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Comparative Genomics of Microbial Drug Efflux Systems

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Abstract

The complete genome sequences of 36 microorganisms have now been published and this wealth of genome data has enabled the development of comparative genomic and functional genomic approaches to investigate the biology of these organisms. Comparative genomic analyses of membrane transport systems have revealed that transporter substrate specificities correlate with an organism's lifestyle. The types and numbers of predicted drug efflux systems vary dramatically amongst sequenced organisms. Microarray and gene knockout studies to date have suggested that predicted drug efflux genes often appear to be a) non-essential and b) expressed at detectable levels under standard laboratory growth conditions.

Introduction

The publication in 1995 of the first complete genome sequence of a free living organism, *Haemophilus influenzae*, by TIGR (Fleischmann *et al*., 1995) has led to a new "genomic " era in biology . As at 15 October 2000, the complete genome sequences of 36 microrganisms have been published (Figure 1). This includes the sequences of important model organisms such as *Escherichia coli*, *Bacillus subtilis* and *Saccharomyces cerivisiae*, important pathogens such as *Vibrio cholerae* and *Mycobacterium tuberculosis*, and archaea such as *Methanococcus jannaschii*. There are currently over 100 genome sequencing projects underway (http://www.tigr.org/tdb/mdb/mdb.html) and given the current accelerating rate of publication of genome sequences (Figure 1) it seems likely that several hundred additional microbial genomes will be completed in the next 5-10 years. In addition to microbial genome sequencing, draft/complete genome sequences of *Caenorhabditis elegans*, *Drosophila melanogaster* and *Arabidopsis thaliana* have been published (*C. elegans* Sequencing Consortium, 1998; Adams *et al*., 2000; *Arabidopsis* Genome Initiative, 2000), and a draft sequence of the human genome has been announced.

This is leading to an increasing "mountain" of genomic data that makes possible both comparative genomic analyses and has spurred the development of a range of post-genomic technologies (for review, see Nelson *et al*., 2000). These functional genomic technologies utilize high-throughput or large-scale approaches that investigate the roles of large numbers of genes or proteins in a systematic fashion, rather than more traditional approaches that investigate the role of a single gene or protein. These include microarray expression analyses (Wilson *et al*., 1999), proteomics using 2 dimensional gel electrophoresis and MALDI TOF (Matrix Assisted Desorption/Ionization-Time of Flight) Mass Spectrometry (Traini *et al.,* 1998), and large scale gene knockout or expression studies (Hutchison *et al.,* 1999).

Both comparative genomic/bioinformatic studies of genome data and functional genomic studies are still in their infancy, and have yet to reach their full potential, although they are already yielding novel findings. In this review, we present an overview of comparative genomic studies of microbial membrane transporters, with a focus on drug efflux systems. Additionally, we examine recent functional genomic studies for their relevance to membrane transport and drug efflux.

Comparative Genomic Analysis of Membrane Transport

Membrane transport is an essential physiological process in all living cells. Transport systems are responsible for the uptake of virtually all minerals, nutrients and vitamins and for the efflux of toxic compounds, end products of metabolism, and cell surface macromolecules. They also function in ion homeostasis, communication between cells and their environment and provide essential constituents of energy-generating systems. The importance of membrane transport systems is emphasized by the findings that transporter genes constitute between 5% and 12% of the total number of genes in each sequenced genome (Paulsen *et al.,* 2000).

The availability of complete genome data has enabled a systematic genome wide comparison of the transporter content of each sequenced organism (Paulsen *et al.,* 1998; 2000). A complete catalogue of predicted membrane transporters has been compiled for each completely sequenced organism, with the transporters classified by protein family and transporter type, and with predicted substrate specificities provided (http://www-biology.ucsd.edu/~ipaulsen/transport/). Phylogenetic analyses provided useful aid for prediction of substrate specificity, as transport proteins typically cluster phylogenetically according to function (Paulsen *et al.,* 1998). Transporter proteins were classified into families using the TC (transporter classification) system which has

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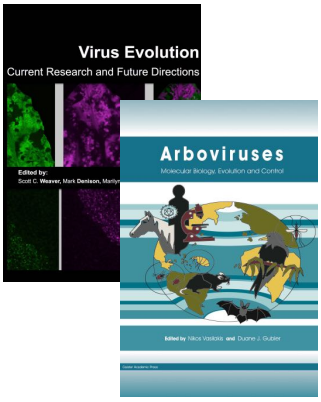

