DRUG DISCOVERY TODAY TARGETS

Functional genomics in antibacterial drug discovery

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Antibacterial drug discovery has experienced a paradigm shift from phenotypic screening for antibacterial activity to rational inhibition of preselected targets. Functional genomics techniques are implemented at various stages of the early drug discovery process and play a central role in target validation and mode of action determination. The spectrum of methods ranges from genetic manipulations (e.g. knockout studies, mutation analyses and the construction of conditional mutants) to transcriptome and proteome expression profiling. Functional genomics supports antibacterial drug discovery by improving knowledge on gene function, bacterial physiology and virulence and the effects of antibiotics on bacterial metabolism.

The tremendous progress in genome analysis in the past decade has had a major impact on all biological sciences, including antibacterial research. Since the deciphering of the genome of *Haemophilus influenzae* in 1995 [1], many other bacterial genomes were sequenced in rapid succession. Complete genome sequences of >200 bacteria are now publicly available, among which are many important human pathogens (e.g. www.ncbi.nlm.nih.gov/genomes/Complete.html). Several pharmaceutical companies have started sequencing programs and have restructured their antibacterial drug discovery process to maximize use of this unprecedented information. Before the genomics era, the search for antibacterial agents relied primarily on antibacterial activity, specifically, growth inhibition of a desired spectrum of bacteria but not eukaryotic cells in laboratory *in vitro* assays. Promising drug candidates were further profiled *in vivo*, and the molecular target was often identified only after the compound had reached the market. This concept proved highly successful in the 1940–1960s because it generated the precursors of almost all antibiotics that are in clinical application today [2].

However, after this 'golden age of antibiotics', it took almost four decades until the oxazolidinone linezolid was marketed as the first representative of a novel chemical class of antibiotics [3]. The ever-increasing number of reports on multidrug-resistant bacterial isolates demonstrates that this approach is now almost exhausted and there is an urgent need for new antibacterial classes not affected by resistance mechanisms already present in the bacterial population [4,5; http://who.int/emc/amrpdfs/WHO_global_strategy_english.pdf; www.idsociety.org]. Thus, the pharmaceutical industry embraced genomic information as the basis for a rational, target-directed antibacterial drug discovery strategy to complement the classic empirical approach. Central to the paradigm shift was the belief that the bacterial genomes harbor a variety of so-far-unexploited targets with the potential for potent and selective antibiotics against a broad spectrum of bacterial pathogens [6–9].

Various functional genomics techniques are implemented in the modern antibacterial drug discovery process (Figure 1). Knockout analyses and mutation studies aid in the selection and validation of potential novel targets by probing their essentiality for

