Microarray analyses of meningococcal genome composition and gene regulation: a review of the recent literature

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Received 21 June 2006; revised 22 August 2006; accepted 26 September 2006. First published online 9 November 2006.

DOI:10.1111/j.1574-6976.2006.00047.x

Editor: Martin Maiden

Keywords
microarray analysis; oligonucleotide array sequence analysis; gene expression profiling; Neisseria meningitidis; genome–bacterial.

Abstract
The development of microarrays for genome comparison and transcriptional profiling along with the public availability of several meningococcal genome sequences has promoted studies elucidating (i) intraspecific and interspecific genomic differences of members of the genus Neisseria, and (ii) the transcriptional response of meningococci to a variety of environmental stresses such as heat shock, iron starvation, serum treatment, and contact with eukaryotic cells. Furthermore, microarray-based finetyping of meningococci is in development. It will remain a difficult, but important, goal to identify sets of genes determining the virulence potential of hypervirulent meningococcal lineages in comparison with apathogenic ones. The recent identification of the meningococcal disease-associated island through the application of microarray analyses has been a step towards this aim. Transcriptional profiling of meningococci has brought about the compilation of large datasets, which also provide information about several regulons. Meningococcal microarray analysis has established a basis for studies clarifying the function of previously unknown genes, and has supported the identification of interesting vaccine candidates. However, harmonization of protocols and tools, as well as central databases are needed to foster the comparability of studies and the integration of knowledge.

Introduction

Over the past 30 years a number of powerful molecular typing techniques have been developed for the analysis of bacterial population structure and taxonomy (reviewed in van Belkum et al., 2001). For example, multilocus sequence typing (MLST) resulted in the establishment of a now widely accepted typing scheme for Neisseria meningitidis isolates and in the generation of a publicly accessible database containing a large number of meningococcal sequence types (STs) (Maiden et al., 1998; Chan et al., 2001). However, all these techniques only allow the examination of a limited number of loci, giving no sense either of genome-wide variation or of the full impact of horizontal gene transfer (Ochman et al., 2000). The advent of high-throughput microbial genome sequencing and annotation has dramatically changed the field and has fuelled the concomitant development of analytical tools that take advantage of these extensive genome-sequence data.

One such innovation that is beginning to transform our understanding of meningococcal population genetics on a genome-wide scale is DNA-array technology (DNA microarrays) (reviewed in Joyce et al., 2002; Bryant et al., 2004). DNA microarrays enable a ‘bird’s-eye view’ of all the genes absent or present in a given genome compared with the reference genome on the microarray. In principle, a DNA microarray is a solid substrate such as a specifically coated glass slide on which DNA or oligonucleotides are arrayed in a grid formation. These nucleic acids are complementary to thousands of genes from the bacterial genome(s) under study and are designed to minimize cross-hybridization. For genome comparison, genomic DNA from a test strain is labelled with a fluorescent marker and is competitively hybridized with genomic DNA from a control strain that has been labelled with a second fluorescent marker. The control DNA usually is from the strain used to design the array, as this should hybridize to all reporter elements on the microarray. A comparison of the levels of test and control DNA bound to each reporter element on the array can be performed, and statistical methods are utilized to determine if the coding sequence in question is present, absent or highly divergent in the test strain (Kim et al., 2002).

With regard to the type of DNA used, two variants of DNA microarrays are available: PCR-product-based and
oligonucleotide arrays. Both PCR-product-based and oligonucleotide arrays are generated by robotic spotting of the respective nucleic acids onto surface-pretreated glass slides. An alternative, although costly, format exists for oligonucleotide arrays in the form of the Affymetrix (www.affymetrix.com) format. Here, oligonucleotides usually 20–25 bases in length are synthesized in situ on a derivatized glass surface using a combination of photolithography and combinatorial chemistry. An important difference between PCR-product-based and all types of oligonucleotide arrays is that the manufacture of oligonucleotide chips relies entirely on pre-existing sequence information, whereas PCR-product-based chips can be generated using, for example, nonannotated clones from uncharacterized genomic libraries, and therefore can be used for de novo gene discovery (see below). However, oligonucleotide-based microarrays offer many advantages over PCR-product-based microarrays, including a reduction in cross-hybridization and an increase in the differentiation of overlapping genes or highly homologous regions. For example, mutant alleles or single nucleotide polymorphisms can be detected using such oligonucleotide microarrays owing to the shorter probe size compared with PCR-product-based microarrays.

