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Novel RNA polymerase inhibitor found in soil extracts provides hope for future antibacterial drugs

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“the rationale for the PUM project was to seek inhibitors of bacterial RNA polymerase that function through the nucleotide-addition site in order to take advantage of its predicted low propensity for resistance, and to do so by screening microbial-extract libraries to identify inhibitors of bacterial RNA polymerase and mutationally de-replicating hits, rapidly identifying those that are new compounds and that function through the nucleotide addition site.”

Richard H Ebright talks to Rachel Coleby, Commissioning Editor: Ebright is at Rutgers University (New Brunswick, NJ, USA), where he performs research on the structure, mechanism, and regulation of bacterial transcription and on antibacterial drug discovery targeting bacterial transcription. He has received research awards, including the Searle Scholar Award, the Walter J Johnson Prize, the Schering-Plough Award of the American Society for Biochemistry and Molecular Biology, the Waksman Award of the Theobald Smith Society, the MERIT Award of the National Institutes of Health, and membership in the American Academy of Arts and Science. He has more than 130 publications in peer-reviewed journals and more than 30 issued and pending patents.

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Please can you introduce yourself & tell us a little bit about your career to date?

I am Board of Governors Professor of Chemistry and Chemical Biology at Rutgers University and Laboratory Director at the Waksman Institute of Microbiology. I received my PhD in Microbiology and Molecular Genetics at Harvard in 1987, where I performed doctoral research on protein–DNA and protein–protein interactions in the regulation of bacterial gene expression. After that, I joined Rutgers University, where I first continued research on regulation of bacterial gene expression and then transitioned to a primary focus on the structural biology and structural biophysics of the enzyme responsible for bacterial RNA synthesis, namely bacterial RNA polymerase, and on antibacterial drug discovery targeting that enzyme.

What are the topics & research projects that you are working on in your lab currently?

One half of my lab performs basic research on the structure and mechanism of bacterial RNA polymerase. RNA polymerase is a ‘molecular machine’ that carries out a complex series of reactions in transcription initiation, elongation, and termination. We use tools of structural biology, including x-ray crystallography and, recently, cryo-electron microscopy, to determine structures of bacterial RNA polymerase in different functional states, and we use tools of single-molecule biophysics, including single-molecule fluorescence and single-molecule nanomanipulation with magnetic tweezers and nanopore tweezers, to monitor transitions between functional states.

The other half of my lab uses information from our and our colleagues’ work on the structure and function of bacterial RNA polymerase to develop new antibacterial compounds that target RNA polymerase, seeking new antibacterial drugs that kill bacteria resistant to current antibacterial drugs. Four features of bacterial RNA

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polymerase make it an attractive target for antibacterial drugs. First, bacterial RNA polymerase is essential for bacterial viability; compounds that inhibit it can kill bacteria. Second, bacterial RNA polymerase is essential for the viability of both replicating bacteria and nonreplicating, dormant bacteria (something that is true for very few other bacterial enzymes); compounds that inhibit it can kill both replicating bacteria and nonreplicating, dormant bacteria. Third, bacterial RNA polymerase is conserved across bacterial species; compounds that inhibit it can have broad-spectrum antibacterial activity. Fourth, bacterial RNA polymerase sequences differ from human RNA polymerase sequences; compounds can selectively inhibit bacterial but not human RNA polymerases. Bacterial RNA polymerase is a proven target for antibacterial drugs. Two current classes of antibacterial drugs: the rifamycins (rifampin, rifapentine, rifabutin, and rifaximin) and the lipiarmycins (fidaxomicin) function by inhibiting bacterial RNA polymerase. However, bacterial pathogens have arisen that are resistant to current antibacterial drugs that target bacterial RNA polymerase. The resistance results from mutations that alter the parts of bacterial RNA polymerase that serve as binding sites for the drug. Our approach to overcome this problem is to find new inhibitor binding sites on bacterial RNA polymerase – inhibitor binding sites that do not overlap known drug binding sites and therefore are unaffected by mutations that alter known drug binding sites – and then to find new inhibitors that function through the new inhibitor binding sites.

Talking specifically about the just-published pseudouridimycin project, what was the rationale behind this research that led to the paper?