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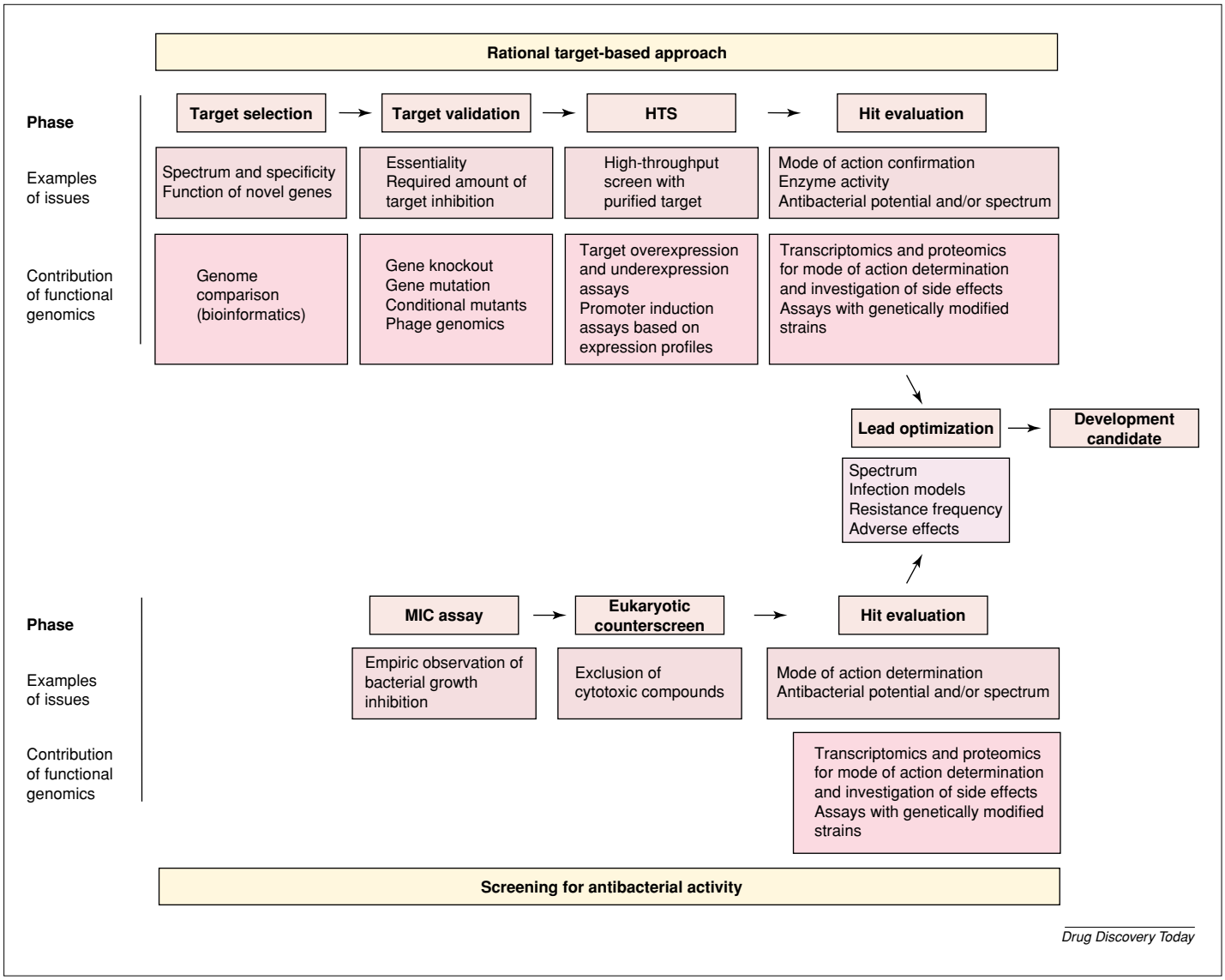

FIGURE 1

Contribution of functional genomics to the antibacterial drug discovery process. Abbreviation: MIC, minimal inhibitory concentration as a measure for antibacterial activity.

bacterial survival. Regulated gene expression in conditional mutants indicates the degree of target inhibition required for growth arrest. The use of genetically modified bacteria, as well as holistic approaches such as transcriptome and proteome profiling, has proven instrumental in validating the mode of action (MOA) of screening hits from target assays. Functional genomics methods also provide crucial hints on the MOA of hits identified using the empiric approach of screening for antibacterial activity. In addition to these direct applications in the drug discovery process, functional genomics techniques help to broaden our knowledge on bacterial physiology and pathogenicity in general and have become the key methods to investigate the role of genes of unknown function. Here, an overview of functional genomics methodologies is presented and their benefits and limitations are discussed. In addition, the potential of this technology in antibacterial drug discovery is illustrated.

Functional characterization of novel genes and identification of novel targets

The availability of complete genome sequences raised the hope of identifying a large number of proteins suitable as novel targets that could be used in screening for novel inhibitory compounds. Indeed, comparison of genomes enables the identification of proteins that are conserved across the medically important pathogens. However, comparative genomics also revealed the high genetic diversity among microbes. Moreover, broad conservation does not necessarily mean that each respective protein performs a function that is indispensable for cellular life. Genetic analyses have shown that the same gene can encode a

TABLE 1

Number of potential essential genes identified in genome-wide gene inactivation studies

Organism	Total no. of genes	No. of (potentially) essential genes[a]	Method	Refs
Bacillus subtilis	4101	271	Plasmid insertion mutagenesis Conditional mutants Estimations derived from literature study	[20]
Escherichia coli	4279	620	Transposon mutagenesis	[16]
Haemophilus influenzae	1709	256	Transposon mutagenesis	[19]
Helicobacter pylori	1552	344	Transposon mutagenesis	[17]
Mycoplasma genitalium	484	256–350	Transposon mutagenesis	[14]
Staphylococcus aureus	2595	150–658	Antisense RNA expression	[26,27]
Streptococcus pneumoniae	2043	113 out of 347 examined genes	Plasmid insertion mutagenesis	[21]

