A Protein Antibiotic in the Phage Qβ Virion: Diversity in Lysis Targets

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A_2, a capsid protein of RNA phage Qβ, is also responsible for host lysis. A_2 blocked synthesis of murein precursors in vivo by inhibiting MurA, the catalyst of the committed step of murein biosynthesis. An A_2-resistance mutation mapped to an exposed surface near the substrate-binding cleft of MurA. Moreover, purified Qβ virions inhibited wild-type MurA, but not the mutant MurA, in vitro. Thus, the two small phages characterized for their lysis strategy, Qβ and the small DNA phage ϕX174, effect host lysis by targeting different enzymes in the multistep, universally conserved pathway of cell wall biosynthesis.

Double-stranded DNA phages encode at least two and as many as five proteins, including a muralytic enzyme, to effect a precisely scheduled host lysis. At a genetically programmed time, one protein, the holin, acts to permeabilize the membrane, allowing the muralytic enzyme to degrade the cell wall. Other proteins serve as negative regulators of the holin or as agents to destabilize the outer membrane (*1*). In contrast, the *Microviridae*, *Leviviridae*, and *Alloleviridae*, which are lytic phages with small, single-stranded nucleic acid genomes, have only a single gene required for lysis and do not produce a muralytic activity (*2*). The *Microvirus* [single-stranded DNA (ssDNA)] ϕX174 has only 10 genes and produces a single lysis protein, E, which is encoded by a 91-codon reading frame embedded in the +1 register within the essential morphogene *D*. Similarly, the *Levivirus* (ssRNA) MS2 has only four genes; the lysis gene *L*, overlapping the coat and replicase genes in the +1 register, encodes a 75–amino acid membrane protein. In contrast, there is no separate lysis gene in the *Allolevivirus* (ssRNA) Qβ. Instead, synthesis of the maturation or A_2 protein, a single-copy virion protein responsible for absorption to the sex pilus and protection of the virion RNA against external ribonuclease, is also necessary and sufficient for lysis (Fig. 1A). Because of the absence of a muralytic activity, the mode of action of these single-gene lysis systems has been mysterious and controversial (*2*, *3*). Recently, we demonstrated that E is lytic for the same reason that the fungal cell wall antibiotic, mureidomycin, is lytic; E causes lysis by acting as a specific inhibitor of MraY, a membrane-embedded enzyme, conserved throughout eubacteria, that catalyzes the synthesis of the first lipid-linked intermediate in peptidoglycan synthesis (*4*, *5*) (Fig. 1B). This finding led us to wonder whether other simple phage lysis systems also encode proteins that function as cell wall antibiotics and, if so, at which step they act. To address these questions, we investigated the lytic mechanism of A_2 using a combined genetic and biochemical approach.

In E-mediated lysis, incorporation of the specific peptidoglycan precursor [^{3}H]diaminopimelate (DAP) into murein is blocked long before lysis. Labeling cells expressing a cloned A_2 gene gives similar results, with incorporation stopping at least 20 min before lysis is detectable (Fig. 2). However, unlike E, A_2 also causes a subsequent degradation of newly synthesized peptidoglycan. Also unlike E, which by blocking MraY causes uridine 5′-diphosphate–*N*-acetylmuramic acid (UDP-MurNAc)–pentapeptide, the last cytoplasmic precursor in the peptidoglycan synthesis pathway, to accumulate (*5*), no DAP-containing precursors accumulate in A_2-inhibited cells (Table 1). Thus, MraY is not the target of A_2, which instead must block either the step where DAP is added to the oligopeptide or one of the five steps preceding it; the enzymes catalyzing these steps are encoded by *murA*, *murB*, *murC*, *murD*, *murE*, and *murI* (Fig. 1B).

