latter mechanism (5).

Mutants resistant to transglycosylase inhibitors were obtained by growing *E. coli imp* (10) on plates impregnated with chlorobiphenyl vancomycin, desleucyl chlorobiphenyl vancomycin, or moenomycin. Three mutants were isolated that were resistant to each of these antibiotics (BE101, BE102, and BE103; Table 1). The muta-

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tions do not cause a significant change in growth rates (11). Nevertheless, each mutant was resistant not only to the transglycosylase inhibitor it was raised on, but also to both of the other two antibiotics. For example, relative to the wild-type strain, moenomycin was less active in all three of

the resistant strains by a factor of 27 to 100, whereas chlorobiphenyl vancomycin was less active by a factor of about 10. The mutants were also resistant to the glycopeptide antibiotic teicoplanin (Table 1), another inhibitor of the transglycosylation step of peptidoglycan synthesis (12). However, the mutants did

not show any resistance to vancomycin.

The mutants remained as sensitive as the parent strain BE100 to antibiotics that inhibit peptidoglycan synthesis but do not block transglycosylation, such as the transpeptidase inhibitors ampicillin, penicillin, or cefoxitin (β -lactams) (Table 1); they also remained

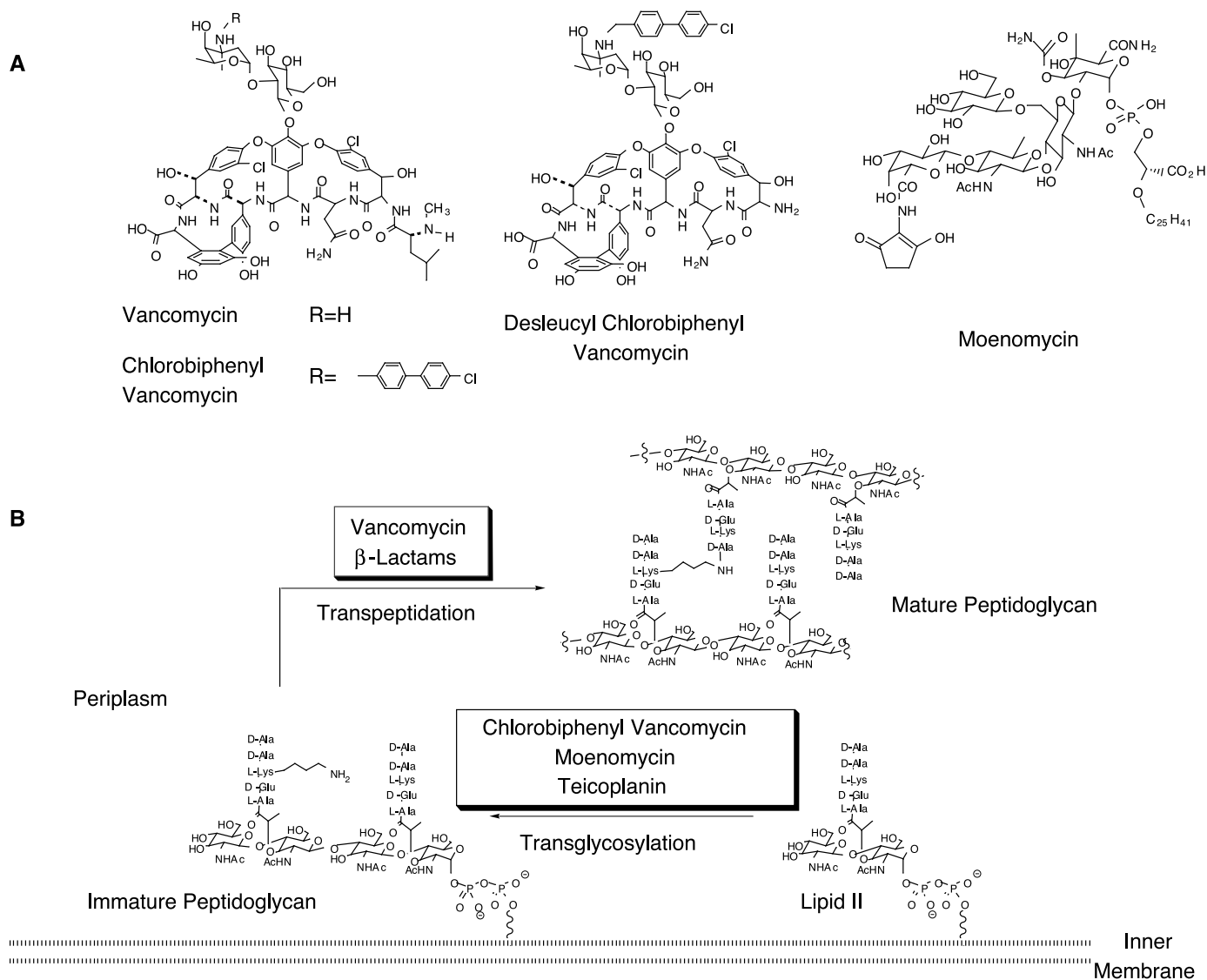
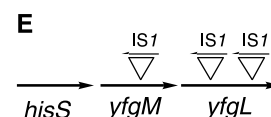
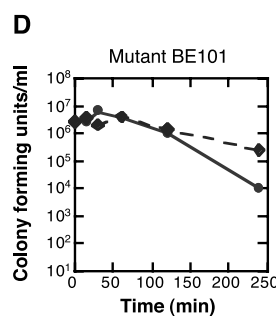
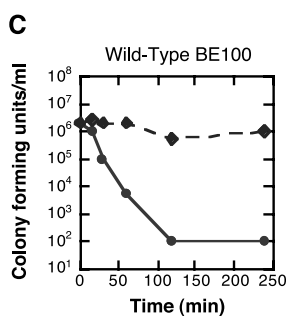