In work to date, we have identified six new inhibitor binding sites on bacterial RNA polymerase. For each of the six new binding sites, we have identified new compounds that function through the binding site, exhibit antibacterial activity and exhibit no cross-resistance with previous or current antibacterial drugs. For three of the new binding sites, we are carrying out active lead optimization programs.

Pseudouridimycin (PUM) represents one of those cases: a new inhibitor binding site, a new inhibitor and an active lead optimization project [1].

The PUM project started with a potentially useful new inhibitor binding site on bacterial RNA polymerase that seemed especially promising: namely, the RNA polymerase ‘nucleotide-addition site’, the part of RNA polymerase that binds nucleoside triphosphates (NTPs), the chemical building blocks that RNA polymerase uses to synthesize RNA. Like other potentially useful new inhibitor binding sites, the nucleotide-addition site is crucial for RNA polymerase function (providing a basis for inhibitor efficacy), is conserved across bacterial species (providing a basis for inhibitor broad-spectrum activity), is different in human RNA polymerases (providing a basis for inhibitor therapeutic selectivity) and does not overlap known drug binding sites and known drug resistance substitutions (providing a basis for absence of cross-resistance to current drugs). However, the nucleotide-addition site offers an additional favorable feature that no other potentially useful new inhibitor binding site offers: namely, the nucleotide-addition site consists almost entirely of amino acid residues that are essential for RNA polymerase function and bacterial viability and cannot be substituted without loss of RNA polymerase function and bacterial viability. We hypothesized that the nucleotide-addition site could be a ‘privileged binding site’, a binding site that not only could be useful but also could be refractory to development of resistance. Accordingly, we focused our attention on the nucleotide-addition site. Working in collaboration with the Milan-based drug discovery company NAICONS Srl. (New Anti-Infectives Consortium. Srl.), we screened microbial-extract libraries for RNA polymerase inhibitors. We then used a powerful approach we term ‘mutational de-replication’ to winnow hits from the screen, first identifying the subset of hits that differed from previously identified RNA polymerase inhibitors, and then identifying the subset of that subset of hits that functioned through the nucleotide-addition site. The screen and mutational de-replication yielded PUM.

In short, the rationale for the PUM project was to seek inhibitors of bacterial RNA polymerase that function through the nucleotide-addition site in order to take advantage of its predicted low propensity for resistance, and to do so by screening microbial-extract libraries to identify inhibitors of bacterial RNA polymerase and mutationally de-replicating hits, rapidly identifying those that are new compounds and that function through the nucleotide addition site.

Can you tell us the key findings of this pseudouridimycin project?

There are three key findings.

The first key finding, of course, is PUM itself. PUM is a new antibiotic effective against drug-resistant bacteria [1]. PUM is produced by a microbe found in a soil sample collected in Italy (a soil sample collected under one of the

cypress trees in the famous Viale de Cipressi between Bolgheri and San Guido in Tuscany) and was discovered by systematically screening microbes from soil samples. PUM potently kills a broad-spectrum of drug-sensitive and drug-resistant bacteria in a test tube and potently cures bacterial infections in mice. PUM functions by inhibiting bacterial RNA polymerase through a binding site and mechanism different from those of the current classes of antibacterial drugs that target bacterial RNA polymerase, the rifamycins and the lipiarmycins. Because PUM inhibits through a new binding site and mechanism, it has no cross-resistance with rifamycins and lipiarmycins, it functions additively when co-administered with rifamycins and lipiarmycins, and, perhaps most important, it has a spontaneous resistance rate that is only one-tenth the spontaneous resistance rate of rifamycins and lipiarmycins.