The Neisserial Research Community has been in a privileged position in that a variety of genomes have been publicly available since the year 2000, namely the sequences of the serogroup A strain Z2491 (Parkhill et al., 2000) and the serogroup B strain MC58 (Tettelin et al., 2000), as well as the preliminary annotations of the serogroup C strain FAM18 (available at the Wellcome Trust Sanger Institute website), and the genome sequence of the gonococcal strain FA1090 (available at the website of the Los Alamos National Laboratory). Thus, a variety of microarrays including pan-Neisseria microarrays have been used for a variety of genomic and gene regulation projects. For example, the pan-Neisseria microarray currently in use in our laboratory comprises sequences representing MC58, Z2491, FAM18, and the apathogenic carrier isolate α-14 characterized by the presence of the capsule null locus (unpublished genome sequence, Institute for Hygiene and Microbiology, Würzburg, Germany), and was developed in collaboration with Operon Biotechnologies (www.operon.com). In this review we cite and discuss studies utilizing these microarrays to analyze (1) gene content differences among meningococcal strains and, more generally, neisserial species, as well as (2) transcriptional profiles of meningococci under various experimental conditions. Studies engaging other neisserial species are discussed if they provide comparative data.

**Genome comparison**

The first application of DNA microarrays to the study of the genomic differences between N. meningitidis and commensal Neisseria species was performed by Tinsley and coworkers (Perrin et al., 2002; Table 1). Based on the genome sequence of N. meningitidis Z2491 (Parkhill et al., 2000), they generated gene-specific PCR products of about 1 kb in length that were spotted onto nylon membranes and hybridized with a defined panel of virulent strains of N. meningitidis. In particular, the DNA arrays were hybridized with genomic DNAs extracted from eight meningococcal strains belonging to four different STs, from three strains of Neisseria gonorrhoeae, and from two strains of Neisseria lactamica.

The majority of the amplicons on the DNA array reacted with sequences in each of the tested chromosomes. However, compared with the genome of strain Z2491, between 3% and 8% of the total for N. meningitidis, about 10% for N. gonorrhoeae, and between 15% and 20% for N. lactamica, respectively, were found to be absent by this technique. Comparison of meningococci alone showed that 89% of the chromosome of Z2491 was shared with the other strains, while strain-specific differences characterized the remaining 11%. 46 kb or about 2% of the sequences that were shared by all virulent strains of N. meningitidis were strictly meningococcus-specific, i.e. present in all strains of invasive meningococci and absent from all the N. gonorrhoeae and N. lactamica isolates. 73 kb (3.3%) were found to be pathogen-specific, and thus present in all N. meningitidis and N. gonorrhoeae isolates but absent from all N. lactamica strains.

Surprisingly, among the meningococcus-specific sequences, only seven were >2 kb long. As expected, one of these regions corresponds to the cps locus specifying the production of capsular polysaccharide, two other meningococcus-specific regions encode the production and secretion of a repeat in toxin (RTX) toxin family protein. The largest of these regions showed homology to proteins with homology to the filamentous hemagglutinin of Bordetella pertussis. As for meningococcus-specific regions, pathogen-specific sequences, which were present in larger numbers than the meningococcus-specific regions, were found to be scattered as small islands around the chromosome. Despite their considerable number, only three of these genes have been shown to play roles in pathogenesis, namely those for the immunoglobulin A protease, the PilC adhesin, and the haemoglobin receptor.

The small sizes of these differential regions are in contrast to what is observed in, for example, Enterobacteriaceae, in which large (20–200 kb), horizontally acquired pathogenicity islands (PAIs) containing genes involved in the pathogenic process are inserted into the core genome (Hacker et al., 1997). However, in face of the lifestyle of meningococci as inhabitants of the upper respiratory tract, PAIs would not provide a selective advantage, because meningitis is a deadly disease that does not favour transmission. Therefore, the absence of such large, complex islands might
support the notion that *N. meningitidis* is essentially a commensal species.