Fig. 1. (A) Structures of antibiotics. **(B)** Final stage of peptidoglycan biosynthesis. Transglycosylase enzymes polymerize lipid II into long polysaccharide chains (immature peptidoglycan), which are then cross-linked by transpeptidases. Chlorobiphenyl vancomycin, moenomycin, and teicoplanin inhibit the transglycosylation step; vancomycin and β -lactams inhibit the transpeptidation step. **(C and D)** Time-kill curves of wild-type BE100 (C) and mutant strain BE101 (D) in the presence of antibiotics at their MICs. A standard microdilution assay was performed in LB. Samples were removed and serial dilutions were plated onto antibiotic-free agar after 15, 30, 60, 120, and 240 min of exposure to each antibiotic. Drug concentrations were 0.8 μ g/ml and 1.6 μ g/ml for vancomycin (\blacklozenge) and 0.3 μ g/ml and 3.2 μ g/ml for chlorobiphenyl vancomycin (\bullet) for BE100 and BE101, respectively. **(E)** Genetic map of the *hisS* operon. The mutant strains BE101, BE102, and BE103 were obtained from parent strain BE100 by plating onto LB agar containing 5 times the MIC for a given antibiotic. Individual colonies were purified. Genetic mapping and DNA sequence analysis revealed insertion mutations in *yfgM* and *yfgL*. The insertions are IS1E elements located at base 342 in *yfgM* (BE101) and at bases 171 (BE102) and 1024 (BE103) in *yfgL* (where +1 refers to the A of their predicted ATG start codons). Arrows indicate direction of transcription.



