1997 Grand Prize Winner

Pharmacia Biotech and *Science* are pleased to announce the 1997 grand prize winner of the Pharmacia Biotech & *Science* Prize for Young Scientists. The winner of the 1997 grand prize in molecular biology was chosen from among the regional winners from four geographical areas: North America, Europe, Japan, and all other countries. The grand prize has been awarded to a regional winner from Europe, Christine Jacobs, for her essay on β-lactam antibiotic resistance and cell wall sensing in Gram-negative bacteria. The essay, reprinted below, describes her doctoral research under the shared supervision of Jean-Marie Frère (Center for Protein Engineering, University of Liège, Belgium) and Staffan Normark (Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden).

Christine Jacobs

Dr. Jacobs was born on 27 December 1968 in Liège, Belgium. In 1991, she was awarded an MS in biochemistry at the University of Liège, Belgium. For her masters thesis on β-lactamase kinetics, she spent 6 months in the laboratory of Staffan Normark at the Department of Molecular Microbiology, Washington University Medical School, St. Louis, where she became interested in bacterial resistance to antibiotics. She received her doctoral training in three different laboratories, taking advantage of the expertise and skills of each. She spent the first year in the laboratory of Staffan Normark at Washington University Medical School, St. Louis. Then, to extend her knowledge in protein chemistry, she went back to the laboratory of Jean-Marie Frère at the University of Liège. Later, in a very fruitful scientific collaboration with James T. Park, she spent 5 months at the Department of Molecular Biology and Microbiology, Tufts University Health Sciences Campus, Boston, to study the relation between β-lactamase regulation and cell wall metabolism. She completed her doctoral work at Karolinska Institute, Stockholm, in the laboratory of Staffan Normark. From 1991 to 1996, she was supported by a Belgian American Educational Foundation fellowship in St. Louis, a short-term European Molecular Biology Organization (EMBO) fellowship at Boston, and a 4-year fellowship from the Fonds National de la Recherche Scientifique in Liège and Stockholm. Her Ph.D. in biochemistry was awarded by the University of Liège in 1996. She is currently an EMBO postdoctoral fellow in the laboratory of Lucy Shapiro at Stanford University Medical School's Department of Developmental Biology.

Regional Winners

Europe: **Georg Halder** for his essay "Development and Evolution of the Eye: Pax-6 and the Compound Eye of *Drosophila Melanogaster*," which is based on his research conducted in the laboratory of Walter Gehring, University of Basel, Switzerland.

North America: **James Brownell** for his essay, "The Identification of GCN5-Related Proteins as Histone Acetyltransferases Links Chromatin Acetylation and Gene Activation," which is based on his doctoral research in the Department of Biology at Syracuse University in the laboratory of David Allis.

Japan: **Mitsuharu Hattori** for his essay "Platelet Activating Factor and Convolutions of the Brain," which describes research conducted in the laboratory of Keizo Inoue in the Department of Pharmaceutical Sciences at the University of Tokyo.

The other finalists were as follows: from North America, **Dirksen Bussiere, William John Feaver, Su Guo,** and **Anita Sil**; from Europe, **Stig Kjaer Hansen** and **Thorsten Melcher**; and from all other countries, **Cheryl Brown, Natalia Koudinova,** and **Michael Packer.**

The full text of the essays written by the regional winners can be seen in *Science* Online in the Special Features/Beyond the Printed Page section: http://www.sciencemag.org/feature/data/pharmacia/1997.shl

Life in the Balance: Cell Walls and Antibiotic Resistance

Christine Jacobs

As a result of the advent of antibiotics, we have been largely victorious in fighting bacterial infectious disease. However, the rapid development and spread of mechanisms of bacterial resistance are making virtually all antibiotics obsolete. One wonders if a return to the preantibiotic era is in our immediate future.