In an extension of their previous study, Tinsley and coworkers performed whole-genome comparisons between a larger set of representatives of the major hyperinvasive meningococcal clonal complexes that are responsible for disease worldwide (29 isolates belonging to nine STs) and those belonging to clonal groups that have no association with disease (20 isolates belonging to nine STs, Bille *et al*., 2005). Of the 1950 amplicons (representing 92% of the 2121 predicted ORFs for isolate Z2491) represented on the arrays, 1532 (79%) were present in all meningococcal isolates examined that might be an approximation to the core meningococcal genome. Isolates with the same ST had similar genomic content profiles and demonstrated congruence between the DNA array technique and the clonal complexes defined by MLST. Remarkably, only a single group of genes (NMA1792–NMA1800) of 8 kb was associated significantly with the hypervirulent isolates, and no other gene satisfied the requirement that it should be present in all 29 invasive isolates but in none of the 20 noninvasive isolates. This gene cluster was present in 100% (29/29) of the disease isolates and absent from 90% (18/20) of the noninvasive isolates. A multivariate statistical analysis of the results revealed that the element was associated very significantly with disease ($P = 0.0013$), even after taking into account the confounding clonal association with invasive complexes. In support of the idea that the gene cluster constitutes a genomic island, the cluster was shown to have a low G+C content and similarity in size and arrangement of coding sequences to filamentous bacteriophages of the *f1* family, which is characterized by the *Vibrio cholerae* phage CTXφ, which carries the cholera toxin. Sequences within a 20-bp inverted repeat called dRS3 present several hundred times in the meningococcal chromosome (Parkhill *et al*., 2000) constitute the insertion site of the island. Further experimental evidence suggested that this element corresponds to an integrated phage genome that is able to excise from the chromosome and is secreted from the bacteria by means of the type IV pilin secretin (PilQ). *Bille et al*. therefore termed it a ‘meningococcal disease associated (MDA) island.’ It is still unclear, however, how this island contributes to the pathogenicity of the meningococcus because it seems not to contain genes coding for known virulence factors. The intriguing finding that disease caused by MDA-positive strains was 30-fold more common in toddlers and children than in young adults has been discussed in a recent review (Moxon & Jansen, 2005). The authors suggest that the phage increases transmission rates, thereby increasing disease incidence. Increased transmission rates are counter-balanced by slightly reduced fitness and carriage duration. In this situation, it was suggested that the phage itself is maintained in the species only because it regularly encounters MDA-negative carrier strains acting as phage recipients, which occurs much more often in adolescents than in young children. This attractive theory by Moxon and Jansen awaits epidemiological proof. Furthermore, the age dependence of MDA presence needs to be confirmed for other strain collections.

By constructing a PCR-product-based ‘pan-Neisseria’ microarray including every gene in the four genomes of *N. meningitidis* Z2491 (Parkhill *et al*., 2000), *N. meningitidis* MC58 (Tettelin *et al*., 2000), *N. meningitidis* FAM18, and *N. gonorrhoeae* FA1090, Hinds and coworkers focused on genes present in serogroup B meningococci, but not in commensal *Neisseria* spp., for targetted vaccine design against serogroup B meningococci that did not affect potentially beneficial commensal *Neisseria*. They hybridized their array with genomic DNA from a total of 38 pathogenic as well as nonpathogenic *Neisseria* species, including 26 *N. meningitidis* strains belonging to different STs comprising 16 invasive, two unspecified and eight carrier isolates (Stabler *et al*., 2005).

In contrast to the number estimated by Tinsley and colleagues (Bille *et al*., 2005), hybridization of the 2907 genes included on the microarray under high stringency conditions indicated that the neisserial core genome might comprise only 862 genes. One thousand twenty four genes were found to be common to all serogroup B isolates as well as to all commensal strains tested, and 55 genes were present in all serogroup B strains but absent from all commensal strains. Twenty three of the serogroup B-specific genes encoded for conserved/hypothetical proteins. The majority of the known genes have some role on the bacterial cell surface, including, for example, pilin (* pilE* and *pilS* regions). The 1499 genes shared by all serogroup B meningococci were also found in at least one of the nonserogroup B meningococci or *N. gonorrhoeae*. With the exception of the capsule gene cluster, no gene set was clearly restricted to pathogenic strains and absent from all the commensal strains and species, which is in accordance with the findings of Bille *et al*., 2005.

