7. C. J. Sherr, *Science* **274**, 1672 (1996).
8. G. I. Shapiro and J. W. Harper, *J. Clin. Invest.* **104**, 1645 (1999).
9. W. C. Hahn *et al.*, *Nature Med.* **5**, 1164 (1999).
10. B. S. Herbert *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14276 (1999).
11. G. Neufeld, T. Cohen, S. Gengrinovitch, Z. Poltorak, *FASEB J.* **13**, 9 (1999).
12. T. de Lange and T. Jacks, *Cell* **98**, 273 (1999).
13. J. Holash, S. J. Wiegand, G. D. Yancopoulos, *Oncogene* **18**, 5356 (1999).
14. J. B. Gibbs and A. Oliff, *Cell* **79**, 193 (1994).
15. T. K. Sawyer, *Biopolymers* **47**, 243 (1998).
16. J. M. Hamby and H. D. H. Showalter, *Pharmacol. Ther.* **82**, 169 (1999).
17. B. P. Eliceiri and D. A. Cheresh, *J. Clin. Invest.* **103**, 1227 (1999).
18. D. C. Heimbrook, A. Oliff, J. B. Gibbs, in (*6*), pp. 35–45.
19. L. H. Hartwell, P. Szankasi, C. J. Roberts, A. W. Murray, S. H. Friend, *Science* **278**, 1064 (1997).
20. M. J. Marton *et al.*, *Nature Med.* **4**, 1293 (1998).
21. D. Fambrough, K. McClure, A. Kazlauskas, E. S. Lander, *Cell* **97**, 727 (1999).
22. J. S. Lanni, S. W. Lowe, E. J. Licitra, J. O. Liu, T. Jacks, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9679 (1997).
23. P. K. Sorger, M. Dobles, R. Tournebize, A. A. Hyman, *Curr. Opin. Cell Biol.* **9**, 807 (1997).
24. C. L. Vogel and J. M. Nabholtz, *Oncologist* **4**, 17 (1999).
25. I. Ojima *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4256 (1999).
26. T. U. Mayer *et al.*, *Science* **286**, 971 (1999).
27. V. C. Jordan, *Trends Endocrinol. Metab.* **10**, 312 (1999).
28. A. S. Levenson and V. C. Jordan, *Eur. J. Cancer* **35**, 1628 (1999).
29. D. P. McDonnell, *Trends Endocrinol. Metab.* **10**, 301 (1999).
30. M. M. Goldenberg, *Clin. Ther.* **21**, 309 (1999).
31. J. Baselga, L. Norton, J. Albanell, Y. M. Kim, J. Mendelsohn, *Cancer Res.* **58**, 2825 (1998).
32. M. X. Sliwkowski *et al.*, *Semin. Oncol.* **26**, 60 (1999).
33. S. Shak, *Semin. Oncol.* **26**, 71 (1999).
34. M. S. Ewer, H. R. Gibbs, J. Swafford, R. S. Benjamin, *Semin. Oncol.* **26**, 96 (1999).
35. B. J. Druker and N. B. Lydon, *J. Clin. Invest.* **105**, 3 (2000).
36. J. D. Moyer *et al.*, *Cancer Res.* **57**, 4838 (1997).
37. D. H. Boschelli, *Drugs Future* **24**, 515 (1999).
38. J. B. Gibbs, *J. Clin. Invest.* **105**, 9 (2000).
39. A. Gescher, *Gen. Pharmacol.* **31**, 721 (1998).
40. A. Oliff, *Biochim. Biophys. Acta* **1423**, C19 (1999).
41. T. M. Williams, *Expert Opin. Ther. Patents* **9**, 1263 (1999).
42. D. M. Leonard and J. S. Sebolt-Leopold, *Drugs Future* **24**, 1099 (1999).
43. R. P. Beckett and M. Whittaker, *Experts Opin. Ther. Patents* **8**, 259 (1998).
44. Z. Fan and J. Mendelsohn, *Curr. Opin. Oncol.* **10**, 67 (1998).
45. E. Keshet and S. A. Ben-Sasson, *J. Clin. Invest.* **104**, 1497 (1999).
46. J. T. Holmlund, B. P. Monia, T. J. Kwoh, F. A. Dorr, *Curr. Opin. Mol. Ther.* **1**, 372 (1999).
47. F. E. Cotter, *Semin. Hematol.* **36**, 9 (1999).
48. W. R. Sellers and D. E. Fisher, *J. Clin. Invest.* **104**, 1655 (1999).
49. F. McCormick, *Cancer J. Sci. Am.* **5**, 139 (1999).
50. C. Heise *et al.*, *Nature Med.* **3**, 639 (1997).
51. B. A. Foster, H. A. Coffey, M. J. Morin, F. Rastinejad, *Science* **286**, 2507 (1999).
52. G. J. Kelloff, C. C. Sigman, P. Greenwald, *Eur. J. Cancer* **35**, 1755 (1999).