Since the discovery of penicillin, β-lactam antibiotics have been the most important family of antibacterial agents. How β-lactam antibiotics kill bacteria is still something of a mystery. They all inhibit the final stage of murein synthesis, which somehow triggers the autolytic activities of murein hydrolases. Murein is an essential heteropolymer that protects the bacterium from osmotic rupture, dictates cell shape, and is intimately involved in cell growth and division. To maintain cell integrity and viability, the murein must remain physically continuous during the bacterial cell cycle. Yet, bonds have to be broken to allow insertion of new material during cell growth. This must be done in a manner that does not endanger the osmotic stability of the cell. Temporal and topological controls must exist that keep wall assembly and turnover in balance and ensure synchronization of cell wall growth and the cell cycle. This view suggests a bidirectional communication between the exterior wall and the transcriptional machinery. The fact that general inhibition of murein synthesis caused by the presence of β-lactam antibiotics results in cell lysis suggests that these drugs disrupt the delicate balance between murein synthetic and degradative activities. Therefore, the elucidation of the mechanisms that upset the control of the murein hydrolases should help identify the control devices themselves, which in turn has important consequences for the discovery of new antibacterial drugs.

The major mechanism of resistance to β-lactam antibiotics is the synthesis of a bacterial enzyme, β-lactamase, that cleaves the β-lactam ring and renders the antibiotic inactive. Some bacterial species have refined this defense mechanism by making the β-lactamase synthesis inducible in the presence of β-lactam antibiotic. In many Gram-negative bacteria, the inducible β-lactamase gene *ampC* is transcriptionally controlled by a regulator encoded by *ampR*, which belongs to the LysR family of transcriptional regulators (*1*, *2*). Mutations in another locus, *ampD*, result in constitutive hyperproduction of the AmpC β-lactamase even in the absence of β-lactam antibiotics. The *ampD* mutants are therefore highly resistant to β-lactam antibiotics (*3*).

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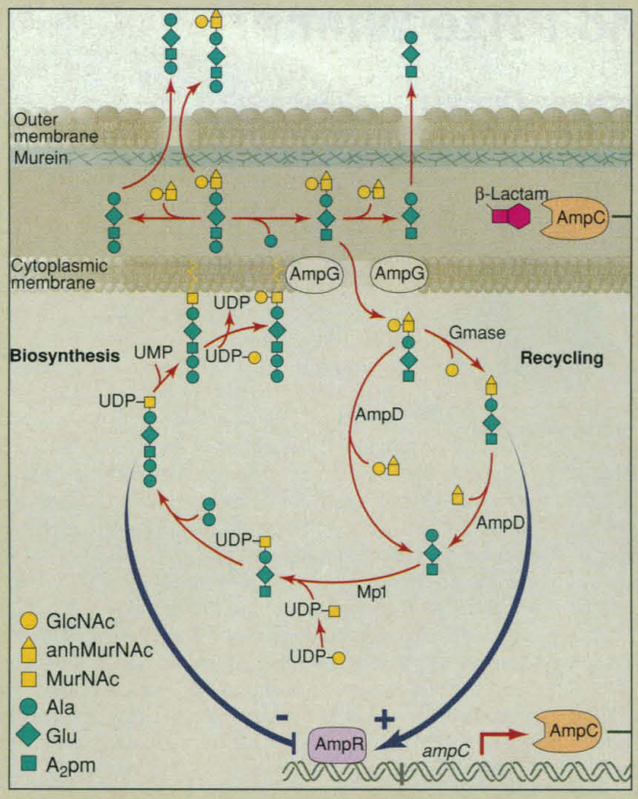
Another gene required for induction of β-lactamase is *ampG*, encoding the AmpG transmembrane protein (*4*). Interestingly, the *ampG* and *ampD* genes are also found in noninducible β-lactamase strains (*3*). We are therefore left with the following questions: What are the cellular functions of AmpG and AmpD? And how is the β-lactam antibiotic attack on the exterior cell wall signaled to the genetic apparatus?