In a similar study using a so-called ‘pan-Neisseria’ microarray-2’, Snyder and Saunders investigated the virulence gene content of 13 strains of *N. lactamica* as well as of a number of other commensal *Neisseria* species (Snyder & Saunders, 2006). The majority of genes in the pathogenic *Neisseria* species are present in nonpathogenic *N. lactamica*, including those designated as virulence genes. Their pan-Neisseria microarray-2 contained 2,845 unique probes for the genes from the genome sequences of *N. gonorrhoeae* strain FA1090, *N. meningitidis* strain MC58, *N. meningitidis* strain Z2491, *N. meningitidis* strain FAM18, and for the genes from the *N. gonorrhoeae* strain MS11 gonococcal genetic island, neisserial genes from GenBank/European molecular biology laboratory (EMBL) that have not yet been covered by probes, and newly identified genes from

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minimal mobile elements. They found that, among the 127 putative virulence genes assessed, 85 were also present in the nonpathogenic *N. lactamica*, and that, of the remaining 42, only 11 were present in all four sequenced genomes of the pathogenic *Neisseria* spp. They concluded that the vast majority of genes present in the pathogens are also present in *N. lactamica*, and that therefore the differences that make a particular species pathogenic or not are not as great as might be supposed on the basis of their distinct behaviours and previous lists of virulence genes. In turn, it might be more the ‘genetic personality’ of a particular *Neisseria* sp., deriving from the combinations of genes and/or differences in their regulation, rather than the mere presence or absence of genes that underlies their different pathogenic potentials.

In a direct reply worth reading, Stabler and Hinds emphasize the importance of the different microarray analysis methods (intensity-based vs. ratio-based analysis) and study designs (loop vs. common reference design) employed to explain the discrepancies with regard to genes absent in distinct species in these otherwise very similar studies. Stabler & Hinds (2006) conclude that it ‘is important to appreciate that the application of apparently similar experimental approaches can lead to very different conclusions’.

**Fine-typing of meningococci using DNA microarrays**

Excellent tools for DNA-sequence-based meningococcal finetyping have been developed in recent years, including multilocus sequence typing as well as antigen sequence typing, i.e. sequence determination of variable regions identified in genes encoding immunodominant outer membrane proteins (Maiden *et al.*, 1998; Vogel *et al.*, 2000; Thompson *et al.*, 2003; Russell *et al.*, 2004). The advent of oligonucleotide microarrays, especially of high-density arrays employing the tiling technique already used for resequencing projects (Chee *et al.*, 1996), inspired molecular epidemiologists to create bacterial typing arrays, such as those established recently for multilocus sequence typing of *Staphylococcus aureus* (van Leeuwen *et al.*, 2003).

Corless *et al.* (pers. commun.) in collaboration with Affymetrix (www.affymetrix.com) successfully used a CustomSeq™ resequencing array containing genomic sequences to type *N. meningitidis*. The resequencing arrays provided results in 48 h, and proved to be highly accurate. Not only did they correctly type strains with a known finetype, but they also identified previously unknown gene alleles (Ray Borrow, Manchester, personal communication).

Swiderek *et al.* (2005) followed a different approach, with the rationale of involving a lower number of probes and of employing an in-house system. The microarrays for MLST were based on a polymorphism-directed oligonucleotide design. The approach was considered as feasible for meningococci because of the profound knowledge of the polymorphic sites occurring at the MLST loci in meningococci (http://pubmlst.org/neisseria/). However, despite highly specific hybridization protocols, the rate of misidentification of oligonucleotides remained unacceptably high, most likely because of the high density of polymorphic sites and the extensive GC-content variations at *N. meningitidis* MLST loci. Thus, for this highly variable organism, a microarray resequencing approach seems to be the only suitable choice.

The presumed advantage of microarrays over DNA sequencing is the rapid and simultaneous assessment of polymorphic sites of many target genes. However, there are a number of technical problems that are obviously difficult to address, for example the development of multiplex PCRs and of efficient labelling technology for genomic DNA, probe design, reduction of process time, portability, and validation of procedures. Furthermore, costs for DNA labelling, microarrays, and technical equipment are still a substantial issue. Thus doubts remain over whether, even for reference laboratories, microarray-based typing can serve as an alternative for DNA sequence typing.