To discriminate between these candidates, we took a genetic approach. After induction of a culture of cells carrying a plasmid-borne A_2 gene, spontaneous survivor colonies were isolated at a frequency of 10^{-6}. Two of 90 survivor colonies were found to be Qβ^r, as judged by cross-streaking and plating tests, and were designated *rat1* and *rat2* (resistance to *A*-two) (*6*). *rat1* cells accumulate plaque-forming virions at a rate indistinguishable from that of the parental cells up until the normal time of lysis and continue to accumulate virions beyond that time (Fig. 3A); thus, the defect in *rat* cells is only in phage-mediated lysis and is not due to a gross defect in A_2 expression. To determine whether the spontaneous *rat* mutations mapped to one of the six *mur* genes implicated by the labeling data, we took advantage of the dispersal of these genes in three clusters: *murA* at 71.8 min; *murB* and *murI* at 89.7 min; and *murC*, *murD*, and *murE* in the cluster of murein synthesis and cell-division genes at 2.1 min (*7*). When the Rat mutants were transduced to kanamycin resistance (Kan^r) with P1 lysates prepared from donor strains with Tn*10kan* markers at 1.8, 72.5, and 90.4 min, Qβ-sensitive transductants were obtained only in the case of the 72.5-min insertion, with ~20% linkage, consistent only with *rat1* being allelic to *murA* (*8*). Sequence analysis of the *murA* gene from *rat1* revealed a single missense change, Leu^{138}→Gln; the identical change was found in *rat2*, whereas no change was found in the parental sequence (*9*). Multicopy clones of *murA* delay lysis after infection with Qβ (Fig. 3B). The basal level of expression of *murA*$^+$ results in a considerable delay of lysis, whereas induction of the *murA*$^+$ clone abolishes macroscopic lysis entirely. In contrast, even the basal level of the *murA*^{rat} allele abolishes lysis. Similar results were obtained for inductions of a plasmid clone of A_2 in which the phage gene is under the control of the *tac* promoter; lysis begins about 20 min after induction in cells carrying only the chromosomal copy of *murA*, but is not observed with a multicopy clone of *murA* or if a single copy of *murA*^{rat1} is present (*10*). These data indicate that the A_2 protein effects lysis by titrating MurA, which catalyzes the

Table 1. Cell wall and precursor labeling in vivo.

Strain	cpm*		
	Cell wall	Nucleotide	Lipid
ET505 pJFlacZK	205,400 ± 3,500	58,900 ± 3,400	3,800 ± 200
ET505 pEmycZK (*10*)	2,000 ± 200	64,600 ± 4,600	500 ± 30
ET505 pA2	8,900 ± 290	2,300 ± 140	590 ± 90

*[^{3}H]DAP-labeled cell wall and precursors were separated by paper chromatography and counted as described (*5*). The results are the mean ± SD of three samples.

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committed step of murein biosynthesis. Consistent with this finding, the residue altered in *rat1* is exposed at the surface near the substrate-binding cleft of MurA (*11*) and is thus available for direct contact with the A_2 protein without requiring conformational changes in MurA.

To confirm the genetic analysis, we compared the sugar nucleotide pool from A_2-inhibited cells, prepared by gel-filtration and ion-exchange chromatography (*12*), to that of control cells. Upon ion-exchange chromatography, ~70% of the *N*-acetyl sugar from the A_2-inhibited culture was recovered in a single peak that was barely detectable in identically prepared extracts from a control culture. The elution position of this peak was identical to that of authentic UDP–*N*-acetylglucosamine (UDP-GlcNAc) and eluted at a much lower salt concentration than the more acidic UDP-MurNAc derivatives involved in cell wall biosynthesis. The material in this peak was subjected to mild acid hydrolysis, and the sugar composition of the hydrosylate was analyzed by paper chromatography. A single reducing sugar was found that comigrated with authentic GlcNAc and stained purple with the aminosugar-specific Elson-Morgan reagent, as expected for an *N*-acetylated aminosugar. The hydrolysate was also examined by thin-layer chromatography, which revealed the presence of single ultraviolet-absorbing constituent comigrating with uridine 5′-monophosphate in both neutral and acidic solvent systems (*10*). Based on an extinction coefficient of 10,000 for uracil, the sugar nucleotide that accumulates after A_2 induction had an *N*-acetylsugar/uracil ratio of 0.86.

Taken with the genetic and physiological data, the finding that UDP-GlcNAc accumulates provides conclusive evidence that MurA is inhibited during A_2-mediated lysis in vivo. Estimates based on the absorbance at 262 nm (A_{262}) of the peak fractions indicated that the concentration of this UDP-GlcNAc pool was ~100 μM in the control cells and about 600 μM in the inhibited cells.