[a]For most species, the conclusion that genes might be essential has been drawn because gene inactivation was not achieved. Therefore, the genes need to be regarded as being potentially essential. Validation of essentiality will reduce the number of essential genes. The essentiality of genes has been studied *in vitro* in complex medium. Genes validated this way are also considered to be probably indispensable *in vivo*. The best-validated essentiality study has been performed in *B. subtilis* and, in this case, the number of essential genes seems to be realistic.

function that is essential in one organism but not in another. Such a phenomenon can be explained by the presence of biochemical bypasses or additional analogous enzymes. For example, the broadly conserved glutamate racemase MurI, which produces the essential component D-glutamic acid for bacterial cell walls, is necessary for the viability of *Streptococcus pneumoniae* [10]. However, in *Staphylococcus aureus*, this protein appears to be dispensable because D-glutamic acid is synthesized via an alternative route [11]. In addition to such a classical example of a bypass, bacterial fatty acid biosynthesis (FAB) provides a famous example for analogous gene replacement. The enoyl-acyl carrier protein reductase isoforms FabK and FabI are two structurally unrelated enzymes that catalyze the same biochemical reaction [12]. Whereas the triclosan-sensitive FabI is conserved among many bacterial species, the enzyme FabK, which is insensitive to triclosan, has been identified in important pathogens such as *S. pneumoniae* and enterococci. Such examples demonstrate the importance of applying comparative and functional genomics in various bacterial species and even in different strains of the same species for target validation. An essential prerequisite for the study of species-immanent target variations is the sequencing of whole genomes of multiple strains from each important pathogen [13].

A gene is regarded as being essential when the bacterium cannot survive its genetic inactivation. Several methods are applied to study the essentiality of genes on a genomic scale. Often, mobile DNA cassettes called transposons are used to inactivate genes by random insertion [14–19]. Genetic footprinting using diverse hybridization and PCR techniques enables mapping of insertion sites in the genomes. Other genome-wide gene inactivation studies apply homologous recombination methods [18–22]. In these studies, resistance markers are normally introduced into the genomes. However, marker-less gene deletions represent the most precise way of gene inactivation. Such techniques are more elaborate and are mainly reported from *Escherichia coli* [23,24]. Furthermore, the observation that a gene cannot be inactivated is not final proof of its essentiality – there might be experimental reasons for this phenomenon. Only conditional mutants, such as temperature-sensitive (ts) mutations and controlled gene expression systems, are able to demonstrate the essential role of a gene. Currently, genes of interest or gene-specific antisense RNA are controlled by regulable promoter systems. In most cases, inducible promoters regulated by arabinose, rhamnose, tetracycline or lactose derivatives are used in Gram-negative species, and promoters regulated by tetracycline, lactose derivatives, xylose, fucose or acetamide are used in Gram-positive species, including mycobacteria [25]. By conditional expression of random genomic fragments and then screening for fragments that when expressed block growth, Forsyth *et al.* [26] and Ji *et al.* [27] applied the naturally occurring RNA antisense principle for genome-wide gene silencing in *S. aureus*, which is one of the most important Gram-positive pathogens. Genome-wide conditional or non-conditional gene inactivation studies enable an estimate of the number of genes essential for growth in different bacterial species (Table 1). The confirmation that the same gene product ('ortholog') is essential in different species greatly increases the confidence in such a target. Comparison of the essential genes from different species leads to the conclusion that the number of genes encoding putative broad-spectrum targets is <200 for major Gram-positive and Gram-negative pathogens or only Gram-positive pathogens (Table 2).

Most of the essential genes are functionally characterized and can be classified according to, for example, enzymatic class and physiological role. For target prioritization, different criteria, such as 'screenability', druggability and physiological context, must be considered. However, this information is often not available and some targets are not yet functionally characterized. The number of under-explored targets is particularly high when focusing on functions conserved in a limited number of pathogens

(narrowed spectra of bacteria). Growth studies with various supplements, cytological evaluation, metabolic labeling experiments and transcriptome and proteome analyses with conditional mutants under semi-permissive conditions might aid in the functional characterization of respective genes.

