ORFs that are homologous across a very diverse range of species. Unassigned ORFs, that are specific to bacteria and demonstrated to be essential for infection, would comprise truly novel classes of antibiotic targets. Obviously, the major challenge in exploiting such targets would be the effective application of advanced computational and biochemical analyses to determine their precise cellular function.

Paralogous and orthologous genes

Second, sequence comparison methodologies often have difficulty sorting paralogous and orthologous gene relationships. Orthologous genes are those which are related by ancestry between species or organisms, while paralogous genes are related to genes in the same organism as well as genes in different organisms. At different cut-off values of similarity, automated procedures will find either orthologous or orthologous and paralogous members of a particular protein family. Many proteins have internal paralogous segments because they evolved through successive rounds of gene duplication, fusion and divergence, while some functionally distinct proteins may also share similar domains (i.e. GTP-binding proteins involved in translation [Cousineau, B. *et al. J. Mol. Evol.* (1997) 45, 661–670]. However, the levels of similarity between orthologous and paralogous proteins vary greatly between protein families making it difficult to develop generalized procedures for distinguishing between family members.

Horizontal gene transfer

Third, horizontal gene transfer between distantly related species can result in some very surprising species and gene relationships. Of course, horizontal gene transfer has long been recognized as playing a role in the proliferation of antibiotic resistance genes between bacteria. However, evolutionary biologists now believe that the eukaryotic genome itself has many genes obtained via horizontal gene transfer from bacteria [Reviewed by Brown, J.R. and Doolittle, W.F. *Microbiol. Mol. Biol. Rev.* (1997) 61, 456–502]. Some eukaryotic genes, and perhaps entire metabolic pathways, show greater similarity to bacterial versions than to archaebacterial homologs, the presumed prokaryotic progenitor of eukaryotes. The ramifications with respect to potential drug cytotoxicity are clear – it might be more difficult to find a specific antibacterial compound against a bacterial target that has a close human homolog.

Summary

Even discounting these exceptional cases, however, the prioritization of *S. aureus* and *S. pneumoniae* ORFs has proven to be very useful by eliminating hundreds of costly anti-infective 'dead-ends' while highlighting many targets that may have escaped the experienced researcher's intuition. To improve the historical description or evolutionary relationships of the proteins in the SB database, more sensitive tools are applied that incorporate more information inherent in each protein, such as conserved amino acid residues or conserved secondary and tertiary structural domains. Also, phylogenetic analyses are used to resolve evolutionary relationships among different species as well as to distinguish between levels of orthology and paralogy. However, these techniques are not yet automated and still rely heavily on the computational biologist's intuition.

Molecular evolution and anti-infectives research seem to be an unlikely winning combination in today's competitive pharmaceutical environment. In practice, however, the application of molecular evolutionary principles has helped to direct experimental efforts towards molecular targets that are more likely to be sustainable in the drug development pipeline. In addition, the methods of evolutionary biology and comparative genomics will continue to play an important role in expanding our general knowledge about the biology of pathogenic bacteria.

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High-throughput screening

Patent strategies

The need for pharmaceutical companies to launch new drugs more frequently and thus maintain a lead over competitors, requires R&D management to improve productivity and focus on more efficient means of rapidly identifying new drug candidates. This has led to more focused R&D portfolio strategies, the development of high-throughput screening (HTS) systems and more aggressive approaches to protection of intellectual property. The development of HTS systems for the selection of drug candidates has driven the establishment of an active patent literature in this field.

In a recent patent analysis Jakobsen, P.H., Kurtzhals, P. and Poulson, F. have reviewed patent strategies for HTS assays [*Exp. Opin. Ther. Patents* (1998) 8, 1157–1165]. These patents cover various aspects of HTS, including specific use of equipment and reagents, and assay design. From a biological perspective applications often focus on the use of specific protein/receptor interactions or other cellular effects in the assay. Other approaches include protecting the *in vitro* methods developed for screening factors that modulate gene expression, inhibit protein or mRNA biosynthesis or modulate intracellular transduction of an extracellular signal. In many case the patent applications contain broad functional claims that have yet to be legally challenged to establish their validity. There will be an increase in the filing of patent applications in this field. Given the broad diversity of the patent literature and the rapidity of developments within this field, it is incumbent on users of HTS systems to maintain an awareness of the developing HTS patent literature.

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