The second key finding is that PUM functions as a 'nucleoside-analog inhibitor' of bacterial RNA polymerase [1], meaning that PUM functions by mimicking an NTP. PUM binds to the NTP binding pocket within the RNA polymerase nucleotide-addition site, and, by occupying the NTP binding pocket, PUM prevents NTPs from binding and being used for RNA synthesis. PUM is the first nucleoside-analog inhibitor that selectively inhibits bacterial RNA polymerase but not human RNA polymerases. The nucleotide-addition sites of bacterial RNA polymerase and human RNA polymerases are very similar, and therefore most researchers thought it would be impossible for a nucleoside-analog inhibitor to selectively inhibit bacterial RNA polymerase but not human RNA polymerases. But PUM contains a side chain that 'reaches' outside the NTP binding site and 'touches' an adjacent site that is present in bacterial RNA polymerase but not human RNA polymerases, and, as a result, PUM binds more tightly to bacterial RNA polymerase than to human RNA polymerases. The fact that PUM functions as a nucleoside-analog inhibitor that binds to the nucleotide-addition site explains the low rate of emergence of resistance to PUM. PUM interacts almost exclusively with amino acid residues of the RNA polymerase nucleotide-addition site that cannot be altered without losing RNA polymerase activity and bacterial viability. Mutations that alter the RNA polymerase nucleotide-addition site in a manner that compromise PUM binding also compromise RNA polymerase activity and bacterial viability. It is important to note that nucleoside-analog inhibitors of viral nucleotide polymerases are the cornerstone of current treatment of HIV infection and hepatitis C virus infection. PUM, and future nucleoside-analog inhibitors of bacterial RNA polymerase similar to PUM, potentially could have an analogous impact in treatment of bacterial infections.

The third key finding is that we discovered PUM by screening microbial extracts [1]. Microbial-extract screening has not been exhausted as a source of new antibacterial compounds. There are many new antibacterial compounds waiting to be discovered by microbial-extract screening.

The new compound pseudouridimycin has shown activity against drug-resistant bacteria, how can you ensure that resistance will not develop towards this compound? Should it be used in combination therapy?

Resistance to PUM arises, but it arises in an order of magnitude less frequently than resistance to current antibacterial drugs that target bacterial RNA polymerase [1].

Resistance is inevitable. Bacteria always will find a way.

The only long-term solution to bacterial resistance will be to transition from monotherapy in treatment of bacterial infections to combination therapy in treatment of bacterial infections. Current treatment of bacterial infections other than tuberculosis almost always involves monotherapy, the administration of a single antibacterial drug. This is a mistake. A grave mistake. Because resistance to a single antibacterial drug requires only a single mutational event or a single gene-transfer event, monotherapy risks the emergence of resistance within individual patients and practically ensures the emergence of resistance within populations of patients. In contrast, because resistance to a combination of multiple antibacterial drugs requires multiple mutational events or multiple-gene transfer events, combination therapy can avoid the emergence of resistance. Combination therapy currently is the standard of care in treatment of the bacterial infectious disease tuberculosis and the viral infectious diseases HIV-AIDS and hepatitis C. Combination therapy needs to be made the standard of care in treatment of all bacterial infectious diseases.

Apropos combination therapy and PUM, we showed in our paper that administering a combination of PUM and a rifamycin suppresses the emergence of resistance by multiple orders of magnitude, to levels below the limit of detection [1]. Combination therapy works, both in general and for PUM in particular.

What are the next steps for this compound in moving it toward clinical trials?

In principle, we could advance PUM to investigational new drug (IND) enabling studies and clinical trials in its present form. The field refers to this as ‘going directly from bug to drug’, since this involves advancing directly from a microbial natural product to a drug. However, we probably first will undertake one to three years of additional lead optimization, seeking ‘semi-synthetic’ PUM analogs having higher potency and/or higher stability. This will entail preparing PUM by fermentation of the soil microbe that produces it (using procedures that our colleagues at NAICONS already have optimized and scaled up), chemically modifying PUM to produce semi-synthetic analogs (using procedures developed and validated in our paper [1]), and then testing potencies and stabilities of those semi-synthetic analogs.

Apart from drug resistance, what do you find the greatest challenge in this field at the moment? (& how do you think we need to go about addressing these issues?)

The greatest current challenge in antibacterial drug development is advancing new antibacterial compounds through clinical studies and regulatory approvals. The challenge is not scientific. It is socio-economic and political.

Pharma has concluded it cannot make money by introducing new antibacterial drugs. If Pharma introduces a new antibacterial drug effective against bacteria resistant to current first-line drugs, the new antibacterial drug will be put on a ‘reserve list’ to be administered only to patients with infections resistant to current first-line drugs, resulting in low annual sales and low annual revenues. The new antibacterial drug will remain on the reserve list until resistance to current first-line drugs becomes universal, and, by that time, the new antibacterial drug likely will have lost patent exclusivity, resulting in high annual sales but low annual revenues. With low revenues initially and low revenues subsequently, Pharma cannot recoup the tens to hundreds of millions of dollars required to for clinical trials and regulatory approvals.