53. I thank my colleagues at Merck for stimulating discussions. In addition, S. Yao, G. Rodan, and N. Kohl provided helpful comments to the manuscript and J. Campbell skillfully prepared Fig. 1.

REVIEW

Harnessing the Power of the Genome in the Search for New Antibiotics

John Rosamond and Aileen Allsop*

Over the past 40 years, the search for new antibiotics has been largely restricted to well-known compound classes active against a standard set of drug targets. Although many effective compounds have been discovered, insufficient chemical variability has been generated to prevent a serious escalation in clinical resistance. Recent advances in genomics have provided an opportunity to expand the range of potential drug targets and have facilitated a fundamental shift from direct antimicrobial screening programs toward rational target-based strategies. The application of genome-based technologies such as expression profiling and proteomics will lead to further changes in the drug discovery paradigm by combining the strengths and advantages of both screening strategies in a single program.

The science of genomics has largely been driven by the desire to understand the organization and function of the human genome. However, determination and characterization of smaller, less complex genomes, notably bacteria and yeast, has preceded that of the human genome, providing a testing ground for high-throughout screening procedures. For example, the *Saccharomyces cerevisiae* genome project, which delivered the first complete eukaryotic genome with 16 chromosomes and about 6200 genes (*1*), provides a model for ways in which DNA sequence information can be used to direct the subsequent systematic study of biochemical and functional processes (*2, 3*). Furthermore, new approaches are being developed for extracting information concerning gene expression, protein levels, subcellular localization and functionality (*4, 5*), providing for the first time a "genome view" of how an organism grows, reproduces, and responds to its environment.

Many of the complete genomes determined so far are of microorganisms, and further microbial genomes are being sequenced (Fig. 1). Microbial genomics are already revolutionizing the pharmaceutical industry's capability for antimicrobial drug hunting—and none too soon. Most antibiotic drugs used today are derivatives of agents which have been in the clinic for more than 30 years (or even longer in nature as natural products). This in itself would not be a problem, were it not for the remarkable ability of microorganisms to evolve and adapt. The biggest threat is antibiotic resistance (*6–8*). This has always been an issue, but in the early years of penicillin use pathogens depended on a single resistance mechanism, whereas many strains found in the clinic today have acquired multiple systems to reduce or avoid the action of an antibiotic (*9, 10*). Most threatening of these are the mechanisms that involve changes in the target site for antibiotic interaction, conferring levels of resistance to all compounds with that same mechanism of action. Furthermore, the DNA coding for these processes can be transferred between related strains, and the short generation time of many microorganisms facilitates the opportunity for gene selection even during a short course of drug treatment (*11*).

Resistance is not the only problem, however. As clinical practice changes to encompass greater use of invasive procedures and patients live longer, more and more individuals are becoming dependent on adequate antimicrobial cover. This is particularly relevant in the case of immunocompromised patients, who may be infected even by normally nonpathogenic organisms. Unfortunately, the use of antibiotics can select for such infections which are not sensitive to standard therapies. For example, in Europe, 10% of infections in intensive therapy units involve *Acinetobacter sp.* highly resistant but previously rare pathogens (*12*). In this way not only is resistance escalating, but also a new range of organisms have to be considered as potential pathogens.

There is therefore a need for a range of new drugs with new mechanisms of action, not susceptible to existing resistance mechanisms and in sufficient numbers to reduce reliance on a small number of chemical classes. Almost all antimicrobial compounds in the clinic today have come from semi-rational optimization programs based on compounds, often natural products, identified by whole-cell, antimicrobi-

AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK.