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sensitive to compounds that inhibit cytoplasmic steps of peptidoglycan synthesis, such as fosfomycin and cycloserine. The mutants were also sensitive to antibiotics that target other essential cellular functions, including erythromycin (a macrolide) and kanamycin or gentamicin (aminoglycosides). The mutants showed increased minimum inhibitory concentrations (MICs) for molecules that block the transglycosylation step of peptidoglycan synthesis, but not for antibiotics with other modes of action; this finding rules out nonspecific mechanisms of resistance, such as altered membrane permeability. A plausible interpretation of the results is that inhibition of transglycosylation in sensitive cells activates a common pathway that leads to rapid cell death. This pathway may be blocked in the mutants. If so, one would predict that the mutants should resist rapid cell death upon treatment with transglycosylase inhibitors, even at the increased MICs.

To test this prediction, we measured the survival rates of resistant and sensitive bacteria when exposed to vancomycin and the three transglycosylase inhibitors. Vancomycin at its MIC prevented growth of all the strains but did not kill them (Fig. 1, C and D). Chlorobiphenyl vancomycin, in contrast, caused the number of colony-forming units (CFUs) of the wild-type strain to decrease by four orders of magnitude in 2 hours (Fig. 1C). However, it did not significantly reduce the CFUs of the mutant strain in 2 hours even at a concentration 10 times that used against the wild-type strain (Fig. 1D). The other transglycosylase inhibitors also showed a significant decrease in their ability to kill mutant strains. The bacteriostatic activity of the transglycosylase inhibitors against the mutant strains is consistent with the inactivation of one or more genes that would otherwise activate a bactericidal response. A prediction that follows from this is that the mutation that causes resistance should be a recessive null mutation.

Our next goal was to identify the mutat-

ed gene(s) that lead to resistance. Using standard genetic methods, we mapped the mutations in all three strains to minute 56.8 in the *E. coli* genome (13). Sequencing of this region showed that IS/E elements (14) had inserted into either *yfgL* or *yfgM* in the *hisS* operon in all three mutants (Fig. 1E). To verify that the mutations in *yfgL* or *yfgM* were responsible for the resistant phenotype, we carried out complementation experiments. The chromosomal region encoding the wild-type *hisS*, *yfgM*, and *yfgL* genes (with their promoter region) was inserted into a plasmid and introduced into both the wild-type and mutant strains (15). The mutant strains carrying the wild-type genes in trans regained sensitivity to transglycosylase inhibitors (as judged by MIC) and showed a bactericidal response similar to that of the wild-type strain. The wild-type strains carrying either the mutant or the wild-type genes in trans retained the wild-type phenotype. Thus, the loss of a functioning copy of *yfgM* or *yfgL* (or both) in the mutant strains leads to the resistant phenotype.

These results clearly implicate the *yfgL* gene product in the bactericidal response to compounds that inhibit transglycosylation. However, because an IS insertion in *yfgM* prevents expression of *yfgL* by polarity, the role of *yfgM* remained ambiguous. To determine whether *yfgM* plays any role in cell death upon transglycosylase inhibition, we cloned *yfgL* and *yfgM* separately into an inducible vector and introduced it into the mutant strains. The mutants became sensitive to transglycosylase inhibitors only if *yfgL* production was induced. In the absence of inducer, or when *yfgM* alone was expressed, the mutant strains remained resistant. Thus, the bactericidal effects of transglycosylase inhibition require a functional *yfgL* gene.

Sequence analysis of the gene products suggests that YfgL is a lipoprotein located on the inner surface of the outer membrane of the bacterial cell (16). We do not yet know

the mechanism by which YfgL causes cell death in the presence of transglycosylase inhibitors, but one possibility is that it affects the regulation of lytic transglycosylases (autolysins) (17). These enzymes break peptidoglycan bonds to permit the incorporation of new peptidoglycan during growth and cell division (7). Some lytic transglycosylases are located on the inner surface of the outer membrane and could cause rapid cell death if they are inappropriately activated (18). We have found that bacterial strains that do not produce the wild-type *yfgL* gene product make considerably more peptidoglycan than do the parent strains (19), consistent with the hypothesis that the normal function of YfgL is to up-regulate lytic transglycosylases.

