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Chemical genomics reveals mechanistic hypotheses for uncharacterized bioactive molecules in bacteria

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In an effort to combat the perpetual emergence of new antibiotic-resistant human pathogens, research in industry and academe aims to find new means of controlling infection. The discovery of new antimicrobial chemicals is not the bottleneck in an era where high-throughput screening rapidly uncovers new bioactive compounds. Rather, the rate-limiting step in antimicrobial discovery pipelines is identifying mechanisms of action (MOA) of bioactive molecules produced by these increasingly large-scale efforts. Chemical genomics has proven to be of high value in providing mechanistic hypotheses for novel bioactive chemical matter. Several techniques fall under this blanket term, including interactions with deletion or transposon libraries, fluorescent or luminescent reporter library profiles, or deep sequencing approaches. Each of these provide unique and complementary outputs, and have high value in generating target lists for chemical screens, or assisting in downstream MOA discovery. We review here the broad usefulness of this technique to aid in MOA determination, to identify targets for new lead molecules, and to expand our mechanistic understanding of existing drugs.

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Introduction

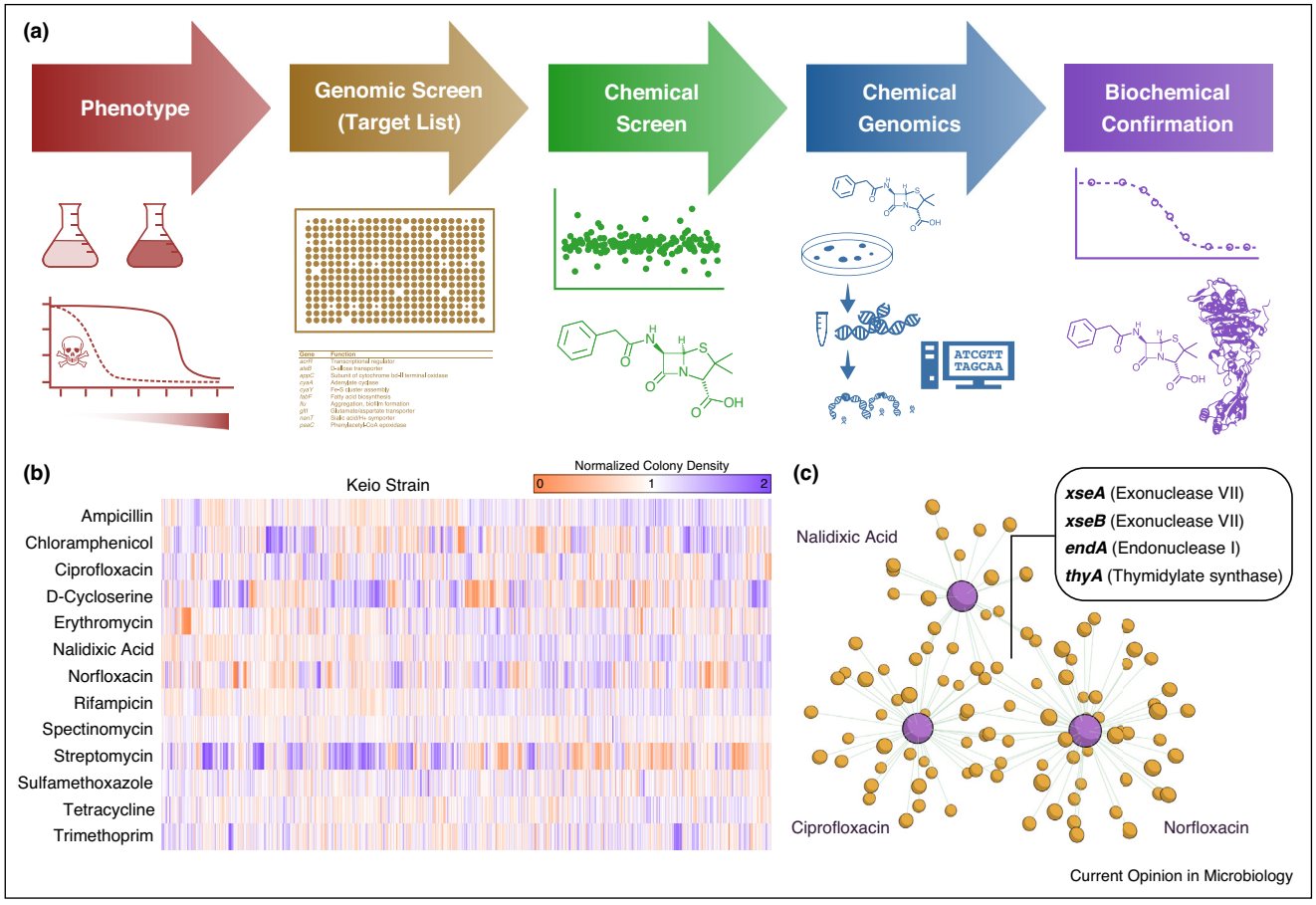
Much of what we know about the complicated biology of bacteria comes from genetic perturbation. Recent advances in high-throughput bacterial transformation and mutagenesis have ushered in an era of genomic libraries, rapidly queried using high-density arraying tools [1–3]. Synthetic genetic arrays that systematically query the interactions of pairs of genetic mutations have proven to be effective in charting genetic networks and answering specific questions regarding bacterial biology [4]. Arrays such as this are, however, typically only possible with non-essential mutations, given that essential genes are not commonly targeted in such mutant collections. This is where a chemical genomics approach has a sizeable advantage, revealing genetic interactions with a chemical probe in a doseable manner [5]. Contrasting with an all-or-nothing genetic perturbation, probing with an antibiotic at subinhibitory concentrations reveals physiological responses to perturbations of essential cell processes. Indeed, chemical–genetic interactions can provide readily testable hypotheses into the mechanistic underpinnings of bioactive chemical matter in bacteria [6].