*To whom correspondence should be addressed. E-mail: Aileen.Allsop@AstraZeneca.com

al screening. In the past 10 years, pharmaceutical research has concentrated on more selective approaches in searching for novel antibiotics, using target-based technology. This involves screening for inhibitors of a specific biochemical reaction or intermolecular interaction (the drug target) as a means of identifying compounds with pharmaceutical potential. Antimicrobial targets therefore should be required for at least microbial growth and preferably cell survival.

The overall advantages of the target-based strategies are obvious (Table 1), and target selection can be greatly enhanced by the information generated from microbial genomics. Furthermore the latest technologies based upon genomic information are creating new opportunities in the search for more effective antibiotic drugs, for example gene expression analysis is currently being used in two separate areas: (i) investigation of the importance of specific genes in defined tissues and organs during the course of a real infection (rather than artificial, laboratory conditions) and (ii) functional analysis, which involves exploring the consequences of impairing specific functions, in terms of expression of other genes.

The desire to identify genes associated with pathogenic processes has resulted in the development of at least three different approaches—IVET, DFI, and STM—which have all been used in this context (*13*) because they each provide a different quality of information. IVET (in vivo expression technology) is designed to identify genes specifically induced during an infection and can be used to generate temporal information (*14*); DFI (differential fluoresence induction) uncouples metabolic requirements from selection parameters, thus focusing on infection specific processes, whereas STM (signature-tagged mutagenesis) identifies genes required for the establishment and maintenance of an infection (*15*). In addition isolating bacterial mRNA sequences directly from infected tissues and amplifying them by RT-PCR is proving a useful tool in understanding bacterial gene expression during a real infections (*16*).

All mRNA-based approaches suffer from the well-known technical problems associated with isolating these molecules from bacteria, but a number of groups have solved these difficulties sufficiently to determine meaningful information (*17*). However the importance of pathogenicity data to the drug-hunting process remains to be established. Such information may certainly highlight new mechanisms by which bacteria react to their host, but it remains to be shown that such mechanisms can be developed into viable therapeutic intervention strategies.

There are some early examples for the use of gene expression profiling (microarray) in microbes which suggest that this application can be used to complement the search for antibiotics (*17*). In particular, the ability to investigate the mechanism by which a compound kills a cell and to compare this data with the effects of mutation in target genes could prove extremely valuable, as will be discussed later.

Having selected a target, whether essential to cell survival or important for establishing pathogenic growth, the next stage of the process is to identify compounds. Drug-hunting usually involves high through-put screening of compound banks, analysis of hits and rapid expansion of active chemical series to establish true lead compounds with antimicrobial activity (Fig. 2). Alternatively, rational design guided by structural information can lead to the same outcome. Initial exploration of chemical space is focused on increasing potency, as measured by target interaction. Lead optimization then methodically selects for improvements in drug-associated properties, such as antimicrobial activity, spectrum, bioavailability, and pharmacokinetics, until development candidates are identified. It is particularly important that from the outset that the relationship between inhibition of the biochemical target and antimicrobial action is established, if these properties are not related optimization can easily lead to toxic or ineffective compounds. Even with state-of-the-

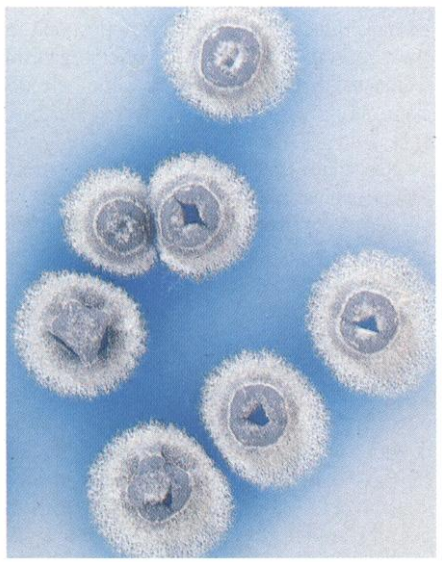

Fig. 1. *Streptomyces coelicolor* colonies with aerial mycelium and spores. A collaboration between the Sanger Centre and D. Hopwood of the John Innes Centre aims at sequencing the chromosome of this organism by 2001. The area of the picture represents about 2 cm by 3 cm. The blue haloes around the colonies are secreted actinorhodin, an antibiotic not used clinically. Actinorhodin is blue under alkaline conditions and red under acidic conditions. It is a polyketide made by multiple condensations of acetate by a Type II polyketide synthase. Photo provided by D. Hopwood, John Innes Centre.