MurA activity can be detected in vitro as UDP-GlcNAc–dependent release of inorganic phosphate (P_i) from phosphoenolpyruvate (PEP) (*13*). Efforts to obtain purified A_2 protein were unsuccessful owing to the insolubility of the overproduced protein. Unexpectedly, however, inhibition of MurA could be demonstrated with Qβ virions purified in a CsCl gradient. Addition of purified virions abolished MurA activity in extracts prepared from cells expressing $murA^+$ from a plasmid (Fig. 4) (*14*). In extracts prepared from cells expressing $murA^{rat1}$ from a plasmid and $murA^+$ from the chromosome, less than 20% inhibition was observed, reflecting the presence of A_2-sensitive MurA molecules from the chromosomal locus. We conclude that the resistance of the mutant MurA to A_2 inhibition provides the cell with resistance to A_2-induced lysis.

The mode of inhibition by A_2 remains to be determined. The simplest notion is that

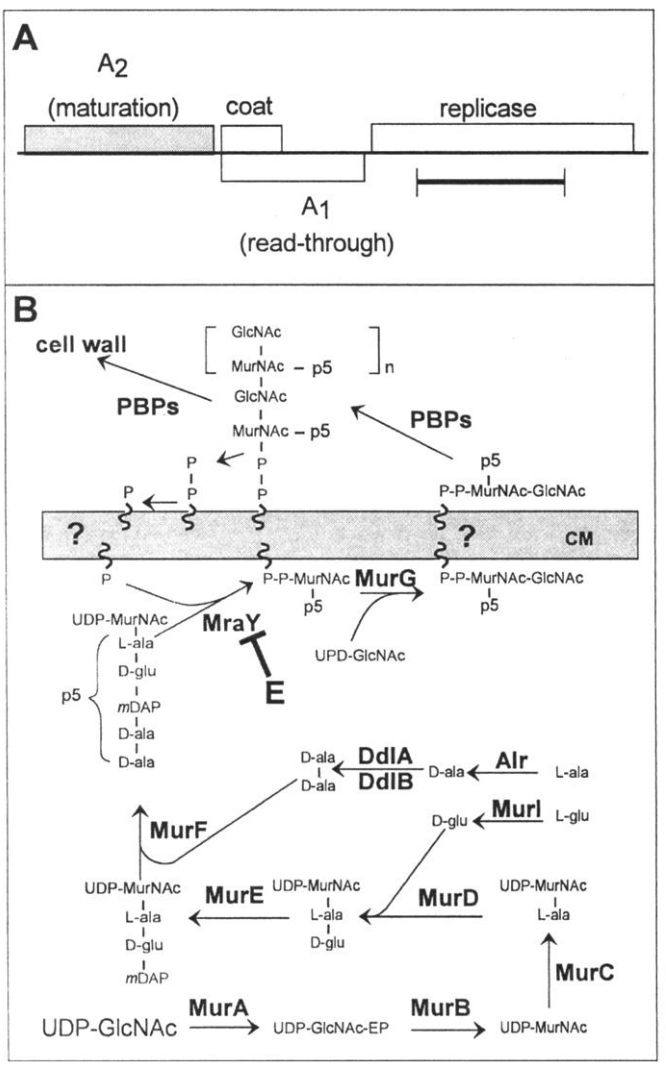

Fig. 1. (**A**) The genome of the ssRNA phage Qβ. The coat protein is the major virion structural protein; A_1 is a product of read-through of the leaky UGA stop codon of the coat protein and is a minor component of the virion. Replicase is the viral-encoded subunit of the RNA-dependent RNA polymerase. A_2, or maturation protein, is present in one copy per virion; it is required for adsorption to the sex pilus of the host and is also responsible for cell lysis (*28*). Bar, 1 kb. (**B**) Pathway for murein biosynthesis [adapted from Nanninga (*29*)]. The committed step, the addition of the pyruvyl moiety to UDP-GlcNAc, is catalyzed by the product of the *murA* gene, UDP-*N*-acetylglucosamine enolpyruvyl transferase. The externalization of the undecaprenol-pyrophospho-MurNAc–GlcNAc disaccharide pentapeptide is catalyzed by an unknown flippase activity, indicated by a question mark. The pathway by which the disaccharide pentapeptide is covalently linked into the murein is catalyzed by the high molecular weight penicillin binding proteins (PBPs), although the molecular details of the pathway are uncertain. The undecaprenol pyrophosphate generated by the PBPs is recycled back to undecaprenol phosphate and then flipped to the cytoplasmic face, again by an unknown flippase activity (*30*). The undecaprenyl moiety is represented by a wavy line embedded in the cytoplasmic membrane (CM). The enzyme inhibited by the φX174 E protein, MraY, is indicated by the blunt arrow.