Homologs of *yfgL* can be identified in many Gram-negative organisms, but not in Gram-positive bacterial species. This may not be surprising because the latter do not have an outer membrane. Nevertheless, enterococcal strains, which are Gram-positive, behave similarly to permeable *E. coli* in that they die very rapidly upon exposure to glycolipid derivatives of vancomycin (6). The bactericidal behavior suggests that an analogous signaling pathway exists in enterococcal strains, even though homologous proteins have not been identified. Thus, transglycosylases are particularly good targets for the design of new antibiotics because they are extracytoplasmic and because inhibiting them provokes a bactericidal response.

The discovery that *yfgL* confers resistance to glycolipid derivatives of vancomycin, but not to vancomycin itself, establishes a genetic basis for the activity differences between these compounds. We could not have discovered *yfgL* easily using traditional genetics alone because cells can survive without it. The *yfgL* gene gives rise to a discernable phenotype only in the presence of small molecules that perturb the transglycosylation step of peptidoglycan synthesis. We presume that YfgL is involved in regulating peptidoglycan synthesis at some level. Small molecules have been used previously to identify regulatory networks (20). The classic example involves the use of cyclosporin to elucidate the cyclophilin/calcineurin network in mammalian cells (21, 22). Microbial regulatory networks are simpler to study using a chemical genetics approach because it is easier to select for mutants and straightforward to analyze them genetically. Small-molecule probes of various kinds should be useful for identifying other "nonessential" genes that regulate essential enzymes in bacteria. In the meantime, understanding how *yfgL* and its putative counterparts in other organisms trigger cell death upon exposure to transglycosylase inhibitors may lead to a better understanding of the regulatory networks involved in bacterial cell growth and division.

Table 1. Minimum inhibitory concentrations for wild-type and mutant strains. MICs were determined against strains grown in LB medium in a standard microdilution format. The MIC is defined as the lowest antibiotic concentration that resulted in no visible growth after incubation at 35°C for 22 hours. For desleucyl chlorobiphenyl vancomycin, higher numbers could not be obtained because of low solubility. Mutant BE101 was raised on chlorobiphenyl vancomycin, BE102 on desleucyl chlorobiphenyl vancomycin, and BE103 on moenomycin.

Antibiotic	MIC ($\mu\text{g/ml}$)			
	Wild type (BE100)	Mutant BE101	Mutant BE102	Mutant BE103
Vancomycin	0.8	1.6	1.6	1.6
Chlorobiphenyl vancomycin	0.2	3.2	1.6	3.2
Desleucyl chlorobiphenyl vancomycin	25	>125	>125	>125
Moenomycin	0.03	1.6	0.8	3.2
Teicoplanin	0.25	16	3	4
Penicillin G	3.2	3.2	1.6	3.2