Table 1. Comparison of the screening strategies for novel antimicrobial compounds.

Whole-cell screening (looking directly for compounds which kill microorganisms)	Target-based screening (looking for biochemical inhibitors)
Advantages	
Selection for compounds which penetrate cells	More sensitive (can detect weak or poorly penetrating compounds suitable for chemical optimization)
Antimicrobial properties established	Easy screening
Highly reproducible	Different approach
Has been used successfully historically	Can target new areas of biology
	Facilitates rational drug design
Disadvantages	
Insensitive	Need to turn an in vitro inhibitor into an antibacterial drug (complicated by penetration issues)
Most active compounds are toxic	Genetic validation of targets (by gene knockout or reduced expression) can be misleading
No rational basis for compound optimization (target unknown)	
Mixed mechanisms of action	
In recent years has failed to deliver	

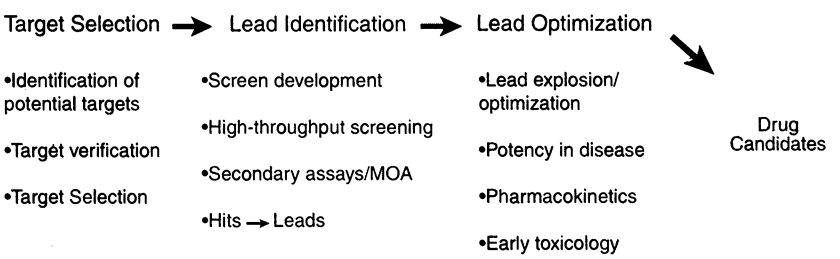

Fig. 2. The drug hunting process. MOA, mechanism of action.

art chemical technologies such as combinatorial chemistry or multiple parallel synthesis (*18*), chemical diversity cannot always deliver the desired compound profile and failure is a constant risk.

Microbial Genomics and Target Selection

Until recently, the major barrier to target-based screening was the small number of potential targets which was limited by the number of cloned and characterized genes. This barrier has in effect been removed as a consequence of the comprehensive genome sequencing projects. Since the publication of the complete yeast genome sequence (*1*), more than 20 other complete genome sequences have been determined, and all but two of these are genomes of model or pathogenic bacteria. Consequently, all genes in these organisms are now available as potential targets and can be prioritized on the basis of various user-defined criteria. For example, ideal antimicrobial targets should be essential to microbial cell survival, highly conserved in a range of pathogens, and absent or radically different in humans.

Usually, another essential requirement of a target is an understanding of the function of the gene product, ideally at the level of its biochemical activity. Genome sequence comparison using any one of a number of bioinformatic platforms conventionally allows the assignment of a function to a gene identified through DNA sequencing via similarity to a characterized protein, involving linear comparisons of DNA and protein sequences (*19*). This has several limitations, most notably the inability to assign function to proteins that lack an obvious homolog, and establishing the functional relationship between weakly related proteins. Consequently, a significant proportion of each complete genome is functionally unannotated.

Recently however, two new computational methods have been described that infer protein function on the basis of properties other than amino-acid sequence similarity (*20, 21*). These methods make use of both theoretical prediction (phylogeny and domain fusion) and experimental data (expression profiles) to identify functional linkages between proteins. The usefulness of these approaches has been demonstrated by the assignment of a general function to about half of the 2500 uncharacterized yeast proteins. The use of domain fusion analysis alone, however, in analysis of bacterial genomes may be more limited. The number and accuracy of these methods will be improved as further complete genome sequences are released. Ultimately, these methods will be superseded by direct comparison of three-dimensional (3D) protein structures, because the function of a protein is more directly a consequence of its shape than its sequence. Although relatively few structures are currently available, the rate of structure determination has increased dramatically, and current structural genomics projects will impact significantly in this area by providing sufficient information to allow most other protein sequences to be modeled accurately (*22*).