**Profiling meningococcal transcription**

In the first publication of meningococcal transcriptome analyses, the heat shock response of *N. meningitidis* was used as a model for a comparative analysis of the suitability of oligonucleotide and PCR-product-based arrays for transcriptional profiling (Guckenberger *et al.*, 2002). It was shown that both technology platforms were highly suitable for this purpose. The heat shock regulated 55 ORFs comprising typical heat shock and stress response genes like those encoding chaperons and proteases, which protect stressed cells against protein misfolding.

Grafantini *et al.* used DNA microarrays based on the meningococcal genome sequence of strain MC58 to study the transcriptome of serogroup B meningococci. Host-cell contact of serogroup B meningococci with cultured human bronchial epithelial cells (16HBE14) induced changes in the expression of 347 genes in comparison to planktonic bacteria (Grafantini *et al.*, 2002b). In order to identify genes that are specifically regulated in pathogenic *Neisseria* upon contact with human bronchial epithelial cells, transcriptome analysis was also carried out with the apathogenic commensal species *N. lactamica* (Grafantini *et al.*, 2002a). Compared with 347 genes in *N. meningitidis*, 285 genes were differentially regulated in *N. lactamica*. Only 167 of the regulated genes were common to both species, indicating that the different behaviour of the two species probably resides in the genes regulated specifically in meningococci and *N. lactamica*, respectively. The activation of transport systems involved in transmembrane trafficking of compounds, such as amino acids and iron, was a common event in both...
species, but the upregulation of transporters appeared to be more pronounced in meningococci. Genes encoding transporters of ammonium, chloride or sulphate were regulated in meningococci. For reasons as yet unknown, the activation of the sulphate transport system, which is strictly linked to sulphur-containing amino acid metabolism, was the most evident difference between meningococci and N. lactamica after interaction with epithelial cells.

Dietrich et al. (2003) investigated the transcriptome of the unencapsulated serogroup B strain MC58 in response to interaction with other cell types, namely HEP-2 epithelial cells and human brain microvascular endothelial cells (HBMEC). Seventy-two genes were found to be regulated in meningococci after contact with epithelial cells. Of these, 21 genes were also found by Grifantini et al. (2002b) to be regulated in the experiments with 16HE14 bronchial epithelial cells. Forty-eight genes were altered in gene expression after contact with endothelial HBMEC cells. Only 13 genes were regulated in both experiments. The regulated genes belonged to five major categories: adhesion genes, host-pathogen crosstalk genes, amino acid biosynthesis genes, genes involved in DNA metabolism, and genes encoding hypothetical proteins.

A large number of the upregulated genes encoded membrane proteins and transporters, suggesting that, upon cell contact, N. meningitidis undergoes substantial surface remodelling. Flow cytometry analysis by Grifantini et al. of a selection of 12 upregulated genes revealed that four proteins were detectable on the bacterial surface only after adhesion to the cells. The expression of five proteins increased upon interaction with host cells, and three proteins were equally present on the surface of both adhering and nonadhering bacteria (Grifantini et al., 2002b). Furthermore, mouse antisera directed against these 12 proteins were tested for their ability to mediate complement-dependent killing of serogroup B meningococci, and indeed five of the sera showed bactericidal activity (e.g. the sera against MafA, and a MIP-related protein). This finding is promising as it demonstrates that microarray analyses have the potential to provide testable hypotheses for further analysis of antigens, which are only expressed in vivo.

The exposure of meningococci to human serum served as a model system of bacteraemia (Kurz et al., 2003). Comparison of meningococci treated with serum with meningococci incubated in phosphate-buffered saline (PBS) by microarray analysis revealed 279 differentially regulated genes, with 134 genes being upregulated and 145 genes being downregulated. One-third of the regulated genes encoded proteins with unknown function. The majority of the remaining genes could be grouped in the following functional categories: metabolism, translation, membrane proteins, and transporters. In addition, 34 of the regulated genes could be classified as known virulence factors. Thirty-nine genes were deregulated both by treatment with human serum and by contact with human cell lines (Dietrich et al., 2003).