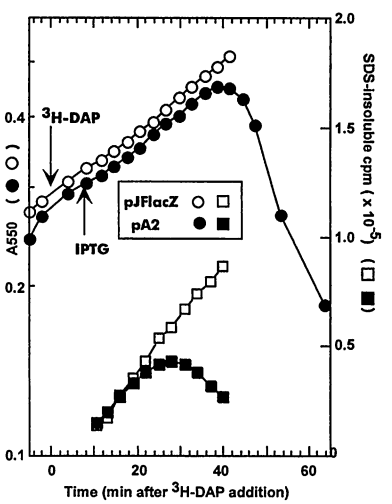

Fig. 2. A_2 expression blocks murein synthesis before lysis. ET505 cells carrying either pA2, with the A_2 gene under *tacPO* control, or the isogenic *lacZ* plasmid, pJFlacZK (*25*), were labeled and induced as described (*5*). Briefly, cells were grown in minimal M9 glucose media in 250-ml culture flasks at 37°C to an A_{550} of 0.3. A portion of each culture was transferred with constant aeration to a prewarmed 50-ml flask containing [^{3}H]DAP at a final activity of 5 μCi/ml. After a 10-min prelabeling period, both labeled and unlabeled cultures were induced with 1 mM IPTG. Culture growth was monitored as A_{550} in the unlabeled culture, and [^{3}H]DAP incorporation into cell wall was monitored in the labeled culture as radioactivity insoluble in boiling 4% SDS, as described (*5*).

the virion-associated A_2 binds to MurA and that the *rat* mutation destabilizes the complex. On the basis of the established turnover number for MurA (*13*), each 50-μl reaction contained about 2.5×10^{11} MurA enzyme molecules. Inhibition was quantitative with the addition of 3×10^{12} virions, but was not observed with 10-fold fewer virions (*15*), which indicates that the dissociation constant of the virion-MurA complex would be in the 10 nM range. This tight binding would result in a titration of MurA activity as virions accumulate. Kozak and Nathans (*16*) showed that the maturation protein of RNA viruses accompanies the RNA into the cell, whereas the rest of the virion is discarded in the medium. Thus, the A_2 protein, like fungal cell-wall antibiotics, can come from outside the cell to inhibit the cytoplasmic synthesis of murein precursors. However, based on the fraction of A_2-sensitive MurA activity observed above (Fig. 4), we estimate that cells with a single copy of *murA* contain about 1500 MurA molecules; this ensures that even at a high multiplicity of infection (MOI), where 30 or more virions can infect a single host (*17*), substantial inhibition of cell wall synthesis by the virion-associated A_2 does not occur until virion production is advanced well beyond the eclipse period.

We have recently isolated Qβ *por* (plates on rat) mutants that overcome the $murA^{rat}$ plating block (*18*). Potentially, these mutants may help distinguish which regions of A_2 are involved in MurA inhibition. Moreover, the ease of isolating these mutants raises the possibility that allele-specific inhibitors can be obtained, if more *rat* alleles are isolated.

Now two single-gene lysis systems have been analyzed in detail; one, φX174 *E*, inhibits MraY, which catalyzes the formation of the first lipid-linked murein precursor, and the second, Qβ A_2, inhibits MurA, which catalyzes the committed step in the murein biosynthesis pathway. We propose that this is a general strategy, and that, in the absence of a muralytic enzyme, the only way for phage to compromise the murein sacculus sufficiently to effect host lysis is to interfere with peptidoglycan synthesis during growth. We predict that an analogous mode of action will be found for the remaining unresolved single-gene lysis system known for male-specific coliphages: the MS2 *L* gene. Preliminary results in this laboratory indicate that the target of *L* is a different gene in the same pathway (*19*). Small-genome phages thus appear to accomplish host lysis by elaborating polypeptides that inhibit murein synthesis at different steps. This raises the attractive possibility that DNA-encoded oligopeptide antibiotics, subject to facile genetic manipulation, might be designed on the basis of these lysis proteins. Moreover, given that all three prototype small-genome phages attack three different steps of the murein synthesis pathway, it also suggests that a search for new classes of small, lytic bacteriophages, not only of *Escherichia coli* but also for other bacteria, is in order.