A computational process similar to that used for the annotation of genome sequences by simultaneous comparison can be used to identify candidate targets within the genome and prioritize them for antimicrobial screening. For broad-spectrum agents, for example, bioinformatic analysis of genome sequences can be used to identify proteins that are highly conserved in the appropriate range of pathogens associated with a particular clinical indication. This relies on the assumption that proteins with highly conserved amino-acid sequences will have more closely related 3D structures than will proteins with relatively dissimilar primary sequences. This becomes even more important when the same bioinformatic tools are used to identify highly conserved bacterial genes that lack a close human counterpart. Clearly, such a comparison will be incomplete until a full human genome sequence is available, but the current, rapidly expanding human sequence databases already provide valuable analyses. Comparative genomics therefore provides, through simple computational analysis, a list of potential targets with useful bacterial spectrum and possible selectivity over humans (Fig. 3) (*23*).

In addition to highly conserved targets which offer the opportunity to develop broad spectrum antibiotics, comparison of microbial genome sequences has also shown that a significant proportion of each genome encodes proteins that are functionally unknown, some of which are specific to that organism (*1*). These provide the opportunity to develop antibiotics with a high degree of specificity for a single organism (or a small set of related bacterial species) such as *Mycobacterium tuberculosis* or *Helicobacter pylori*. Such narrow specificity potentially offers long-term benefits by reducing problems arising from cross resistance. However, there are two significant limitations to this. First, most of these genes are unannotated and have no known or obvious function, making it difficult to progress target-based screens. Second, there are currently no rapid, accurate, and sensitive tools to identify the specific causative agent for many infections. However, organism-specific genes may not only provide the potential targets for novel therapeutic agents but also the principal components of rapid, PCR-based diagnostic tools (*24*). This potential will undoubtedly be realized in the near future.

Microbial Genomics and Drug Discovery

As well as defining the genetic complement of the cell, the complete genome sequence of an organism provides the opportunity to investigate the biology of that organism. Transcript profiling using DNA microarrays provides a rapid and systematic method for the high-throughput analysis of gene expression at the level of the whole genome (*25, 26*), providing a specific analysis of expression of each individual gene monitored by mRNA concentrations. From the perspective of drug discovery, the patterns generated from the parallel analysis of all genes in an organism using microarrays can give clues to the function of previously uncharacterized genes (target identification), as well as providing information about cellular responses to treatments with small inhibitor molecules (mechanism of action studies at all discovery phases). If microarrays can identify reproducible and statistically significant changes in global gene expression, this information would significantly streamline most phases of drug discovery and development. Several recent lines of evidence support the view that

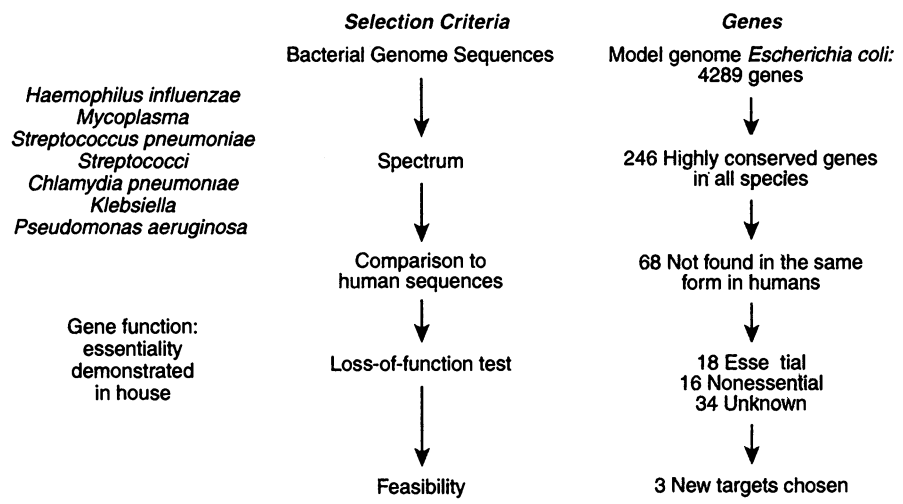

Fig. 3. An example of a target identification cascade for a respiratory tract antibacterial drug. Among the 18 known essential targets are the molecular targets of both the quinolone and macrolide antibiotics. These established drug classes are known to have the desired antimicrobial spectrum.