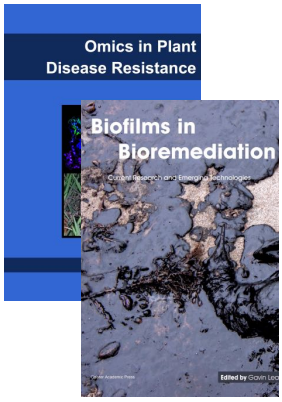

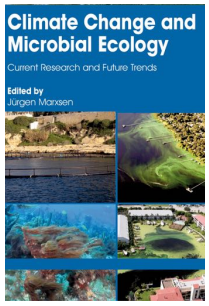

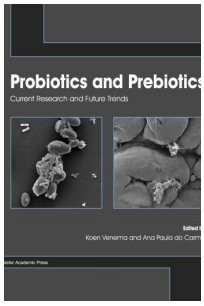

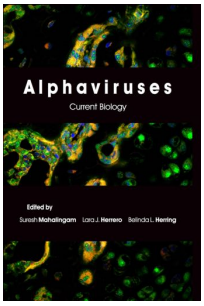

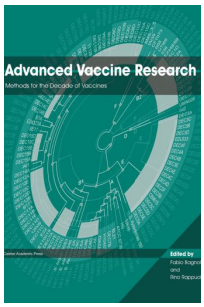

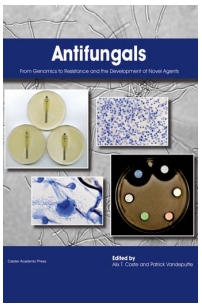

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been developed for transport proteins (Paulsen *et al.,* 1998; Saier, 2000; http://www-biology.ucsd.edu/~msaier/transport/). This classification system has defined over 150 families of membrane transport proteins.

The number of predicted cytoplasmic membrane transporters present in each sequenced microorganism is displayed in Figure 2A. The number of transport systems varied over fifteen-fold between different microorganisms, with organisms such as *E. coli*, *B. subtilis and Pseudomonas aeruginosa* possessing more than 250 transporters each. Figure 2B displays the number of transporters relative to the genome size of an organism. Most organisms cluster fairly close to the median of 3.1 transporter/100 kb, with exceptions including the model organisms *E. coli* and *B. subtilis*, which appear to possess more extensive transporter capabilities.

A wide variation was observed in the energy coupling mechanisms used to drive transport amongst the various microorganisms (Paulsen *et al.,* 2000). The mycoplasmas, spirochetes and *Thermotoga maritima* were highly dependent on ATP-dependent transport, probably due to the fact that these organisms lack a TCA cycle and an electron transfer chain, and hence can only generate a proton motive force by substrate-level phosphorylation. Photosynthetic organisms such as *Synechocystis* PCC6803 and *Chlorobium tepidum* are also reliant on ATP-dependent transporters, probably due to their ability to synthesize an ATP pool by photosynthesis. Other microorganisms examined were all highly reliant on secondary transporters. Thus, the bioenergetics of membrane transport in microorganisms appears to correlate very well with the overall bioenergetics of the organism.

A wide variation was also observed between the organisms analysed in terms of the overall substrate specificities of their transporters (Paulsen *et al.,* 2000). The archaea and obligate free-living bacteria all possessed a very high percentage of transporters for inorganic ions and a corresponding lack of transporters for organic nutrients. In contrast organisms with a heterotrophic lifestyle had a corresponding emphasis on transporters for organic carbon compounds. The intracellular parasites were deficient in transporters for sugars, but possessed an array of transporters for amino acids, nucleotides and other compounds typically found in an intracellular environment. Thus, the overall substrate specificities of an organism appear to reflect the diversity and concentration of substrates present in their respective environments.

Apart from overall comparisons between organisms, it also becomes possible to develop a detailed overall profile of the transport capabilities of an organism, and to cross correlate the transport capacity of an organism with its corresponding metabolic pathways. One example of such a detailed analysis is the incorporation of the available transporter information on *E. coli* into the EcoCyc database of *E. coli* metabolism (Karp *et al.,* 2000). This has enabled us to start to map the intersection of the set of starting reactants of all metabolic pathways with the complete inventory of compounds transported into the cell (Paulsen and Karp, unpublished data).

Families of Multidrug Transporters

Six cytoplasmic membrane transport protein families have been described that include multidrug efflux systems: the ATP binding cassette (ABC) superfamily (Saurin *et al.,* 1999), the major facilitator superfamily (MFS) (Pao *et al.,* 1998), the resistance/nodulation/cell division (RND) superfamily (Tseng *et al.,* 1999), the small multidrug resistance (SMR) family (Paulsen *et al.,* 1996), the multidrug and toxic compound extrusion (MATE) family (Brown *et al.,* 1999), and the multidrug endosomal transporter (MET) family. Examples of the first four families are discussed in detail in the remainder of this symposium, the MATE and the MET families have not yet been extensively characterized.

The ABC superfamily is a very large, ancient superfamily of ATP-dependent transporters, which currently consists of 52 sub-families. The ABC superfamily includes uptake or efflux systems for a range of substrates including drugs, sugars, amino acids, carboxylates, metal ions, peptides, etc. For more detail on ABC drug efflux systems, see the articles by Van Veen *et al.* and Rogers *et al.,* in this symposium.