Another approach to identify novel targets of high priority is to examine the molecular mechanisms that bacteriophages apply to arrest crucial cellular processes. By sequencing 26 *S. aureus* phages, Liu *et al.* [28] identified 31 novel polypeptide families that inhibited growth when expressed in *S. aureus*. For one of these polypeptides, affinity chromatography identified the target as the essential protein DnaI, which is a helicase loader required for primosome assembly during the initiation of DNA replication. Screening for small molecules that inhibit the interaction of the phage protein with DnaI enabled the identification of antibiotic compounds acting through the helicase loader. Such a phage genomics platform could be expanded to other species and could provide valuable information and screening tools for novel or unexplored targets.

Genetically modified bacterial strains for drug screening and determination of mode of action

Functional genomics techniques are valuable tools to evaluate the pool of targets that could be exploited for the discovery of novel antibiotics. In addition, they have become indispensable tools for accelerating the MOA characterization of unexplored or novel antimicrobial compounds and offer new mechanism-based screening opportunities.

A common method of defining the MOA of an antimicrobial compound is the generation and characterization of resistant mutants, because point mutations in a target gene generally confer drug resistance. However, traditional methods of mutation mapping of random mutants are too time-consuming for characterizing whole compound collections. The first methods for rapid resistance mapping have been developed based on the introduction of random mutations into DNA pieces by error-prone PCR and subsequent transformation of *S. pneumoniae* [29,30]. It has to be taken into account that resistance mutations are not only located in target genes, but also in drug transporter genes, in bypass genes or regulatory genomic regions. Moreover, target-related resistance mutations against compounds with more than one target might not be identifiable.

Conditional mutants with downregulated target gene expression generally exhibit a selective increase in sensitivity to a target-specific inhibitor. Comparing the relative growth inhibition of such strains to the wild-type provides a simple screen for identifying the MOA of antimicrobials, as reported by DeVito *et al.* [31], who used arabinose-regulable expression systems, and Forsyth *et al.* [26], who used antisense RNA. Instead of using individual mutant–wild-type strain pairs, conditional mutants with regulable antisense RNA constructs can also be pooled to identify concomitantly different drug targets of novel antimicrobials. The plasmids containing different antisense RNA-encoding inserts are isolated from mixed cultures. PCR amplification of the inserts and hybridization of the PCR products with microarrays carrying gene-specific DNA probes enabled Yin *et al.* [32] to quantify the relative number of each mutant after growth in the presence or absence of sublethal doses of antibiotics. A proof-of-concept study revealed that strains with increased susceptibility to known antibiotics could be selectively detected within a pool of 78 different strains expressing antisense RNA [32]. Such studies in miniaturized high-throughput scale remain challenging, because the degree of increased susceptibility depends on the extent of target gene repression and growth phase. These parameters might vary for different target genes. Furthermore, particular types of inhibition might not be detected by such assays, such as the formation of toxic enzyme–compound complexes (e.g. as in the case of the topoisomerase II- and IV-targeting quinolones).

In contrast to hyper-susceptibility tests, target overexpression assays represent another tool for MOA identification. For example, Huang and co-workers [33] generated an expression library of 2300 unique open reading frames (ORFs) in *S. aureus*. Overexpression of these ORFs led to reduced antibiotic susceptibility and enabled the identification of targets for antimicrobials and the elucidation of resistance mechanisms. Such tests might be helpful in MOA identification for novel antimicrobials. However,

TABLE 2

Number of bacterial targets present in important Gram-positive and both Gram-positive and Gram-negative pathogens[a]

Functional category of gene product	No. of targets in Gram-positive pathogens	No. of targets in Gram-positive and Gram-negative pathogens
Cell division	12	9
Nucleotide biosynthesis	12	10
Coenzyme biosynthesis	10	7
Fatty acid biosynthesis	14	14
Translation	71	68
Transcription	9	8
Replication	26	25
Cell wall biosynthesis	19	16
Others	10	10
Total number	183	167

[a]The number of genes encoding targets for antibiotic drug discovery are counted and grouped according to functional categories. Genes counted fulfill two criteria: (i) they occur in the important Gram-positive pathogens *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes* and *Enterococcus faecalis* ('Gram-positive' column) or additionally in the Gram-negatives *Escherichia coli* and *Pseudomonas aeruginosa* ('Gram-positive and Gram-negative' column); and (ii) the genes are described as being probably essential in at least two of the mentioned species. Where data for only one strain were available, the essentiality test results from *Bacillus subtilis* were also considered. It is not yet known whether all of these genes are essential in all of the listed organisms. Thus, the number of targets will probably decrease. Genome comparison for counting the genes was performed using the Phylosopher System (Genedata).