Mechanism of action (MOA) studies are among the most challenging aspects of the discovery phase of modern drug development. Although hundreds of thousands of compounds can be screened with relative ease, and large numbers of bioactive compounds can be assembled, prioritizing compounds for in-depth study and identifying the biological target(s) remain daunting hurdles to modern phenotype based screening efforts. In this review, we will discuss the utility of chemical genomics approaches in MOA predictions for unknown molecules. The usage of deletion and transposon mutant libraries, reporter libraries, and deep-sequencing approaches will be discussed herein.

Chemical–genetic interactions using mutant libraries

Bacterial genomic libraries such as the Keio collection [7] of non-essential knockouts in the model bacterium *Escherichia coli*, can be used to probe the MOA of bioactive molecules [5,6,8,9]. Incorporating a chemical probe into growth medium, libraries are arrayed and inoculated in high-throughput, revealing chemical–genetic interactions for a compound of interest (Figure 1a). In this manner, genetic enhancers and suppressors of chemical lethality can be viewed as functionally connected to the machinery that is the target of the chemical (Figure 1b,c). Further, the interactions can reveal interesting phenotypes that may be useful for downstream chemical screening; providing a potential target list for further screening. This was recently demonstrated in a unique manner by Stokes et al. [10•], who reported an idiosyncratic effect of temperature on the growth inhibition by vancomycin in *E. coli*. Although

Figure 1



Example chemical screen, utilizing chemical genomics to generate a target list, and to inform on MOA of a bioactive molecule. A pipeline demonstrating this is shown in **(a)**, where a chemical genomics screen of the phenotype of interest can generate a list of potential targets, pre-validating the screening approach. After a high throughput chemical screen is conducted, chemical genomics again can provide mechanistic hypotheses that are readily testable downstream. Depending on the chemical genomics method used (Table 1), the outputs can provide speculative targets, can inform on regulation, or provide information regarding resistant mechanisms. An example of crosses with a deletion (Keio) library are shown in **(b)**, where data from French *et al*. [5] are displayed in heatmap form, identifying functional fingerprints for some known antibiotics. Fluoroquinolone examples are highlighted in **(c)**, where lethal interactions from (b) are illustrated as an example of how individual interactions can provide mechanistic hypotheses; in this case, for DNA replication inhibitors.

vancomycin has long been thought to be inactive against all Gram-negative bacteria, *E. coli* was found to be exquisitely sensitive to vancomycin at 15 °C. Further, genetic suppressors of this unusual phenotype were found by screening the Keio collection and were greatly enriched for outer membrane-related processes. This is of particular interest, given that the World Health Organization priority list for antibiotic-resistant pathogens are largely Gram-negative organisms [11]. Indeed, this phenotype was leveraged for a high-throughput chemical screen targeting the outer membrane of *E. coli* and led to the identification of pentamidine as a potent potentiator of Gram-positive antibiotics against Gram-negative pathogens [12••]. In addition to the Keio collection in *E. coli*, ordered genomic libraries exist for a number of bacterial pathogens including *Salmonella* Typhimurium [13], *Acinetobacter baylyi* [14], *Pseudomonas aeruginosa* [15], and *Staphylococcus aureus* [16].

The challenge in studying chemical genetic interactions with essential genes has been largely overcome through the development of *trans*-acting molecules to generate conditional mutants in genes otherwise refractory to standard approaches. Querying essential protein targets to identify MOA is therefore possible with interfering/antisense RNA and CRISPR-based libraries. In *S. aureus*, a bactericidal natural product extract was crossed with a library bearing antisense RNA interference plasmids for essential genes [17]. This effort revealed a DNA replication MOA, and a new topoisomerase inhibitor similar to novobiocin. Genomic libraries employing CRISPR interference (CRISPRi) enable the study of chemical–genetic interactions with essential or any other gene of interest [18,19]. An inducible, catalytically inactive Cas9 protein can be targeted to any genomic loci by sgRNAs to inhibit transcription initiation, offering a dose-dependent means