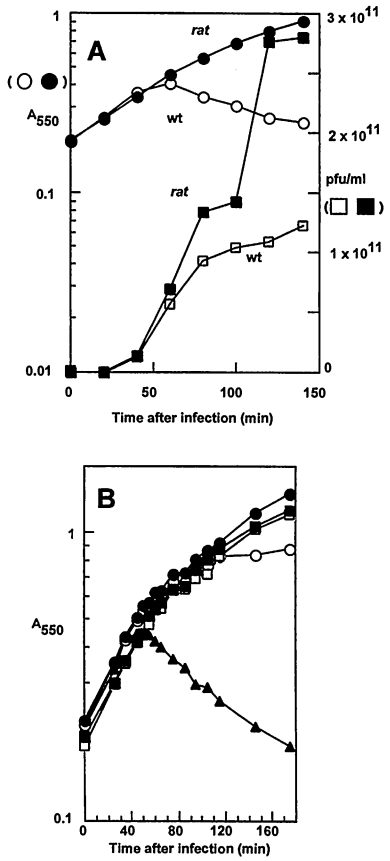

Fig. 3. (**A**) Host lysis, but not virion production, is compromised in the *rat* mutant. $murA^+$ or $murA^{rat1}$ cells were infected with Qβ at time t = 0, and both culture mass and the total intracellular and extracellular production of virions were determined at the indicated times (*31*). Circles: A_{550}; squares: total Qβ titer. Open symbols: parental; solid symbols: *rat1*. (**B**) Multicopy clones of *murA* and $murA^{rat1}$ can block Qβ lysis. Male $murA^+$ cells carrying the plasmid vector pZE12-luc (*24*) or its derivatives pZE12-murA or pZE12-rat1, with $murA^+$ or $murA^{rat1}$ cloned under the control of the hybrid $p_{L\text{-}lac}$ promoter (*9*), were infected at an MOI of 5 at t = 0. IPTG (final concentration of 1 mM) was added when the culture reached an A_{550} of 0.2 to induce the expression of the cloned *murA* locus, where indicated. Triangles: vector. Solid and open circles: induced and uninduced pZE12-murA, respectively. Solid and open squares: induced and uninduced pZE12-rat1, respectively.

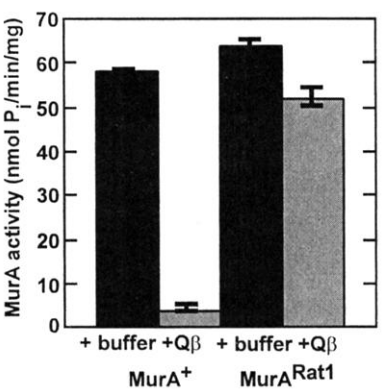

Fig. 4. Purified Qβ virions inhibit MurA, but not $MurA^{Rat1}$. MurA activity was assayed as UDP-GlcNAc–dependent P_i release from PEP in crude, cell-free extracts prepared from cells expressing multicopy $murA^+$ or $murA^{rat1}$, either with buffer or purified Qβ virions added (*14*). The fractional inhibition of the activity in the extracts from the multicopy $murA^{rat1}$ cells presumably reflects the small proportion of wild-type MurA enzyme encoded by the chromosomal *murA* locus in these cells.