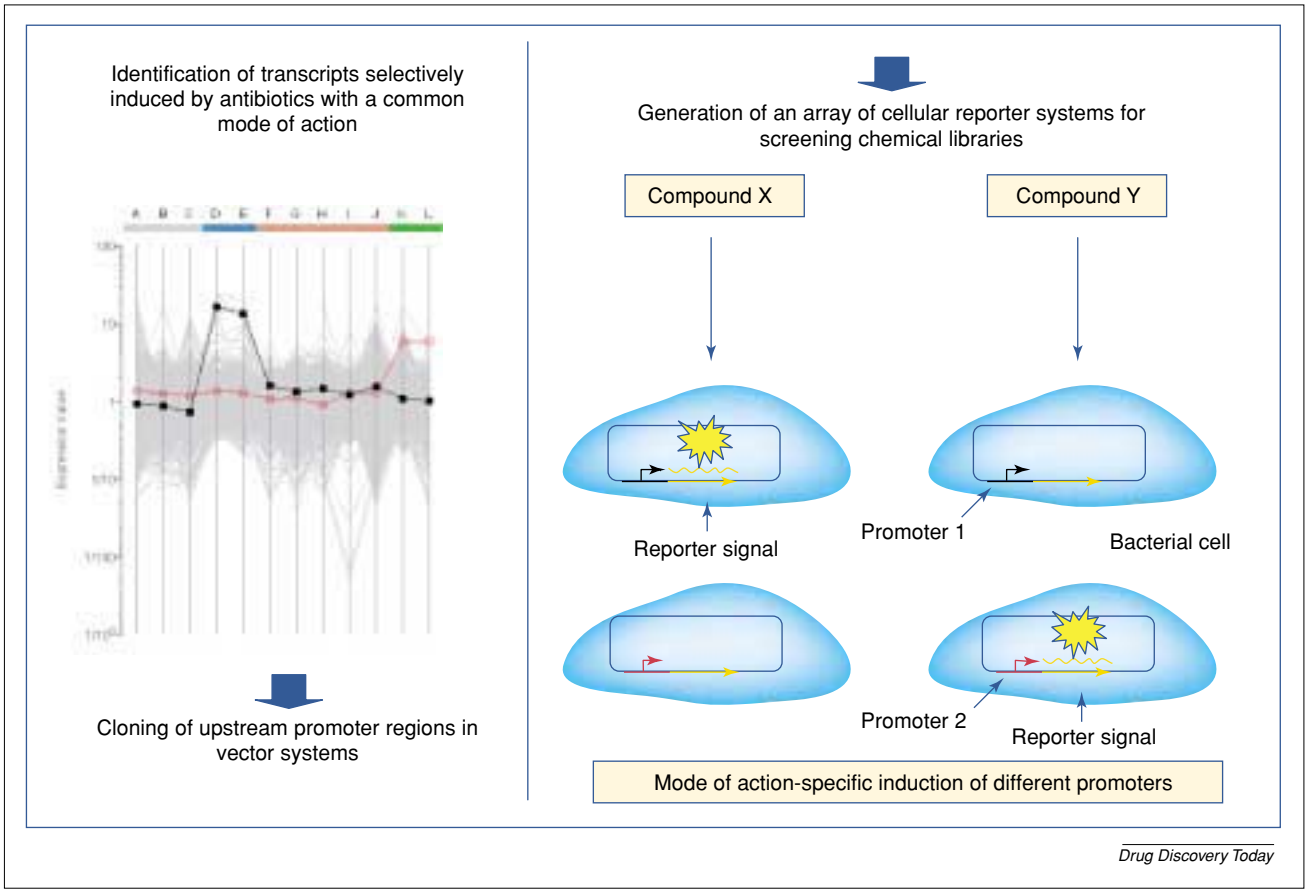

FIGURE 2

Strategy for the design of promoter induction assays based on expression profiling. Transcriptional expression profiles of all genes of a bacterial genome are represented by gray lines. In the presence of several different antibiotics (A–L with color-coded MOA), genes selectively responding to a specific type of growth inhibition can be identified. For example, one transcript is only induced by treatment with compounds D and E (black line), whereas another transcript is selectively induced by compounds K and L (red line). Upstream regions of the corresponding genes are cloned in front of reporter genes, enabling the detection of compounds with MOAs similar to D and E or K and L, respectively. Arrays of promoter induction systems represent helpful tools for the discovery of novel drug candidates.

the low sensitivity of overexpression assays and high amounts of compound needed for testing make such tests unsuitable for screening approaches.