The MFS superfamily is another very large, ancient superfamily, which includes proton, sodium ion and solute driven transporters, and currently consists of 30 distinct sub-families (Pao *et al.,* 1998). The MFS includes uptake or efflux systems for a range of substrates, including sugars, drugs, neurotransmitters, carboxylates, amino acids, osmolites, iron-siderophores, and nucleosides. Several sub-families have been implicated in drug efflux, in particular, the DHA12 and DHA14 families, which consist of proton-driven drug and multidrug efflux proteins. Members of these two families possess either twelve or fourteen transmembrane segments (TMS), respectively (Paulsen and Skurray, 1993), and include well-characterized members such as Bmr from *B. subtilis* and QacA from *Staphylococcus aureus* (see papers by Neyfakh, and Brown and Skurray in this symposium).

The RND superfamily consists of seven distinct families based on phylogeny, the majority of which consist of large proteins with twelve TMS (Tseng *et al.,* 1999). These families include efflux systems for drugs, metal ions, lipooligosaccharides, proteins, and glycolipids. Only one family in the RND superfamily has been demonstrated to include multidrug efflux transporters, examples include AcrB from *E. coli* and MexB from *P. aeruginosa* (see papers by Nikaido and Zgurskaya, and Poole in this symposium). These RND multidrug efflux pumps function via a drug:proton antiport mechanism and have a very broad substrate specificity. Some members of the MFS, ABC and RND families function with accessory proteins belonging to the membrane fusion protein (MFP) and outer membrane factor (OMF) families to enable efflux across both membranes of a Gram-negative bacterial cell envelope (Dinh *et al.,* 1994).

The SMR family consists of small bacterial proteins of approximately 100-110 amino acids with four TMS (Paulsen *et al.,* 1996). The best characterized members of this family are EmrE from *E. coli* and Smr from *S. aureus*. EmrE has been purified and reconstituted as a multidrug:proton antiporter (for more details, see paper by Schuldiner *et al.*

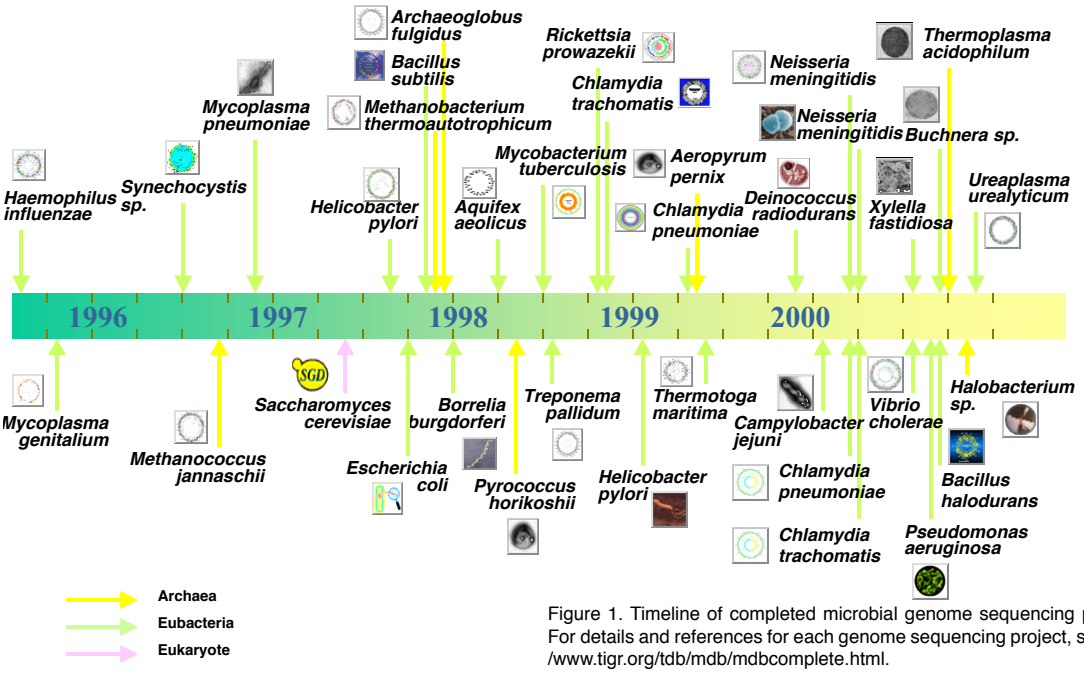

Figure 1. Timeline of completed microbial genome sequencing projects. For details and references for each genome sequencing project, see http://www.tigr.org/tdb/mdb/mdbcomplete.html.