References and Notes

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6. A culture of XL1 Blue *recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 lac* [F'::Tn*10 proA*$^+$*B*$^+$ *laq*I^q D(*lacZ*) M15] (Stratagene) carrying plasmid pGL101(A_2^+) (*20*) was grown to an A_{550} of 0.2, and A_2 expression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Lysis of the culture occurred 20 to 25 min after induction (not shown). About 60 min after induction, 0.1 ml of the culture was plated undiluted and at several 10-fold dilutions on Luria-Bertani (LB)-ampicillin (Amp)-tetracycline (Tet) plates for survivors. A total of 90 survivors were isolated and screened for Qβ resistance (Qβ^r) by cross-streaking. For cross-streaks, ~10^9 plaque-forming units (PFU) of Qβ were spread with a sterile wire loop down the center of a LB-Amp-Tet plate and allowed to dry. Survivor colonies were picked directly from the selection plate and streaked across the phage zone. A streak was scored as positive if there was marked and reproducible growth across the phage zone. LB and minimal growth media have been described (*4*). In LB liquid media, kanamycin (Kan), Tet, chloramphenicol (Cam), and Amp were used at 10, 10, 25, and 100 μg/ml, respectively, except for liquid culture of cells carrying the plasmid pInvRecA-Cm, where the Cam concentration was 25 μg/ml. In LB agar, Kan, Tet, Cam, and Amp were used at 40, 10, 10, and 100 μg/ml, unless otherwise specified.
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8. *rat1* or *rat2* mutants were transduced to Kan^r with P1 lysates prepared on strains (*21*) carrying the transposon insertions *zab3093*::Tn*10kan* (1.8 min), *zhc3168*::Tn*10kan* (72.5 min), or *thi3178*::Tn*10kan* (90.4 min). Preparation of P1 lysates and transduction were done as described by Miller (*22*). Transductant colonies were selected on LB-Kan plates (100 μg/μl Kan), grown to saturation in LB-Kan, and individually scored for Qβ^r by spotting a serial dilution of phage on a softagar lawn. Because XL1 Blue and its *rat* mutant derivatives are *recA*, both were transformed with pInvRecA or pInvRecA-Cm before P1 transduction, as described (*23*). pInvRecA-Cm was constructed by ligating a blunt-ended Aat II–Sac I fragment, which contains the Cam^r determinant from pZA31-luc (*24*), into the blunt-ended Sca I site of pInvRecA. The *recA* plasmids were cured from the transductants by incubation at 37°C, as described (*23*).
9. The *murA* loci from the parental and the *rat* mutants were amplified with Pfu DNA polymerase and prim-

ers that are complementary to sequences upstream and downstream of the *murA* locus and that also carry Kpn I and Xba I restriction sites (*11*). The *murA*, *murA*^{rat1}, and *murA*^{rat2} amplification products were double-digested with Kpn I and Xba I, and cloned into the same sites in pZE12-luc, resulting in pZE12-murA, pZE12-rat1, and pZE12-rat2, respectively. pZE12-luc is a ColE1 replicon conferring Amp^r with the luciferase reporter gene *luc* under the IPTG-inducible hybrid $P_{LlacO-1}$ promoter (*24*). The constructs pZA31-murA and pZA31-murA^{Rat1} were constructed similarly, except that the parental vector is a p15A replicon carrying Cam^r (*24*). All constructs were confirmed by DNA sequencing (*11*).