Our first breakthrough came from the demonstration of a direct link between β-lactamase induction and cell wall metabolism. We discovered that *ampG* and *ampD*, genes essential for β-lactamase induction, were also required for cell wall recycling (*5*). *Escherichia coli* degrades up to 50% of its murein per generation, but most of the liberated murein fragments (muropeptides) are transported from the periplasm into the cytoplasm and recycled for further murein biosynthesis (*6*). Mutations in *ampG* and *ampD* dramatically decrease the ability of the cell to recycle its murein (*5*). An *ampG* mutant releases muropeptides into the external medium, whereas an *ampD* mutant accumulates a novel muropeptide species in its cytoplasm, the anhMurNAc-tripeptide (anhydro-*N*-acetylmuramyl-L-Ala-D-Glu-*m*-, A_2pm being diaminopimelic acid). We further showed in vitro that purified AmpD had *N*-acetylmuramyl amidase activity with a strict requirement for the presence of an anhydro function on the muramic acid residue (*7*). In that way, de novo cell wall precursors, which lack the anhydro bond, will not be degraded by the enzyme.



β-Lactamase control. A model for β-lactamase induction based on sensing the relative levels of murein intermediates in the cytoplasm.

On the basis of these findings, a new recycling scheme was proposed (see figure). The first step is the degradation of murein by specific cell wall hydrolases to yield GlcNAc-anhMurNAc-tripeptide (*N*-acetylglucosamyl-anhMurNAc-tripeptide). This muropeptide is transported into the cytoplasm by the permease AmpG. It is then either directly cleaved by AmpD or first converted into anhMurNAc-tripeptide by a cytosolic β-*N*-acetylglucosaminidase (Gmase) and then hydrolyzed by AmpD. In both cases, the resulting tripeptide is reintroduced into the murein biosynthetic pathway by direct addition to the peptidoglycan precursor uridine 5′-diphosphate (UDP)–MurNAc, as previously proposed (*6*). The gene encoding the tripeptide-adding enzyme responsible for this last step was recently identified as *mpl* (*8*).

Subsequently, using in vitro transcription studies, we showed that purified AmpR, in the absence of any effector, directed *ampC* transcription. This observation was somewhat surprising since other members of the LysR family usually depend on a ligand to activate transcription. Instead, the main murein precursor, UDP-MurNAc-pentapeptide (UDP-MurNAc-L-Ala-D-Glu-*m*-diaminopimelate-D-Ala-D-Ala), was found to inhibit transcription, and this inhibition was reversed by an intermediate in murein recycling, the anhMurNAc-tripeptide (*9*). These results led us to propose that β-lactamase expression is regulated by sensing the relative levels of the two endogenous murein intermediates (see figure).

During normal growth in the absence of a β-lactam antibiotic inducer, the AmpR regulator expressed is maintained in an inactive form by the murein precursor UDP-MurNAc-pentapeptide. This inactivation of AmpR can be relieved by both "knockout" mutations in the *ampD* gene and the presence of β-lactam antibiotics in the culture medium. Inactivation of *ampD*, which encodes the cytosolic amidase specific for the recycling of muropeptides, results in a drastic accumulation of its substrate, the anhMurNAc-tripeptide (*5*). The high concentration of this muropeptide inside the cell is sufficient to displace the UDP-MurNAc-pentapeptide from its AmpR-binding site, thereby reactivating AmpR. In wild-type cells, by impairing cell wall synthesis, the presence of β-lactam antibiotics results in a decrease of the UDP-MurNAc-pentapeptide concentration and an increase in the anhMurNAc-tripeptide concentration in the cytoplasm (*5*). These two effects are likely to be additive, displacing the UDP-MurNAc-pentapeptide repressor from AmpR, resulting in activation of β-lactamase expression.

Because the relative levels of the cytosolic intermediates of murein metabolism are altered by exposure to β-lactam antibiotics, the net result is the sensitive control of a bacterial defense mechanism. In addition, the presence (and maintenance) of this network in strains with noninducible β-lactamase (like *E. coli*) strongly suggests that this system functions as a means of monitoring cell wall integrity and maintaining a proper balance between murein synthesis and degradation during bacterial growth.

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