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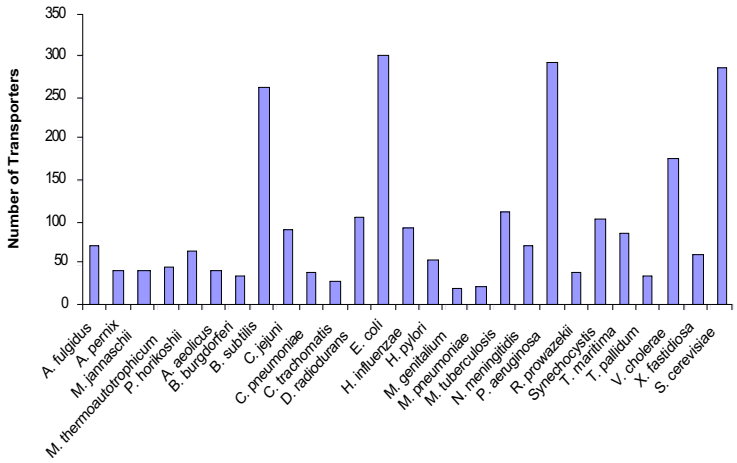

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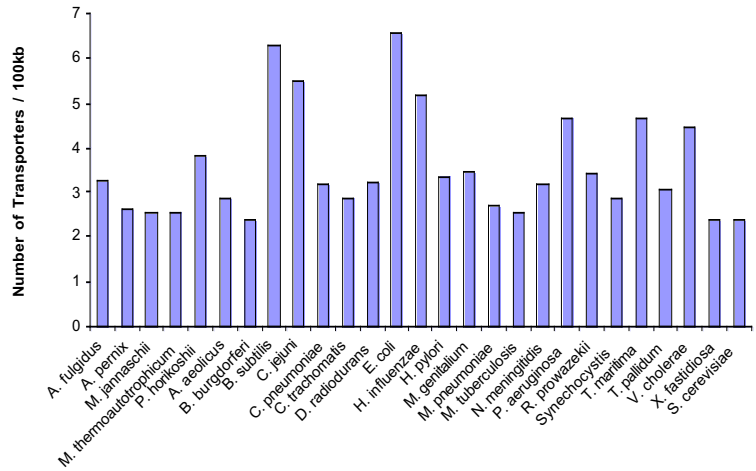

Figure 2. Number of predicted cytoplasmic membrane transporters (A) and number of such transporters per 100 kb of DNA (B) for each of the completely sequenced microorganisms. Transporters were predicted as described by Paulsen et al., 2000. A complete list of all of the transporters for these organisms are available at http://www-biology.ucsd.edu/~ipaulsen/transport.