such gene expression profiles have a key role to play in antimicrobial drug discovery programs, although as yet there is still almost no published information in this area. In one study (*27*), two potent protein kinase inhibitors were analyzed for their effect on global gene expression in yeast by comparing the transcript profile of cells before and after treatment with the compounds. Although the two compounds were thought to act against the same target and showed similar in vitro activity, there were distinct and significant reproducible differences in the changes in gene expression induced by the two compounds, emphasizing the utility of microarray in evaluating the selectivity of drug candidates. In a separate study, yeast cells were treated with the immunosuppressive drug FK506 and a characteristic profile determined. A similar pattern of expression was seen using cells from which the FK506 target had been deleted, demonstrating that depletion of gene function by genetic or chemical treatment can produce the same effect on global gene expression (*28*). This suggests that genetic inhibition of gene function by mutation or deletion can be used as the "gold standard" marker for specific gene inhibition, and that expression patterns generated after treatment with small molecule inhibitors can be related to the "genetic patterns" to define or confirm the target and mechanism of action of the inhibitor. Furthermore, treating cells lacking the FK506 target with FK506 revealed apparent secondary targets for the drug, suggesting that microarrays can also be used to identify secondary (and potentially unwanted) mechanisms of action.

The potential of microarrays to define mechanisms of action is reinforced by our recent work and that of others on the sterol biosynthesis pathway in yeast. These experiments were set in this well-characterized system as a test of the technology on a genome-wide basis. Inhibition of sterol biosynthesis results in increased transcription of some of the genes in the pathway as a result of feedback inhibition (*29*). Using strains in which sterol biosynthesis is inhibited genetically through *ERG1* or *ERG11*, and chemically through treatment with terbinafine (which inhibits squalene monooxygenase, the product of *ERG1*) or fluconazole (which inhibits lanosterol 14α-demethylase, the *ERG11* product), we observed similar and expected changes in the expression of genes in the sterol pathway after all of the treatments. However, using clustering algorithms with the expression data for the whole genome in each case, we found that the pattern of expression observed after treatment with fluconazole most closely resembles that seen after *ERG11* inhibition, whereas the pattern seen after terbinafine treatment is most similar to that of *ERG1* inhibition (*30*). This result reinforces the value of expression data generated from cells that have been perturbed through genetic treatment, and implies that microarrays can not only confirm the mechanism of action of a compound but that their sensitivity may be sufficient to discriminate between compounds acting at different steps in the same metabolic pathway.

The importance of analysing expression data by clustering can be seen further from studies on co-regulated genes. Coexpression may indicate genes whose protein products interact together in a heterologous complex, or which act in concert in the same pathway without direct physical interaction. Transcriptional co-regulation can thus indicate physical or functional interactions between proteins, and associating the regulatory patterns of known proteins with those of unknown function can be used to suggest functional linkages for further analysis (*31, 32*).

Not all cellular processes are controlled at the level of gene expression, and protein profiling thus provides a complementary approach to the use of DNA microarrays. Proteomics (*33*) has progressed substantially from the simple concept of 2D gel electrophoresis, into a technology capable of investigating the total protein content of a cell and its response to changing conditions. The sensitivity and versatility of this approach is increasing as new electrospray mass spectrometry techniques improve resolution, identification, and quantification (*34*). Determination of a cellular response based upon proteomic analysis alone in microrganisms is, however, still problematic, because many regulatory proteins are only present in trace amounts and therefore difficult to identify in gel images (*35*).

All of the above technologies, alongside numerous other strategies, are potential tools in exploring the functionality associated with bacterial genes. An understanding of function is important to facilitate development of suitable screening assays. In time, however, the technology will develop such that screening compounds directly on the basis of transcript profiling, before the detailed biochemistry of a target has been defined, will soon become a reality.

Conclusions

Traditionally, antimicrobial drug discovery has relied upon random screening or semi-rational modification of known structural series. These strategies have failed to deliver sufficient molecular diversity to counteract the constant selection pressures within the clinic, resulting in substantial and increasing drug resistance. In recent years, target based drug discovery has allowed the pharmaceutical industry to focus screening activities into new mechanisms of action and new chemical classes. These activities, however, have been limited to individual examples of established microbial biochemistry and have carried a high risk of failure. Microbial genomics complements and extends the traditional genetic tools in providing, for the first time, a basis for rational drug-target discovery in anti-infectives.