References and Notes

1. J. C. J. Barna, D. H. Williams, *Annu. Rev. Microbiol.* **38**, 339 (1984).
2. T. D. Bugg, S. Dutka-Malen, M. Arthur, P. Courvalin, C. T. Walsh, *Biochemistry* **30**, 2017 (1991).
3. R. Nagarajan et al., *J. Antibiot.* **42**, 63 (1989).
4. Other explanations have been proposed (23).
5. M. Ge et al., *Science* **284**, 507 (1999).
6. S. A. Zelenitsky, J. A. Karlowky, G. G. Zhanel, D. J. Hoban, T. Nicas, *Antimicrob. Agents Chemother.* **41**, 1407 (1997).
7. J.-V. Höltje, *Microbiol. Mol. Biol. Rev.* **62**, 181 (1998).
8. Y. van Heijenoort, M. Leduc, H. Singer, J. van Heijenoort, *J. Gen. Microbiol.* **133**, 667 (1987).
9. W. Vollmer, M. von Rechenberg, J.-V. Höltje, *J. Biol. Chem.* **274**, 6726 (1999).
10. Vancomycin and its analogs do not penetrate the outer membrane of most *E. coli* strains. Nevertheless, *E. coli* is the best understood bacterial species, and *E. coli* membrane preparations have been used for many of the mechanistic studies on vancomycin and its derivatives. Thus, as the test organism for selecting mutants, we chose an *E. coli imp* strain. The *imp* mutation alters the permeability of the outer membrane and confers sensitivity to vancomycin and its carbohydrate derivatives as well as moenomycin. BE100 is an isolate of BAS849 (MC4100 Δ *lambB106 imp-4213*) (24).
11. Some antibiotics, for example the β -lactams, show a decrease in their bactericidal activity toward slow-growing cells (25). In our system, the rate of killing is independent of growth rate.
12. R. Kerns et al., *J. Am. Chem. Soc.* **122**, 12608 (2000).
13. Strains containing *Tn10* insertions in nearby genes *purC* and *yfhS* were obtained from the collection of Singer et al. (26). Strains containing kanamycin resistance cassettes in *pbpC* and *yfgJ* were constructed using the method recently described by Yu et al. (27). Linkage between these markers and the mutation conferring resistance to the transglycosylase inhibitors was determined by generalized transduction using P1vir (28).
14. M. Umeda, E. Ohtsubo, *Gene* **98**, 1 (1991).
15. We used the polymerase chain reaction (PCR) to amplify *hisS*, *yfgM*, *yfgL*, and the region 100 base pairs upstream of *hisS* from the chromosomes of BE100 and BE103 with the primers yfgM-N1 (5'-AAAGAATCCCGTGTATGATGAACCCG-3') and yfgL-1 (5'-TACACCGTCTCTGTGCCA-3'). The PCR products were purified, digested with EcoR I and Kpn I, and cloned into the multicloning site of pUC19. To reduce copy number, we cloned an EcoR I to Hind III fragment from this pUC19 derivative into the EcoR I to Hind III sites of pBR322. *yfgL* alone was PCR-amplified from the chromosome of BE100 with the primers LEco-N (5'-AAAGAATTCGAGAGGGACCCGATGCAA-3') and LHin-C (5'-AAAAGCTTGATTAACGTGTAATAGAGTACA-3'), digested with Hind III and EcoR I, and cloned into the multicloning site of pBAD18; *recA::kan* was moved into BE100 and the mutants by transduction. Plasmids containing either the intact or mutated *hisS* operon or *yfgL* alone were transformed (28) into the recombination-deficient strains, and transformants were selected on LB plates with ampicillin (5 μ g/ml). The strains containing the pBAD vector were induced with 0.1% arabinose. The transformants were grown in liquid LB with ampicillin (5 μ g/ml) and their resistance phenotypes were determined by MIC.
16. K. Yamaguchi, F. Yu, M. Inouye, *Cell* **53**, 423 (1988).
17. It is thought that the bactericidal action of antibiotics in *Streptococcus pneumoniae* is related to the autolysin LytA (29). Recently, it was suggested that a two-component signaling system controls death in this organism (30). Other lysis pathways are also possible (31).
18. We do not think that YfgL is a lytic transglycosylase itself because it has no homology to any known lytic transglycosylase (32).
19. Incorporation of 14 C-labeled uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) into the peptidoglycan in membrane preparations of BE100 and the mutants was measured as described (33). The mutant strains were able to incorporate about 18%