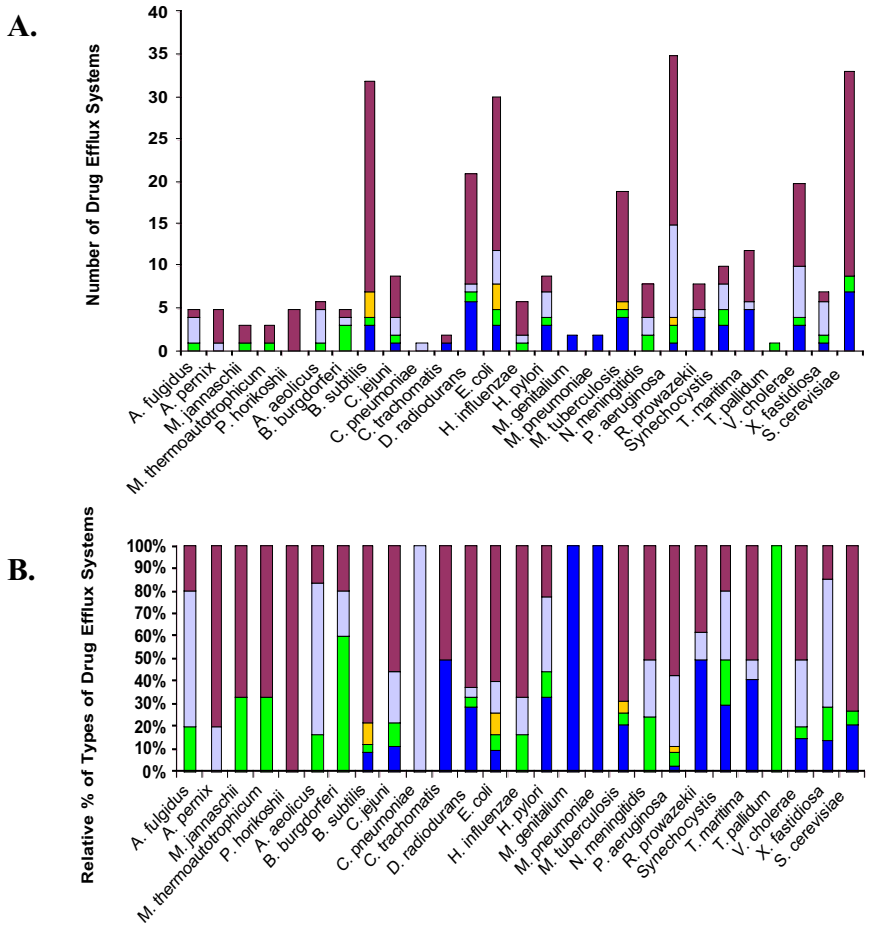

Figure 3. (A) Numbers of predicted drug efflux systems belonging to the MFS (brown), RND (light blue), SMR (yellow), MATE (green) and ABC (dark blue) transporter families for each of the completely sequenced microorganisms. Details of the predicted drug efflux systems present in each organism are available at http://www-biology.ucsd.edu/~ipaulsen/transport/. (B) Percentage of drug efflux systems from each family displayed relative to the total number of drug efflux transporters.

in this symposium). The SMR family appears to be related to a larger superfamily of transporters, the drug/metabolite (DMT) superfamily that also includes transporters for sugars, purines and other metabolites (Jack and Saier, unpublished data).

The MATE family includes proteins from bacteria, yeast and plants, which have twelve predicted TMS (Brown *et al.,* 1999). This family includes two characterized multidrug efflux proteins, NorM from *Vibrio parahaemolyticus* and an orthologue of NorM in *E. coli* (Morita *et al.,* 1998). NorM confers resistance to compounds such as norfloxacin, ciprofloxacin, ethidium, kanamycin, and streptomycin and appears to function via a drug:Na^+ antiport mechanism (Morita *et al.,* 2000).

Members of the MET family are found in animals, typically located in late endosomal, Golgi and lysosomal membranes, and have four predicted TMS. The mouse MTP protein is the only characterized protein in this family and can transport thymidine, nucleobase and nucleoside analogues, antibiotics, anthracyclines, ionophores and steroid hormones into intracellular compartments (Hogue *et al.,* 1999).

Comparative Genomic Analysis of Multidrug Transporters

Figure 3 shows the distribution in each of the sequenced organisms of probable drug efflux transporters belonging to the five families of multidrug efflux systems found in microorganisms (the MET family appears restricted to higher eukaryotes). There is a wide variation in the number and types of predicted drug efflux systems between these organisms. The number of drug efflux systems does not correlate very well with the genome size of the organism or with the total number of transporters (data not shown). All of the organisms examined did include at least one predicted drug efflux system, even in the organisms with small genome sizes, lending credence to the notion that drug efflux systems are essentially ubiquitous.

The highest numbers of predicted drug efflux systems are in the soil or environmental bacteria: *B. subtilis*, *P. aeruginosa* and *Deinococcus radiodurans*, as well as *E. coli* and *S. cerevisiae*. This correlation lends a little weight to the concept that drug efflux systems may have originated as excretion systems for secondary metabolites or as

mechanisms of defense against such antibiotics. However, other organisms such as the intracellular organisms, *M. tuberculosis* and *Rickettsia prowazekii*, which would not be expected to encounter antibiotics in their environments, also have relatively large numbers of predicted drug efflux systems. Thus, the abundances of drug efflux systems in these different organisms do not necessarily clarify the natural physiological roles of these transporters.

There is a great deal of variation in terms of the types of putative drug efflux systems amongst the organisms examined. For instance, the archaea predominantly use MFS-type drug efflux systems and thus far completely lack ABC-type drug efflux systems. Organisms such as the mycoplasmas and *T. maritima* are largely or entirely reliant on ABC drug efflux systems, and this may reflect their overall preference for primary transporters due to their lack of a TCA cycle and electron transfer chain (Paulsen *et al.,* 1998; 2000). However, the spirochetes *Treponema pallidum* and *Borrelia burgdorferi* that also are generally reliant on primary transporters, have no ABC drug efflux systems, but instead are the only organisms where the majority of their putative drug efflux systems belong to the MATE family. While the intracellular parasites *M. tuberculosis* and *R. prowazekii* have a relatively high number of efflux systems (see above), the intracellular chlamydia parasites are almost devoid of putative drug transporters.

As might be expected the two most predominant types of drug efflux systems across the broad swathe of microorganisms examined were those of the ABC and MFS superfamilies. The RND-type transporters were common in both γ- and β-proteobacteria consistent with their capacity to mediate transport across two membranes in conjunction with MFP and OMF proteins. However, RND drug efflux systems were also commonly found in phylogenetically diverse organisms such as *Aquifex aeolicus* and *Archaeoglobus fulgidus* and *Synechocystis* PCC6803. SMR drug efflux proteins were present only in a small minority of organisms and in relatively low numbers. Putative drug efflux proteins from the MATE family were widely distributed, but with the exception of the spirochetes made up only a small fraction of efflux systems present in most organisms.