10. T. Bernhardt, I. Wang, D. Struck, R. Young, data not shown.
11. Supplemental data, details of methods, and a figure showing the location of the altered residue on the structure of MurA are available on *Science* Online at www.sciencemag.org/cgi/content/full/292/5525/2326/DC1.
12. ET505 cells carrying either the A_2 plasmid (pA2) (*25*) or the vector (pJFlacZK) were grown in LBKan to an A_{550} of 0.8 and induced with IPTG at $t = 0$. Immediately before induction, $MgCl_2$ was added to a final concentration of 0.1 M to stabilize lysing cells (*4*). At $t = 45$ min, the cells were harvested, and total sugar nucleotides were extracted and purified through a Sephadex G-25 column as described (*5*). The *N*-acetylsugar–containing fractions were pooled and further fractionated on a 1-ml Hi-Trap Source Q anion-exchange column (Pharmacia) with a 0 to 0.4 M gradient of NH_4HCO_3. To determine the major sugars present in individual peaks from the ion-exchange column, we pooled the appropriate fractions, lyophilized them, and subjected them to hydrolysis with 0.02 N HCl at 90°C for 20 min. The hydrolysate was analyzed by paper chromatography with butanol:pyridine:water (6:4:3) as the solvent system. Dried paper chromatograms were stained with alkaline silver nitrate to detect reducing sugars or with the Elson-Morgan reagent to detect aminosugars (*26*).
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14. MurA and MurA^{Rat1} extracts were prepared from cultures of BL21(DE3) *slyD1 zhd*::Tn*10* containing the plasmids pZA31-murA and pZA31-murA^{Rat1}, respectively. (The host contains the T7 gene *1*, but neither plasmid contains a promoter for T7 RNA polymerase.) The cultures (35 ml) were induced with 1 mM IPTG at an A_{550} of 0.2, grown for 2.5 hours (final A_{550} of 2), and harvested by centrifugation (5000 rpm for 5 min in a JA20 rotor). The cells were washed with 30 ml of 50 mM tris (pH 8), resuspended in 1 ml of 50 mM tris (pH 8.0), 5 mM dithiothreitol, and disrupted in a SLM-Aminco French pressure cell at 16,000 psi. The extracts were cleared of whole cells and cell debris by centrifugation at maximum speed in a microcentrifuge for 10 min at 4°C. MurA activity was monitored as UDP-GlcNAc–dependent release of P_i from PEP as described by Marquart *et al.* (*13*). Reaction mixtures contained 5 μl of 1 M tris (pH 8.0), 5 μl 10 mM UDP-GlcNAc, 5 μl of 10 mM PEP, 30 μl of buffer A [50 mM tris (pH 8.0), 100 mM NaCl, 5 mM EDTA] or 30 μl of purifed Qβ phage in buffer A (30 μg of purified particles), and 5 μl of diluted MurA or MurA^{Rat1}extract (2 μg of total protein). Qβ particles were purified exactly as described by Strauss and Sinsheimer (*27*). The phage preparation was free of contaminating proteins as judged by SDS–polyacrylamide gel electrophoresis followed by Coomassie blue staining. The resulting preparation had a viable titer of 10^{13} PFU/ml and a physical titer of 10^{14} particles/ml determined from a protein concentration of 1 mg/ml.
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25. ET505 is *E. coli* W3110 *lysA*::Tn*10*, obtained from the *E. coli* Genetic Stock Center (New Haven, CT; http://cgsc.biology.yale.edu/). A *lysA* strain is required to prevent the conversion of added [^{3}H]DAP to lysine, so that [^{3}H]DAP can be incorporated only into the cell wall and its precursors. The plasmid pA2 is a derivative of pJFlacZK (*5*) and contains the A_2 gene and the *lacZ* gene in tandem, under control of the IPTG-inducible *tac* promoter.
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31. Isogenic *murA*$^+$ and *murA*^{rat1} transductants of XL1 Blue *zhc-3168*::Tn*10kan*, constructed by P1 transduction with the original *rat1* mutant as donor, were grown to an A_{550} of 0.2 at 37°C and infected at an MOI of 5 with Qβ. One milliliter of culture was withdrawn, vortexed with 1% $CHCl_3$, and chilled on ice at the times indicated. To estimate total Qβ production, the sample, diluted with 3 ml of LB, was subjected to disruption in a French pressure cell at 16,000 psi and then titered by plating serial dilutions on XL1 Blue.
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The Human Nuclear Xenobiotic Receptor PXR: Structural Determinants of Directed Promiscuity

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The human nuclear pregnane X receptor (hPXR) activates cytochrome P450-3A expression in response to a wide variety of xenobiotics and plays a critical role in mediating dangerous drug-drug interactions. We present the crystal structures of the ligand-binding domain of hPXR both alone and in complex with the cholesterol-lowering drug SR12813 at resolutions of 2.5 and 2.75 angstroms, respectively. The hydrophobic ligand-binding cavity of hPXR contains a small number of polar residues, permitting SR12813 to bind in three distinct orientations. The position and nature of these polar residues were found to be critical for establishing the precise pharmacologic activation profile of PXR. Our findings provide important insights into how hPXR detects xenobiotics and may prove useful in predicting and avoiding drug-drug interactions.

The pregnane X receptor (PXR; also known as NR1I2), a member of the nuclear receptor family of ligand-activated transcription factors, is a key regulator of cytochrome P450-3A (*CYP3A*) gene expression in mammalian liver and small intestine (*1–5*). The *CYP3A* gene products are heme-containing proteins that metabolize a wide variety of chemicals, including >50% of all prescription drugs (*6*). PXR is activated by most of the xenobiotics (exogenous chemicals) that are known to induce *CYP3A* gene expression, including the commonly used antibiotic rifampicin, the glucocorticoid dexamethasone, and the herbal antidepressant St. John's wort (*1–4, 7, 8*). Like other nuclear receptors, PXR contains both a DNA-binding domain and a ligand-binding domain. PXR binds to the xenobiotic DNA response elements in the regulatory regions of *CYP3A* genes as a heterodimer with the 9-cis retinoic acid receptor, also known as the retinoid X receptor (RXR) (*1, 2, 4*).

PXR can mediate dangerous drug-drug interactions. For example, hyperforin, a constituent of St. John's wort, activates PXR and upregulates *CYP3A* expression, which leads to the metabolism of vital drugs including the antiretroviral drug indinavir and the immunosuppressant compound cyclosporin (*8–11*). Unlike the steroid, retinoid, and thyroid hormone recep-

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