- of the radiolabeled UDP-GlcNAc, whereas the wild-type strain incorporated only 5%. This could be due to increased activity of the transglycosylase enzymes or failure of the lytic transglycosylases to degrade peptidoglycan.
20. P. J. Alaimo, M. A. Shogren-Knaak, K. M. Shokat, *Curr. Opin. Chem. Biol.* **5**, 360 (2001).
21. J. Liu et al., *Cell* **66**, 807 (1991).
22. S. L. Schreiber, *Science* **251**, 283 (1991).
23. D. H. Williams, B. Bardsley, *Angew. Chem. Int. Ed.* **38**, 1173 (1999).
24. B. A. Sampson, R. Misra, S. A. Benson, *Genetics* **122**, 491 (1989).
25. E. Tuomanen, R. Cosenz, W. Tosch, O. Zak, A. Tomasz, *J. Gen. Microbiol.* **132**, 1297 (1986).
26. M. Singer et al., *Microbiol. Rev.* **53**, 1 (1989).
27. D. Yu et al., *Proc. Natl. Acad. Sci. U.S.A.* **97**, 5978 (2000).
28. T. J. Silhavy, M. L. Berman, L. W. Enquist, *Experiments*

- with *Gene Fusions* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1984).
29. A. Tomasz, A. Albino, E. Zanati, *Nature* **227**, 138 (1970).
30. R. Novak, B. Henriques, E. Charpetier, S. Normark, E. Tuomanen, *Nature* **399**, 590 (1999).
31. T. G. Bernhardt, I.-N. Wang, D. K. Struck, R. Young, *Science* **292**, 2326 (2001).
32. J. Lommatzsch, M. F. Templin, A. R. Kraft, W. Vollmer, J.-V. Höltje, *J. Bacteriol.* **179**, 5465 (1997).
33. A. A. Branstrom, S. Midha, R. C. Goldman, *FEMS Microbiol. Lett.* **191**, 187 (2000).
34. Supported by grants from Advanced Medicine Inc. and NIH (D.K. and T.J.S.).

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 10.1126/science.1063611
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Regional Magnetic Fields as Navigational Markers for Sea Turtles

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Young loggerhead sea turtles (*Caretta caretta*) from eastern Florida undertake a transoceanic migration in which they gradually circle the north Atlantic Ocean before returning to the North American coast. Here we report that hatchling loggerheads, when exposed to magnetic fields replicating those found in three widely separated oceanic regions, responded by swimming in directions that would, in each case, help keep turtles within the currents of the North Atlantic gyre and facilitate movement along the migratory pathway. These results imply that young loggerheads have a guidance system in which regional magnetic fields function as navigational markers and elicit changes in swimming direction at crucial geographic boundaries.

Hatchling loggerhead sea turtles (*Caretta caretta*) from eastern Florida begin a long-distance migration immediately after entering the sea (1). Turtles swim from the Florida coast to the North Atlantic gyre, the circular current system surrounding the Sargasso Sea, and remain within the gyre for a period of years (2–4). During this time, they gradually migrate around the Atlantic before returning to the North American coast (5, 6).

For young loggerheads, conditions within the North Atlantic gyre are favorable for survival and growth, but straying beyond the latitudinal extremes of the gyre is often fatal (2, 3). As the northern edge of the gyre approaches Portugal, the east-flowing current divides. The northern branch continues past Great Britain and the water temperature decreases rapidly. Loggerheads swept north in this current soon die from the cold (2–4). Similarly, turtles that venture south of the gyre risk being swept into the South Atlantic

current system and carried far from their normal range. An ability to recognize the latitudinal extremes of the gyre, and to respond by orienting in an appropriate direction, might therefore have adaptive value.

Previous experiments have shown that hatchling loggerheads can detect magnetic inclination angle (7) and field intensity (8), two geomagnetic features that vary across Earth's surface and could, in principle, provide positional information to a migrating turtle (9, 10). In these initial experiments, one of the two parameters was held constant while the other was varied. This approach was necessary to demonstrate that turtles can detect each field element. In nature, however, these field elements vary together across Earth's surface. Most pairings of inclination and intensity used in previous studies resulted in fields with combinations of parameters that do not naturally occur in the North Atlantic (7).

To determine whether hatchlings can distinguish among the magnetic fields actually found in different oceanic regions, we subjected hatchling loggerheads to fields replicating those found in three widely separated

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