It should be stressed that these analyses are based on bioinformatic predictions, and very probably will be modified over time in light of experimental data on these transporters. In the case of drug efflux proteins, transporters in general, and indeed for many different types of proteins, a comparative genomic approach finds interesting differences in terms of different families of proteins performing apparently similar jobs in different organisms. However, what is not clear at this stage is the specific rationale for each of these differences. Why do spirochetes prefer MATE family drug efflux proteins? Why do archaea appear to lack ABC drug efflux systems? The reasons for these choices are not readily apparent and are not easily addressed experimentally.

Functional Genomic Analysis of MDR Transporters

Functional genomic approaches, such as microarray expression analysis and large-scale gene knockout or expression studies, are starting to be used to address biological questions. For instance with respect to drug efflux, in this symposium, the papers by Rogers *et al.* and Davis *et al*. describe systematic knockout studies of predicted drug efflux genes in *S. cerevisiae* and *Enterococcus faecalis*. In the case of *E. faecalis*, out of 31 predicted drug efflux pumps, knockouts in only 4 of these transporter genes, all belonging to the ABC superfamily, were found to have a significant effect on drug efflux.

Other larger gene knockout studies are underway elsewhere, for instance there are large community-wide projects systematically targeting all of the genes in *B. subtilis* and *S. cerevisiae*. Hutchison *et al*. (1999) undertook transposon mutagenesis of *Mycoplasma genitalium* and *Mycoplasma pneumoniae*, which have small gene complements, in order to identify non-essential genes in these two organisms under laboratory growth conditions. This analysis suggested that approximately only 265-330 genes were essential in *M. genitalium*, giving insight into what may be the minimal set of essential genes required for life. Knockouts were obtained in several *M. genitalium* transporters, including both of the putative ABC multidrug efflux pumps in this organism.

Another functional genomics approach, microarray expression analysis has been used to study global transcriptional levels in several prokaryotes and has provided some insight into expression of drug efflux genes. For instance, global microarray expression analysis of *B. subtilis*, has indicated that 27 of 31 putative drug efflux transporters in *B. subtilis* appeared to be expressed at detectable levels in LB media, and of these none were apparently regulated in response to glucose (Saier *et al.,* in press). Similarly, microarray studies of *E. coli* expression have indicated that the majority of known and putative drug efflux pumps in this organism are also expressed in conditions of both minimal and rich media (Tao *et al.,* 1999).

The MarA global regulatory protein in *E. coli* is known to control expression of multiple chromosomal genes affecting resistance to antibiotics and other environmental hazards, such as the multidrug efflux genes *acrAB* and *tolC* (Aono, 1998). Surprisingly, global microarray analysis of an *E. coli* strain constitutively expressing MarA only affected expression of two other possible efflux systems: *yadGH*, encoding an ABC efflux system of unknown specificity and *ydeA*, an MFS sugar and drug efflux system (Barbosa and Levy, 2000). Microarray studies of *M. tuberculosis* have shown that the anti-tuberculosis drug isoniazid induced several genes that encode proteins physiologically relevant to the drug's mode of action, and interestingly also induced expression of the putative MFS drug efflux protein Rv2846c (Wilson *et al.,* 1999). Whether this transporter is capable of transporting isoniazid has not been experimentally examined yet.

While only a handful of published studies of microarray expression analysis of prokaryotes are currently available, these isolated examples show the potential of this approach for identifying physiological inducers or regulatory genes involved in expression of multidrug efflux genes and thereby providing clues to the function of these genes. Similarly, there are not many examples of global knockout or gene expression studies published yet, but these also have the potential for illuminating the interplay or functional overlap

of drug efflux transporters in a single organism. The few preliminary examples of functional genomic studies of prokaryotes do suggest though that putative drug efflux pumps may tend to be a) non-essential and b) expressed under common laboratory conditions.

Conclusions

Complete genome sequencing has transformed our ability to address complex biological questions. Both comparative and functional genomics provide approaches that were hitherto not feasible. However, it must be pointed out that genome sequencing and comparative genomics do not necessarily provide the answers to many biological questions, but instead allows us to know what questions we should be asking, and functional genomics gives us new approaches for trying to answer such questions.