Iron plays a prominent role in a variety of metabolic pathways, making it essential for life in most organisms. In mammals, various mechanisms to sequester iron exist. Iron is bound to proteins such as ferritin, lactoferrin, and transferrin. Pathogenic bacteria have therefore evolved iron-acquisition mechanisms, many of which in Neisseria are under the control of the ferric uptake regulator protein Fur (reviewed in Perkins-Balding et al., 2004). In the presence of ferrous iron (Fe\(^{2+}\)), Fur binds as a dimer to DNA regulatory sequences (Fur boxes), which typically results in the repression of transcription of many iron-regulated genes (reviewed in Escolar et al., 1999). To define the Fur regulon of N. meningitidis, the gene expression of bacterial cultures supplemented with ferric nitrate was compared with the gene expression of iron-depleted bacterial cultures (Grifantini et al., 2003). A total of 233 genes were iron-regulated, of which 203 belonged to putative transcriptional units. Only 50% of the iron-regulated genes were found to contain Fur-binding consensus sequences in their promoter region. Several genes unknown to be Fur-regulated were shown to bind Fur by gel-shift analysis. Interestingly, 10 Fur-regulated genes were upregulated on iron addition, demonstrating that Fur can also act as a transcriptional activator. Under iron-depleted conditions, several virulence-associated genes were overexpressed, for example genes involved in toxin production and multidrug resistance as well as genes involved in cell adhesion: the frpC gene, the frpA/C-related genes and others. Several of the iron-regulated genes identified in this study belonged to the group of hypothetical genes. Computational analysis of the genes NMB1436, NMB1437, and NMB1438 revealed homologies to oxidoreductases carrying iron-sulphur clusters. Therefore, deletion mutants of this operon were investigated in more detail, revealing that the operon – although not being regulated by oxidative stress – is required to protect meningococci from hydrogen peroxide-mediated killing (Grifantini et al., 2004). These data are an impressive example of how microarray analysis can foster consecutive functional studies assigning function to previously hypothetical genes.

In a further microarray approach, the meningococcal genes differentially expressed in the presence or absence of the Fur protein and in response to iron limitations were identified (Delany et al., 2006). Eighty three genes whose transcription is controlled by Fur, either by binding directly to their promoters or through indirect mechanisms, were observed. In a fur mutant, heat shock genes were expressed at higher levels, indicating that they are Fur-repressed but independent of iron limitations.

The iron-responsive genes were also investigated for gonococci (Ducey et al., 2005). A comparison of the gonococcal data with the meningococcal data (Grifantini...
et al., 2003) revealed that only a small proportion of genes (c. 20%) were similarly regulated, a finding that might be the result of different experimental conditions. A large number of genes derepressed during iron starvation encoded hypothetical proteins. Of these, 20 genes were unique to meningococci and 30 were unique to gonococci, suggesting a role of these genes for species-specific pathogenesis.

The autoinducer-2 (AI-2) is a quorum-sensing molecule that has been demonstrated to regulate virulence factor expression in both Gram-negative and Gram-positive bacteria. A meningococcal mutant of the luxS gene encoding a protein shown to be involved in the biosynthesis of AI-2 also exhibited an attenuated virulence in the infant rat model of bacteraemia (Winzer et al., 2002). However, subsequent transcriptional profiling using DNA microarray analyses indicated that the AI-2 had no significant regulating effect in N. meningitidis (Dove et al., 2003). The results of this study were confirmed by proteome analysis, which revealed that a meningococcal luxS mutant did not alter protein expression in response to exogenous AI-2 (Schauder et al., 2005).

A two-component regulatory system with homology to the PhoPQ system in salmonella was recently identified in meningococci (Johnson et al., 2001). The meningococcal PhoPQ system was described as a magnesium-sensing two-component regulatory system controlling remodelling of the bacterial surface in the host environment. Magnesium-regulated changes in gene expression were mostly abrogated in the phoP knock-out mutant (Newcombe et al., 2005). Furthermore, a large number of genes were expressed at different levels after growth of the mutant on blood agar compared with the wild-type strain. The results suggest that PhoPQ as a regulator system may contribute significantly to host adaptation by meningococci, similar to what is found in Salmonella spp. By their analysis of the phoPQ knock-out mutant, Newcombe et al. described a regulon, as has been done for the Fur-regulon mentioned above.