Promoter induction assays complement cell-based MOA characterization of antimicrobials. Some studies have already been published describing the coupling of promoters to reporter genes to measure their specific response to particular types of antibiotic stress [34–38]. Large-scale expression profiling techniques, such as transcriptome or proteome analysis, now aid in the identification and characterization of regulatory networks responsive to antibiotics. Such holistic studies of gene expression during antibiotic treatment also provide a knowledge base for the identification of optimal promoters for the construction of pathway-specific reporter assays (Figure 2). Co-regulated genes and operons are generally controlled by the same transcriptional regulator, thus it is probable that they share common regulatory elements. The combination of DNA sequence motif detection algorithms and expression-based correlation analyses enables the systematic prediction of promoters controlling specific bacterial stress responses. For example, Fischer and co-workers [39] identified the promoter regulated by the fatty acid-phospholipid regulator FapR in *Bacillus subtilis*. The promoter selectively and strongly responds to inhibitors of FAB [39]. This promoter was fused to a reporter and a cell line constructed that was applied in HTS, leading to the identification of novel FAB inhibitors. Concomitantly, a panel of novel *B. subtilis* reporter strains indicative of various MOAs was published [40,41]. The promoters used for construction of these strains were also derived from antibiotics-triggered expression profiles of *B. subtilis*. Whole-cell-based reporter assays have limitations, particularly as a result of the limited concentration window in which compounds might be detected as inducing agents. In addition, the subsequent identification of the precise target for inducing agents requires considerable effort. Nevertheless, such assays are elegant tools to detect bioactive compounds interfering with specific pathways.

Functional genomics for the study of bacterial physiology and pathogenicity

Global expression profiling techniques hold the power to analyze bacterial physiology holistically as never before.

TABLE 3

Examples of transcriptomic or proteomic expression profiling in the study of bacterial infection[a]

Pathogen	Infection model, culture condition and sample	Type of model	Functional genomics technique	Refs
Helicobacter pylori	Bacteria grown on serum plates	*In vitro*	Proteomics	[65]
Mycobacterium tuberculosis	Nutrient starvation to induce growth arrest	*In vitro*	Transcriptomics and proteomics	[66]
	Human macrophage cell line THP1	*In vitro* Intracellular	Transcriptomics and proteomics	[67]
Salmonella enterica sv. Typhimurium	Murine macrophage-like cell line J774-A.1	*In vitro* Intracellular	Transcriptomics	[68]
Chlamydia pneumoniae	Human larynx cell line hep-2	*In vitro* Intracellular	Proteomics	[69]
Vibrio cholerae	Human cholera stool	*In vivo*	Transcriptomics	[70]
Borrelia burgdorferi	Rat peritoneum	*In vivo*	Transcriptomics	[71]
Helicobacter heilmannii	Murine stomach	*In vivo*	Transcriptomics	[72]
Uropathogenic *Escherichia coli*	Murine bladder	*In vivo*	Transcriptomics	[73]
Pseudomonas aeruginosa	Biofilm on granite pebbles	*In vitro*	Transcriptomics	[74]
	Murine cornea	*In vivo*	Transcriptomics	[75]

[a]Because selective recovery of the pathogen of interest and sufficient amounts of bacterial RNA or protein from the infected tissue is technically highly challenging and requires elaborate cell lysis, extraction and labeling conditions, most reports rely on *in vitro*-cultivated bacteria grown in minimal media, biofilms or during nutrient starvation to mimic the conditions encountered in the host environment. Also, first reports on the transcriptional status of *in vivo*-derived bacteria are appearing, as well as proteomic studies of intracellular pathogens recovered from infected cells. Some researchers took advantage of samples that are free of host cells and commensal flora, such as from the stomach or from cholera stools.