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References

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., George, R.A., Lewis, S.E., Richards, S., Ashburner, M., Henderson, S.N., Sutton, G.G., Wortman, J.R., Yandell, M.D., Zhang, Q., Chen, L.X., Brandon, R.C., Rogers, Y.H., Blazej, R.G., Champe, M., Pfeiffer, B.D., Wan, K.H., Doyle, C., Baxter, E.G., Helt, G., Nelson, C.R., Gabor Miklos, G.L., Abril, J.F., Agbayani, A., An, H.J., Andrews-Pfannkoch, C., Baldwin, D., Ballew, R.M., Basu, A., Baxendale, J., Bayraktaroglu, L., Beasley, E.M., Beeson, K.Y., Benos, P.V., Berman, B.P., Bhandari, D., Bolshakov, S., Borkova, D., Botchan, M.R., Bouck, J., Brokstein, P., Brottier, P., Burtis, K.C., Busam, D.A., Butler, H., Cadieu, E., Center, A., Chandra, I., Cherry, J.M., Cawley, S., Dahlke, C., Davenport, L.B., Davies, P., de Pablos, B., Delcher, A., Deng, Z., Mays, A.D., Dew, I., Dietz, S.M., Dodson, K., Doup, L.E., Downes, M., Dugan-Rocha, S., Dunkov, B.C., Dunn, P., Durbin, K.J., Evangelista, C.C., Ferraz, C., Ferriera, S., Fleischmann, W., Fosler, C., Gabrielian, A.E., Garg, N.S., Gelbart, W.M., Glasser, K., Glodek, A., Gong, F., Gorrell, J.H., Gu, Z., Guan, P., Harris, M., Harris, N.L., Harvey, D., Heiman, T.J., Hernandez, J.R., Houck, J., Hostin, D., Houston, K.A., Howland, T.J., Wei, M.H., Ibegwam, C., Jalali, M., Kalush, F., Karpen, G.H., Ke, Z., Kennison, J.A., Ketchum, K.A., Kimmel, B.E., Kodira, C.D., Kraft, C., Kravitz, S., Kulp, D., Lai, Z., Lasko, P., Lei, Y., Levitsky, A.A., Li, J., Li, Z., Liang, Y., Lin, X., Liu, X., Mattei, B., McIntosh, T.C., McLeod, M.P., McPherson, D., Merkulov, G., Milshina, N.V., Mobarry, C., Morris, J., Moshrefi, A., Mount, S.M., Moy, M., Murphy, B., Murphy, L., Muzny, D.M., Nelson, D.L., Nelson, D.R., Nelson, K.A., Nixon, K., Nusskern, D.R., Pacleb, J.M., Palazzolo, M., Pittman, G.S., Pan, S., Pollard, J., Puri, V., Reese, M.G., Reinert, K., Remington, K., Saunders, R.D., Scheeler, F., Shen, H., Shue, B.C., Siden-Kiamos, I., Simpson, M., Skupski, M.P., Smith, T., Spier, E., Spradling, A.C., Stapleton, M., Strong, R., Sun, E., Svirskas, R., Tector, C., Turner, R., Venter, E., Wang, A.H., Wang, X., Wang, Z.Y., Wassarman, D.A., Weinstock, G.M., Weissenbach, J., Williams, S.M., Woodage, T., Worley, K.C., Wu, D., Yang, S., Yao, Q.A., Ye, J., Yeh, R.F., Zaveri, J.S., Zhan, M., Zhang, G., Zhao, Q., Zheng, L., Zheng, X.H., Zhong, F.N., Zhong, W., Zhou, X., Zhu, S., Zhu, X., Smith, H.O., Gibbs, R.A., Myers, E.W., Rubin, G.M., and Venter, J.C. 2000. The genome sequence of *Drosophila melanogaster*. Science 287: 2185-2195

Aono, R. 1998. Improvement of organic solvent tolerance level of *Escherichia coli* by overexpression of stress-responsive genes. Extremophiles 2: 239-48.

Barbosa, T.M., and Levy, S.B. 2000. Differential expression of over 60 chromosomal genes in *Escherichia coli* by constitutive expression of MarA. J. Bacteriol. 182: 3467-3474.

Brown, M.H., Paulsen, I.T., and Skurray, R.A. 1999. The multidrug efflux protein NorM is a prototype of a new family of transporters. Molec. Microbiol. 31: 394-395.

Dinh, T., Paulsen, I. T. and Saier, M. H., Jr. 1994. A family of extracytoplasmic proteins that allow transport of large molecules across the outer membranes of Gram-negative bacteria. J. Bacteriol. 176: 3825-3831.

Fleischmann, R.D., Adams, M.D., White, O., Clayton, R.A., Kirkness, E.F., Kerlavage, A.R., Bult, C.J., Tomb, J. –F., Dougherty, B.A., Merrick, J.M., McKenny, K., Sutton, G., Fitzhugh, W., Fields, C., Gocayne, J.D., Scott, J., Shirley, R., Liu, L.-I., Glodek, A., Kelley, J.M., Wiedman, J.F., Phillips, C.A., Spriggs, T., Hedblom, E., Cotton, M.D., Utterback, T.R., Hanna, M.C., Nquyen, D.T., Saudek, D.M., Brandon, R.C.,Fine, L.D., Fritchman, J.L., Fuhrman, J.L., Geoghagen, N.S.M., Gnehm, C.L., McDonald, L.A., Small, K.V., Fraser, C.M., Smith, H.O., and Venter, J.C. 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science 269: 496-512.