### Table 1. Summary of microarray studies discussed in this review

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<th>Type of analysis</th>
<th>Description</th>
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<td>Perrin et al. (2002)</td>
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and the FNR-regulon published very recently (Bartolini et al., 2006).

*Neisseria gonorrhoeae* has developed a variety of mechanisms to detoxify reactive oxygen intermediates produced by phagocytic cells (Seib et al., 2004). To detect genes that are differentially expressed after transient exposure to hydrogen peroxide, the transcriptome response of *N. gonorrhoeae* was analyzed by microarrays (Stohl et al., 2005). Seventy five gonococcal genes were upregulated after H₂O₂ treatment, in contrast to only 10 genes upregulated in meningococci (Grifantini et al., 2004), a result that might, however, be explained by different experimental conditions (H₂O₂ concentration, 5 mM vs. 200 μM; incubation time, 15 min vs. 30 min, respectively). The roles in protection against oxidative damage of two highly upregulated genes with unknown function (NGO554 and NGO1686) and *recN* were characterized in more detail by analysis of the respective deletion mutants. All three gonococcal mutants showed significant increase in sensitivity to H₂O₂ but only minor or no increase in sensitivity to other oxidative damaging agents. Furthermore, the survival of two of the mutants (*recN* and NGO1686) showed a significant decrease in survival after exposure to polymorphonuclear neutrophils relative to the parent strain.

**Conclusions**

DNA microarray technology has already made invaluable contributions to the understanding of meningococcal genome diversity, population genetics and pathogenesis. There are some caveats with using DNA microarrays for genome typing. The results are highly dependent on the experimental set-up, such as chip design (PCR-product-based vs. oligonucleotide-based, glass slides vs. nylon membranes, etc.), hybridization conditions (stringency), the selection of strains, and the proper application of adequate algorithms used for downstream data analysis. Another major shortcoming of the technology is that only the distribution of already known genes can be assessed. Although laborious and time-consuming, and therefore not applicable to larger strain collections, genome comparison by representational difference analysis might still be an alternative approach that enables the identification of novel sequences of DNA (Tinsley & Nassif, 1996; Claus et al., 2000a, b). Thus, competitive hybridization of microarrays containing PCR-amplified random genomic fragments from a reference strain with a collection of test strains allowed for the identification of previously unknown virulence-associated genes in the case of *Haemophilus influenzae* (Fernays et al., 2006). In the long run, it is only by whole-genome sequencing of as many pathogenic as well as nonpathogenic strains as possible that we might be able to catch a glimpse of the as yet unknown virulence-associated genes that might contribute to the meningococcal gene pool (Maiden et al., 1996). Technological advances such as pyrosequencing (Ronaghi, 2001) might foster such efforts.

Transcriptional profiling of meningococci has provided tremendous new insights into the complexity of gene regulation. As for gene content investigations, the comparability of results is still hampered by several factors. A central database such as PRODORIC (Munch et al., 2003), which for *Pseudomonas aeruginosa* and other species assembles published data, laboratory experiments, and computational information in a central database that integrates data into regulatory networks, would help to improve data exploration and retrieval also in the case of meningococcal research. Furthermore, the measurement of protein expression by proteomic tools will put in place a more direct analysis of the biological processes. The serogroup A and B meningococcal proteomes have already been characterized (Bernardini et al., 2004; Mignogna et al., 2005). In addition, the proteomic analysis of a mutant of a lytic transglycosylase has provided important information for the improvement of outer membrane vesicle vaccine design (Ferrari et al., 2006).

**Acknowledgements**

We apologize to all authors whose publications could not be discussed in this context owing to space limitations. We gratefully acknowledge the invaluable technical assistance of Anja Schramm-Glück with regard to our microarray facility. Kerstin Hubert is thanked for critically reading the manuscript. Ray Borrow, Manchester, is thanked for sharing information about unpublished results. The microarray research at the Institute for Hygiene and Microbiology has been funded by the German Research Foundation, the Federal Ministry of Education and Research, and by the European Union.

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