Glycosylated Polyene Macrolides Kill Fungi via a Conserved Sterol Sponge Mechanism of Action

Nicole M. Revie and Leah E. Cowen

Systematic analysis of glycosylated polyene macrolides expands the biosynthetic gene cluster and chemical space and supports the sterol sponge mechanism of antifungal action.

Natural products have historically served as a rich reservoir of bioactive molecules with diverse activities that have had a profound impact on modern medicine. Notably, glycosylated polyene macrolides (GPMs), such as amphotericin B and nystatin, represent a clinically important group of fungicidal small molecules. Despite their use in clinics to treat deadly, invasive fungal infections for over half a century, the mechanisms by which GPMs kill fungi have been shrouded in mystery. Guo and colleagues perform a tour-de-force exploration of the hypothesis that there is a conserved mechanism by which GPMs kill fungi involving self-assembly into a “sterol sponge” that extracts the essential fungal sterol, ergosterol, from cell membranes.1

To test this hypothesis, they first developed a bioinformatic pipeline for comprehensive identification of GPM biosynthetic gene clusters (BGCs), thereby expanding the repertoire of established BGCs by 10-fold. This approach enabled prioritization of structural novelty as predicted by the sequence diversity of AmphI, a conserved enzyme that installs the core carbon framework, as well as sequence diversity of macrolide tailoring enzymes. To accelerate the discovery of new GPMs, targeted detection of the electron-rich substructure present in all GPMs was achieved by co-opting a tetrazine ligation reactivity-based strategy coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). All seven structurally diverse GPMs characterized in this study were shown to directly bind ergosterol, and further, precomplexation with ergosterol impeded their antifungal activity. Together, this supports a conserved sterol sponge GPM mode of action (Figure 1).

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Figure 1. (A) Structures of GPMs including known antifungals (amphotericin B, natamycin, and lucensomycin), as well as the newly identified tetraene-containing GPM from Actinokineospora spheciospongiae, kineosporicin. The conserved polyene substructure is highlighted in blue. (B) Sterol “sponge” mechanism of action. GPMs with a conserved ergosterol binding region, form large, extramembranous aggregates that extract ergosterol from lipid bilayers.
Despite their use in clinics to treat deadly, invasive fungal infections for over half a century, the mechanisms by which GPMs kill fungi have been shrouded in mystery.

This comprehensive approach to map GPMs reveals broad conservation in the sterol sponge mode of action defined for the most prominent example of this class, amphotericin B. These findings challenge the previously prevailing model that fungicidal activity was attributable to ion channel-mediated membrane permeabilization. Previous synthetic efforts yielded amphotericin derivatives that were unable to form ion channels but still able to sequester ergosterol and kill fungi, and thus uncoupling ion channel formation from the proximal mode of antifungal activity. The capacity of GPMs to form extramembranous aggregates that quickly sequester ergosterol has been supported by extensive solid state nuclear magnetic resonance (NMR), transmission electron microscopy (TEM), and cell biological studies. Of the seven GPMs characterized by Guo and colleagues, only amphotericin B formed ion channels, as demonstrated by potassium leakage. However, consistent with the sterol extraction mode of action, each of the seven GPMs directly interacted with ergosterol, as evidenced by isothermal titration calorimetry, and precomplexation with ergosterol diminished antifungal activity. The considerable variance in the magnitude of impact of precomplexation with ergosterol suggests that there may be additional features to explore, which may include reactive oxygen species generation and pleiotropic consequences of membrane perturbation.

Beyond defining conservation of the sterol sponge mode of antifungal activity, Guo and co-workers provide a powerful platform for discovery of new GPMs. All of the relevant BGCs were identified in the phylum Actinobacteria with the majority of clusters coming from soil-dwelling bacteria; however, animal-associated bacteria were also implicated. Pseudonocardiaae emerged as the greatest source of novelty, with 19 uncharacterized BGCs that were predicted to produce structurally distinct GPMs. A pervasive challenge with natural product discovery is that the presence of gene clusters does not necessarily track with production of sufficient isolatable quantities to enable molecular characterization. Tetrizine reactivity-based screening to selectively target the electron-rich polyene, recently deployed to detect isonitrile-containing natural products, proved to be an effective strategy to rapidly identify natural products of interest. This co-opted strategy led to the discovery of a first-in-class methyltetraene- and diepoxide-containing GPM, kineosporicin, with broad-spectrum antifungal activity.

This generalized approach of prioritizing diversity based on BGCs and accelerating small-molecule discovery by leveraging chemoselective enrichment likely has far-reaching utility to overcome challenges that have traditionally hindered natural product discovery. More broadly, there is an opportunity to explore the unknown universe of genomes yet to be sequenced from uncharted environments with the potential to better understand the ecological context of natural product production. There is an ever-growing appreciation of the vast potential of exploring the chemical diversity of molecules that has largely evaded widespread resistance and HIV.7 The recent rate of emergence and spread of multidrug-resistant fungal pathogens threatens the efficacy of our limited arsenal of antifungals, with only three major classes to treat these invasive infections.8

The discovery of new antifungals addresses a growing public health threat posed by fungal pathogens, which take a devastating toll on vulnerable patient populations. Mortality attributed to fungal infections is estimated at approximately 1.5 million people every year, outpacing mortality rates for malaria or breast cancer and on par with that of tuberculosis and HIV.7 The discovery of new antifungals addresses a growing public health threat posed by fungal pathogens, which take a devastating toll on vulnerable patient populations. Mortality attributed to fungal infections is estimated at approximately 1.5 million people every year, outpacing mortality rates for malaria or breast cancer and on par with that of tuberculosis and HIV.7 The recent rate of emergence and spread of multidrug-resistant fungal pathogens threatens the efficacy of our limited arsenal of antifungals, with only three major classes to treat these invasive infections.8

The polyenes represent a broad-spectrum, fungicidal class of molecules that has largely evaded widespread resistance over more than 50 years of clinical use. The primary
mechanisms of polyene resistance are genetic alterations that result in the substitution of ergosterol with alternate sterols, thereby removing the proximal target of polyenes. However, there are profound fitness costs associated with these altered membranes making resistant mutants vulnerable to diverse exogenous stresses and posing an evolutionary constraint limiting the emergence and spread of resistance.\textsuperscript{9} A major liability of polyenes is the host toxicity, which is largely attributable to limited selectivity for ergosterol over cholesterol, the mammalian sterol counterpart. New insights into the mode of action provides an opportunity to optimize fungal selectivity by harvesting chemical diversity in nature or what is accessible through synthetic strategies.\textsuperscript{1,10} Beyond fungal selectivity by harvesting chemical diversity in nature into the mode of action provides an opportunity to optimize resistance-evasive antifungal classes that target essential processes, additional creative approaches to identify fungal-selective, resistance-evasive antifungal classes that target essential processes, resistance mechanisms, and regulators of fungal virulence.

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**Author Information**

**Corresponding Author**
Leah E. Cowen – Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5G 1M1, Canada; orcid.org/0000-0001-5797-0110; Email: leah.cowen@utoronto.ca

**Author**
Nicole M. Revie – Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5G 1M1, Canada

Complete contact information is available at: https://pubs.acs.org/10.1021/acscentsci.1c00520

**Notes**

The authors declare the following competing financial interest(s): L.E.C. is a co-founder and shareholder in Bright Angel Therapeutics, a platform company for development of novel antifungal therapeutics. L.E.C. is a consultant for Boragen, a small-molecule development company focused on leveraging the unique chemical properties of boron chemistry for crop protection and animal health.