Hogue, D.L., Kerby, L. and Ling, V. 1999. A mammalian lysosomal membrane protein confers multidrug resistance upon expression in *Saccharomyces cerevisiae*. J. Biol. Chem. 274: 12877-12882.

Hutchison, C.A., Peterson, S.N., Gill, S.R., Cline, R.T., White, O., Fraser, C.M., Smith, H.O., and Venter, J.C. 1999. Global transposon mutagenesis and a minimal *Mycoplasma* genome. Science 286:2165-2169.

Karp, P.D., Riley, M., Saier, M., Paulsen, I.T., Paley, S.M., and Pellegrini-Toole, A. 2000. The EcoCyc and MetaCyc databases. Nucl. Acids Res. 28: 56-59.

Morita, Y., Kataoka, A., Shiota, S., Mizushima, T., and Tsuchiya, T. 2000. NorM of *Vibrio parahaemolyticus* is an Na(+)-driven multidrug efflux pump. J. Bacteriol. 182: 6694-6697.

Morita, Y., Kodama, K., Shiota, S., Mine, T., Kataoka, A., Mizushima, T., and Tsuchiya, T. 1998. NorM, a putative multidrug efflux protein, of *Vibrio parahaemolyticus* and its homolog in *Escherichia coli*. Antimicrob. Agents Chemother. 42: 1778-1782.

Nelson, K.E., Paulsen, I.T., Heidelberg, J. and Fraser, C.M. 2000. Status of genomic projects of nonpathogenic bacteria and archaea. Nature Biotech. 18: 1049-1054.

Pao, S.S., Paulsen, I.T., and Saier, M.H., Jr. 1998. The major facilitator superfamily. Microbiol. Molec. Biol. Rev. 62: 1-32.

Paulsen, I.T. and Skurray, R.A. 1993. Topology, structure and evolution of two families of proteins involved in antibiotic and antiseptic resistance in eukaryotes and prokaryotes- an analysis. Gene 124: 1-11.

Paulsen, I.T., Nguyen, L., Sliwinski, M.K., Rabus, R., and Saier, M.H., Jr. 2000. Microbial genome analyses: comparative transport capabilities in eighteen prokaryotes. J. Molec. Biol. 301: 75-101.

Paulsen, I.T., Skurray, R.A., Tam, R., Saier, M.H., Jr., Turner, R.J., Weiner, J.H., Goldberg, E.G., and Grinius, L.L. 1996. The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. Molec. Microbiol. 19: 1167-1175.

Paulsen, I.T., Sliwinski, M.K. and Saier, M.H., Jr. 1998. Microbial genome analyses: global comparisons of transport capabilities based on phylogenies, bioenergetics and substrate specificities. J. Molec. Biol. 277: 573-592.

Saier, M.H., Jr. 2000. A functional-phylogenetic classification system for transmembrane solute transporters. Microbiol. Mol. Biol. Rev. 64: 354-411.

Saier, M.H., Jr., Goldman, S.R., Maile, R.R., Moreno, M.S., Weyler, W., Yang, N., and Paulsen, I. T. (in press) Overall transport capabilities of *Bacillus subtilis*: An Overview. *In Bacillus subtilis* and Other Gram-Positive Bacteria (ed. Sonenshein, A.L., Hoch, J. A., Losick, R.). American Society for Microbiology, Washington DC.

Saurin, W., Hofnung, M., and Dassa, E. 1999. Getting in or out: early segregation between importers and exporters in the evolution of ATP-binding cassette (ABC) transporters. J. Mol. Evol. 48: 22-41.

Tao, H., Bausch, C., Richmond, C., Blattner, F.R., and Conway, T. 1999. Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. J. Bacteriol. 181: 6425-6440.

The *C. elegans* Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science 282: 2012-2018.

Traini, M., Gooley, A.A., Ou, K., Wilkins, M.R., Tonella, L., Sanchez, J.C., Hochstrasser, D.F., and Williams, K.L. 1998. Towards an automated approach for protein identification in proteome projects. Electrophoresis. 19: 1941-1949.

Tseng, T.T., Gratwick, K.S., Kollman, J., Park, D., Nies, D.H., Goffeau, A., and Saier, M.H., Jr. 1999. The RND permease superfamily: an ancient, ubiquitous and diverse family that includes human disease and development proteins. J. Mol. Microbiol. Biotechnol. 1: 107-125.

Wilson, M., DeRisi, J., Kristensen, H.H., Imboden, P., Rane, S., Brown, P.O., and Schoolnik, G.K. 1999. Exploring drug-induced alterations in gene expression in *Mycobacterium tuberculosis* by microarray hybridization. Proc. Natl. Acad. Sci., U S A 96: 12833-12838.