Whereas the genome provides the static blueprint of all properties that a cell is able to develop, the transcriptome and proteome are highly dynamic and ideally suited to capture snapshots of bacterial physiology when challenged by environmental changes, including external stress or antibiotic treatment. The ordered arrangement of a DNA microarray enables direct quantification of the mRNA of the respective genes. For proteome analysis by 2D gel electrophoresis, a virtual protein map has to be assembled in a first step that links the identity of each protein (as determined by mass spectrometry) to the spot position on the 2D gel [42]. The expression profiles derived by both complementary techniques disclose the molecular details of the bacterial adaptive response, such as the underlying signal transduction cascade, the regulatory network and the metabolic pathways that affect the new phenotype. *B. subtilis* and *E. coli* have, over the years, advanced to be the model organisms for Gram-positive and Gram-negative bacteria, respectively, and are thus far the leading objects for expression profiling of bacterial physiology. Detailed proteomic and transcriptomic studies have been performed for *B. subtilis* (e.g. during phosphate starvation, glucose and amino acid limitation, heat, ethanol, acid and oxidative stress [43–48]) and similar analyses were reported for *E. coli* [49–51].

In addition to providing insight into bacterial physiology in general, genomics has largely increased understanding of the molecular basis of bacterial pathogenicity. The number of commercially available microarrays of pathogenic bacteria is constantly increasing, as is the collection of protein maps. A comprehensive and up-to-date list of available bacterial proteomes has been compiled (e.g. in the Integr8 database at the European Molecular Biology Laboratory; www.ebi.ac.uk/proteome). Furthermore, a substantial number of microarray and proteome studies have identified gene regulation and cross-talk between pathogen and host in the course of the infection process (Table 3). Although theses studies do not directly apply proteomics or transcriptomics to the antibacterial drug discovery process, they are nonetheless important contributions, because they increase molecular understanding of bacterial physiology and virulence. In the long term, such knowledge provides the basis for the identification of pathogen-specific diagnostic markers [52] as a crucial prerequisite for the application of small-spectrum antibiotics. In addition, such knowledge forms the basis for the rational design of molecularly characterized vaccines to substitute current poorly defined vaccines still relying on killed or life-attenuated pathogens, or extracts thereof, or toxins detoxified by chemical treatment [53–55].

Bacterial expression profiling for determination and validation of mode of action of a drug

Transcriptome and proteome analysis were widely applied in the study of the physiological response of bacteria to different environmental conditions and have proved extremely useful in the investigation of the underlying network of adaptive responses. Such studies have demonstrated that a group of proteins, coined the 'general stress proteins', is induced rather nonspecifically by multiple stress factors, whereas others are expressed selectively to meet the requirements of a specific type of external challenge [45]. For example, heat or ethanol induce the heat-shock proteins, amino acid starvation triggers the stringent

response or DNA damage initiates the SOS response [43,44,56]. Such subsets of mRNAs or proteins, the expression levels of which are characteristic of a defined growth condition, form a designative transcriptomic or proteomic signature. Similarly, stress caused by antibiotics also induces characteristic transcriptomic or proteomic responses, which can be exploited for MOA studies in antibacterial drug discovery. Central to this idea is the observation that antibiotics with different MOAs induce discriminative expression profiles and that the signatures obtained with well-known antibiotics are references for comparison with the profiles induced by a novel antibacterial agent of interest. Published bacterial expression profiles prove that the effects observed on the transcriptional or translational level are indeed related to the cellular function targeted by the respective antibiotics. For example, inhibitors of translational peptidyl transfer (e.g. tetracycline, chloramphenicol, erythromycin or fusidic acid) lead to the increased expression of ribosomal proteins and elongation factors. In addition, antibiotics that cause mistranslation or abort translation (e.g. aminoglycosides and puromycin), which results in the production of defective proteins, induce chaperones and proteases. Furthermore, inhibitors that interfere with tRNA charging (e.g. mupirocin) trigger the stringent response [56]. These examples demonstrate that it is not only possible to assign an antibiotic to inhibition of a global metabolic area, such as protein synthesis, but that further subclassification is feasible, providing first hints of the molecular target. A comprehensive list of published proteomic and transcriptomic studies for MOA determination has been produced [57].