The new technologies based upon microbial genomics not only provide the tools to drive target discovery beyond established biochemistry but also create the ability to compare targets for likely performance in a clinical situation. The technologies are not limited to target selection and are providing new approaches to support compound optimization into early drug development. This not only streamlines accurate compound evaluation but also converts random screening campaigns into rational compound optimizations, thus revolutionizing both antibacterial and antifungal drug screening. We can thus expect a whole variety of novel agents with new mechanisms of action, of unrelated structural classes, to be generated from increasingly efficient drug-hunting programs.

References and Notes

1. B. Dujon, *Trends Genet. Rev.* **12**, 263 (1996).
2. E. B. Basset Jr. *et al.*, *Curr. Opin. Genet. Dev.* **6**, 736 (1996).
3. K. Thomas, *FEBS Lett.* **396**, 1 (1996).
4. E. A. Winzeler and R. W. Davies, *Curr. Opin. Genet. Dev.* **7**, 771 (1997).
5. S. G. Oliver, *Curr. Opin. Genet. Dev.* **7**, 405 (1997).
6. R. N. Jones and M. A. Pfaller, *Diagn. Microbiol. Infect. Dis.* **31**, 379 (1998).
7. T. M. File, *Chest* **115**, 3S (1999).
8. U.N. World Health Organization, *WHO: The World Health Report* (WHO Publications, Geneva, 1996).
9. H. S. Gold and R. C. Moellering, *N. Engl. J. Med.* **335**, 1445 (1996).
10. P. M. Hawkey, *Brit. Med. J.* **317** (1998).
11. J. Davies, *Science* **264**, 375 (1994).
12. S. Brown, C. Banttar, H.-K. Young, S. G. B. Aymes, *Lancet* **351**, 186 (1998).
13. E. J. Strauss and S. Falkow, *Science* **276**, 707 (1997).
14. A. Camilli and J. J. Mekalanos, *Mol. Microbiol.* **18**, 671 (1995).
15. D. M. Heithoff, C. P. Conner, M. Mahan, *Trends Microbiol.* **5**, 509 (1997).
16. M. J. Browne, I. S. Gloger, J. E. Hodgson, J. H. Robinson, *Mol. Med. Today* **1**, 373 (1995).
17. M. Wison *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 12833 (1999).
18. S. L. Schreiber, *Science* **287**, 1964 (2000).
19. E. V. Koonin, R. L. Tatusov, M. Y. Galperin, *Curr. Opin. Struct. Biol.* **3**, 355 (1998).
20. E. M. Marcotte, M. Pellegrini, M. J. Thompson, T. O. Yeates, D. Eisenberg, *Nature* **402**, 83 (1999).
21. A. J. Enright, I. Iliopoulos, N. C. Kyprides, C. A. Ouzounis, *Nature* **402**, 86 (1999).
22. S. K. Burley *et al.*, *Nature Genet.* **23**, 151 (1999).
23. R Illingworth, P. Barth, N. Schnell, J. Rosamond, unpublished data.
24. S. M. Thomas and J. F. Burke, *Expert Opin. Ther. Patents* **8**, 503 (1998).
25. D. D. L. Bowtell, *Nature Genet.* **21**, 25 (1999).
26. P. O. Brown and D. Botstein, *Nature Genet.* **21**, 33 (1999).
27. N. S. Gray *et al.*, *Science* **281**, 533 (1998).
28. M. J. Marton *et al.*, *Nature Med.* **4**, 11 (1998).
29. G. Dixon, D. Scanlon, S. Cooper, P. Broad, *J. Steroid Biochem. Mol. Biol.* **62**, 165 (1997).
30. A. White, J. Chavda, N. Schnell, J. Rosamond, unpublished data.
31. D. E. Bassett Jr., M. B. Eisen, M. S. Boguski, *Nature Genet.* **21**, 51 (1999).
32. R. J. Cho *et al.*, *Mol. Cell* **2**, 65 (1998).
33. R. A. VanBogelen *et al.*, *Electrophoresis* **18**, 1243 (1997).
34. M. Mann and M. Wilm, *Trends Biochem. Sci.* **20**, 219 (1995).
35. C. P. Gray and W. Keck, *Expert Opin. Invest. Drugs* **7**, 147 (1998).