Accordingly, Pharma has zero interest in discovering new antibacterial compounds, essentially zero interest in developing new antibacterial compounds and little interest even in producing and marketing new antibacterial drugs for which all development already has occurred and all risks already have been removed.

It is imperative to find ways to incentivize Pharma to advance new antibacterial compounds through clinical studies and regulatory approvals. If society want new antibacterial drugs, society will need to incentivize companies to introduce new antibacterial drugs. There will be need to either ‘market entry rewards’ that incentivize companies by providing tax credits or payments upon introduction of new antibacterial drugs, or there will have to be ‘transferable exclusivity vouchers’ that incentivize companies by providing extended patent exclusivity terms for other drugs upon introduction of new antibacterial drugs.

Are there any specific technological advances that have revolutionized your work recently or will revolutionize it in the future?

One revolution in technology that is sweeping structural biology and structural biophysics is the ‘resolution revolution’ in cryo-electron microscopy (cryo-EM). Until very recently, the only method able to determine high-resolution structures of a large molecular machine, such as bacterial RNA polymerase, was x-ray crystallography, which entailed the difficult, often impossible step, of crystallizing functional states of interest of the molecular machine, and which typically required years for each functional state. Cryo-EM now enables us to determine high-resolution structures of bacterial RNA polymerase without crystallization and in just weeks to months. Over the past year, we have used cryo-EM to determine a series of structures of bacterial RNA polymerase in different functional states and, most recently, two structures of bacterial RNA polymerase in complex with inhibitors. Cryo-EM is becoming our new method of choice to find where new inhibitors bind to RNA polymerase and how they function on RNA polymerase.

Looking to the future, where do you imagine your work going in the next 5–10 years?

With cryo-EM rapidly providing high-resolution structures of functional states of RNA polymerase, and with single-molecule fluorescence and single-molecule manipulation, particularly nanopore-based single-molecule nanomanipulation, defining transitions between functional states of RNA polymerase, we now can elucidate structures and mechanisms for all steps along the pathway of transcription initiation, elongation, and termination by RNA polymerase. I anticipate that we will essentially fully understand transcription within 5–10 years.

The resulting new information on structure and mechanism will enable us to find more ways to inhibit RNA polymerase and more ways to obtain new antibacterial compounds that target RNA polymerase.

Finally, if you had unlimited money & resources, what would you make it your priority to do in this field?

I would pursue two approaches.

The first approach involves selective nucleoside-analog inhibitors of bacterial RNA polymerase. Having found a first selective nucleoside-analog inhibitor of bacterial RNA polymerase – PUM – I think it likely we can find others. Nucleoside-analog inhibitors have been the cornerstone of treatment of viral infections, including HIV and hepatitis C, and, as a result, thousands of nucleoside-analog inhibitors have been synthesized and tested for antiviral activity. I think it likely that, among the thousands of already existing nucleoside-analog inhibitors, there are selective nucleoside-analog inhibitors of bacterial RNA polymerase waiting to be found. Given unlimited money, resources, and access to Pharma compound collections, I immediately would screen the thousands of already existing nucleoside-analog inhibitors, seeking ones that have bacterial RNA polymerase inhibitory activity and antibacterial activity.

The second approach involves our new strategy for screening microbial extracts and mutationally de-replicating hits. In unpublished work, in collaboration with NAICONS, we have identified a dozen additional microbial extracts that inhibit bacterial RNA polymerase, each containing a new inhibitor of bacterial RNA polymerase, and most containing a new inhibitor of bacterial RNA polymerase that functions through a new binding site on RNA polymerase. I think it likely that at least some of these dozen microbial extracts contain compounds that have antibacterial activity against the highest-priority bacterial pathogens, both Gram-positive and Gram-negative. However, in order to proceed further with any of these dozen additional microbial extracts, we need to fractionate the microbial extract, isolate the active component, determine its structure, and characterize its properties, and that requires approximately US\$10,000–20,000 per microbial extract. This is a rounding error (or less) by the standards of Pharma-based drug discovery in most therapeutic areas, but, as I noted above, Pharma currently has zero interest in antibacterial drug discovery. Given unlimited money and resources, I immediately would proceed with isolation, structure determination, and characterization of the new RNA polymerase inhibitors in these dozen microbial extracts, and, in parallel, I would seek to identify additional active microbial extracts.

Financial & competing interests disclosure

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