Two recent reports [56,58] describe the effects of a broad set of reference antibiotics on a single model organism under standardized assay conditions, thereby establishing reference compendia suitable for comparison with the profiles of novel antibacterial agents with unknown function. Bandow *et al.* [56] investigated the effects of 30 antibacterial compounds and Hutter *et al.* [58] investigated the effects of 37 antibacterial compounds on the *B. subtilis* proteome and transcriptome, respectively. As a proof-of-principle, the MOA of the novel natural-product-derived pyrimidinone antibiotic BAY502369 was correctly predicted as peptidyltransferase inhibition. In addition, the novel class of phenyl-thiazolylurea-sulfonamides, which originates from a lead optimization program on a screening hit from a biochemical target assay, triggered the increased expression of the direct target phenylalanyl-tRNA synthetase, as well as the stringent response on transcriptome and proteome level [59,60]. Analysis of the signatures of the reference antibiotics revealed that the target is often among the upregulated genes and that many of the changes are consistent with established knowledge. However, the expression profiles are complex and many genes not directly related to the function of the target are also affected, which obscures the primary target-related effect. Therefore, it is essential that as many diverse expression profiles as possible are included in such a reference compendium to raise the chance that the profile obtained with a novel compound has its match among the reference set. To validate a completely novel mechanism, it might be necessary to resort to conditional mutants downregulating the desired target. A successful example is the application of a *B. subtilis* mutant downregulating the peptide deformylase. The proteome profile of this mutant correlated nicely with the profile of the wild-type treated with the deformylase inhibitor actinonin [61]. In addition, the target of moiramide B was recently identified as bacterial acetyl-CoA carboxylase (ACC) on the basis of a reference compendium of transcriptome profiles, including a mutant downregulating subunits of the ACC [59,62].

There have been debates as to whether proteome or transcriptome analysis is more relevant for drug discovery purposes. Supporters of proteomics argue that RNA abundance alone does not reflect the protein content, which underlies co-translational, post-translational and degradative modification. By contrast, DNA microarrays yield data on the full genomic equivalent of mRNAs in a single experiment, whereas all currently available proteomics techniques visualize only a subset of the proteome. In our experience, the results obtained by the two techniques complement each other and provide a deeper biological understanding of the action of a compound and the physiological response of a bacterium, if applied in combination.

Conclusions

Microbial genomics has significantly changed the strategies employed for antibacterial drug discovery over the past years. Genomes of dozens of pathogenic bacteria have been analyzed and hundreds of genes evaluated for their potential as novel drug targets. Functional genomics techniques are now in place at various stages of the early drug discovery process and have proven highly successful for *in vitro* target validation and determination of the MOA of novel antibacterial agents. Genetic tools have evolved for a variety of bacterial species to make gene disruption comparatively easy. Substantial technical progress in gene chip production, 2D gel electrophoresis, mass spectrometry and data analysis have largely improved the reproducibility and throughput of transcriptome and proteome analysis. Although direct application of transcriptome and proteome expression profiling for the antibacterial drug discovery process is still in its early days, first reports are emerging, where broad reference datasets have been generated and used for rapid MOA predictions of novel antibacterial agents [39,56,58].

By contrast, it is obvious that, despite significant efforts, only few genomics-derived compounds are currently in clinical development or in later preclinical stages. The reasons why many target-based screening approaches have failed to produce good leads are multifarious [4,25,63,64]. Important issues are that: (i) high-throughput assays based

on purified enzymes yielded hits that often lacked cell penetration; (ii) the majority of compounds in large synthetic libraries were often too hydrophobic or too simple in structure to provide a good starting point for antibiotics; and (iii) target screenability, according to HTS criteria, was sometimes more important than target quality, particularly at the beginning of the genomics and HTS era. However, it should be kept in mind that the target-based approach results in leads that are positioned *a priori* early in the drug discovery pipeline and that functional genomics has been tremendously helpful for rapid assessment of their potential at this early phase. Obstacles that have to be overcome in downstream profiling (e.g. cell penetration, efflux, pharmacokinetic stability and physicochemical profile) are not addressed by functional genomics and, here, the drop-out risk remains undiminished. In addition, it is important to note that the application of functional genomics is not restricted to the target-based approach but also supports the traditional screening strategy by *de novo* MOA predictions for compounds and natural products with interesting antibacterial activity and unknown targets. In summary, there is still good reason to believe that a strategy combining the traditional and genomics-based approach, together with the battery of available novel techniques, will result in the discovery of the new antibacterial classes that are so urgently needed to cope with bacterial resistance development.

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