The impact of expression profiling technologies on antimicrobial target identification and validation ▼

The main objective of anti-infectives research is to develop chemical compounds with a defined antibiotic activity profile, and a low risk for side effects. To identify novel structural classes of antibiotics, many pharmaceutical companies have recently implemented target-based technologies[1,2]. Since the publication of the complete genome sequences of many pharmaceutically relevant pathogens, there has been an increase in the use of advanced gene-expression technologies[3]. The investigation of the complete transcriptome of a pathogen is now possible with the availability of whole genome microarrays, oligo DNA chips and nylon filter technologies for several pathogenic bacteria[4].

From the perspective of anti-microbial drug discovery, three applications of bacterial expression-profiling technologies seem to be particularly promising: (1) mRNA-profile based target identification; (2) mode-of-action (MOA) investigations of small inhibitor molecules, used to prioritize chemical lead development; and (3) developing novel types of whole-cell assays, to directly screen thousands of potential targets in parallel.

Certainly, the most obvious application of mRNA profiling is to obtain information about the function of previously uncharacterized genes. Co-expression of certain groups of genes indicate that the corresponding gene products interact together in some way, be it as part of a heterologous complex or within the same biochemical pathway. Therefore, automated correlation studies provide a systematic approach for identifying additional targets in essential cellular functions, enabling the development of novel target-based assays.

Drug Discovery Today

Figure 1. An analysis of the global transcriptional response of *Escherichia coli* to two antibiotics, based on two different mechanisms-of-action. The first row shows the physical genome map of *E. coli*, whereas the next four rows correspond to the first compound (CAA), a DNA biosynthesis inhibitor, and the last three rows represent the mRNA profiles induced by exposure to a protein biosynthesis inhibitor (RB). Red fields and green fields indicate upregulated and downregulated genes, respectively; black represents a non-responsive behaviour. The characteristic stress-induced operon activation and repression patterns are clearly visible. Integrated bioinformatics systems such as GeneData's GD Expressionist™ and GD Phylosopher™ enable an automated classification of the induced mRNA patterns according to the underlying mechanism of action. Moreover, marker promoters can be identified that enable the development of cell-based assays. As an example, the genetic regulatory elements controlling the purin biosynthesis pathway are shown as red flags on the physical genome map. Note that the location of the upstream regulatory elements correlate perfectly with the expression patterns for the two distinct inhibitory mechanisms.

The second major application of whole-genome expression profiling technologies is the elucidation of cellular responses to treatments with small-molecule inhibitors[5]. Certain patterns of the induced mRNA profiles are characteristic for different types of stress, reflecting the underlying MOA of the inhibitor. A reference compendium approach can be used to classify the MOAs of novel structural classes of antibiotics. Exhaustive reference compendia databases combined with powerful pattern-recognition algorithms are already used for automated MOA predictions. In particular, this approach allows the screening of large libraries of natural compounds for molecules that exhibit antibacterial potential. A multi-level MOA classification even enables the identification of the actual targets by analyzing time and concentration series measurements to separate primary from secondary responses. Based on this kind of evaluation, a compound's potential as a development candidate can be assessed.

Understanding the underlying regulatory modes governing the stress behaviour of pathogens is the next step to be taken. Co-regulated genes and operons representing stress-induced pathways are likely to share common regulatory elements in their genomic upstream regions (e.g. transcription-factor binding sites). A combined approach of DNA sequence-motif detection algorithms and transcriptome correlation analyses enables the identification of common upstream motifs controlling a pathogen's stress response (Fig. 1). The knowledge of stress-specific marker promoters can then be used to develop cell-based

1359-6446/01/$ – see front matter PII: S1359-6446(01)02047-5

assays, using genetically modified cells by coupling marker promoters to reporter genes. These assays enable completely novel MOA-specific screening strategies, which are capable of evaluating thousands of targets at the same time.

It has to be emphasized that the bottleneck for all three strategies lies in the data analysis process, whereas the actual expression data acquisition process no longer seems to be the difficult step. Computational platforms for a thorough data analysis of large-scale expression-profiling series are needed to extract the biologically relevant information. The combined analysis of genome and transcriptome data will significantly streamline drug discovery at all development phases by bridging the gap between biology and chemistry.

References

1 Freiberg, C. (2001) Novel computational methods in anti-microbial target identification. *Drug Discov. Today* 6 (Suppl.), S72–S80

2 Moir, D.T. *et al.* (1999) Genomics and antimicrobial drug discovery. *Antimicrob. Agents Chemother.* 43, 439–446

3 Rosamond, R. and Allsop, A. (2000) Harnessing the power of the genome in the search for new antibiotics. *Science* 287, 1973–1976

4 Wilson, M. *et al.* (1999) Exploring drug-induced alterations in gene expression in *Mycobacterium tuberculosis* by microarray hybridization. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12833–12838

5 Gmuender, H. *et al.* (2001) Gene expression changes triggered by exposure of *H. influenzae* to Novobiocin or Ciprofloxacin: Combined transcription and translation analysis. *Genome Res.* 11, 28–42

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