Minireview

The dynamic proteome of Lyme disease Borrelia

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Abstract

The proteome of the spirochete bacterium Borrelia burgdorferi, the tick-borne agent of Lyme disease, has been characterized by two different approaches using mass spectrometry, providing a launching point for future studies on the dramatic changes in protein expression that occur during transmission of the bacterium between ticks and mammals.

We have all experienced it: the ‘deer in the headlights’ sensation of dumbfounded wonderment and awe when confronted with our first genome sequence. This is a particularly likely response to the genome of the spirochete Borrelia burgdorferi and its relatives - spiral bacteria that are transmitted by deer ticks of the Ixodes ricinus group and that cause the chronic disease Lyme borreliosis in humans and other animals. Although there were previous indications of its unusual characteristics, no one anticipated that the 1.5 megabase genome of B. burgdorferi would contain an odd mixture of approximately 12 linear and 9 circular plasmids, as well as a 0.9 megabase linear chromosome [1,2]. The plasmids, which range from 5 to 56 kilobases, are present consistently in the strains examined so far and contain genes required for the spirochete’s life cycle; these replicons thus could be considered ‘mini-chromosomes’, although the term plasmids is typically used for simplicity. The 833 predicted plasmid-encoded open reading frames (ORFs) include 454 hypothetical genes, many of which are members of 107 paralogous families of unknown function. The plasmids are also rife with pseudogenes, leading to the conclusion that the Lyme disease spirochete genome is ‘in flux’: that is, it is actively evolving [2]. The unique properties of the Borrelia proteome are now starting to be revealed with two recent studies of global protein expression. In the first approach, Jacobs et al. [3] compared the protein profiles of three strains of B. burgdorferi, while in the second, Nowalk et al. [4] examined the proteins present in the soluble and membrane-associated fractions of the bacterium.

Lyme disease is a chronic disease marked by skin lesions, debilitating neurologic symptoms and arthritis. B. burgdorferi is the predominant cause of human Lyme borreliosis in North America, whereas B. burgdorferi and two related Borrelia species, B. garinii and B. afzelii, cause disease in Eurasia. These obligate pathogens have a precarious life cycle in which they alternate between two distinct environments: the tick intestinal tract (midgut) and mammalian (or, in some instances, avian) tissue (Figure 1). In humans, Lyme disease Borrelia causes a local lesion called erythema migrans at the site of the tick bite and then readily disseminates through the bloodstream to other tissues, setting up an infection that can last for months to years. The bacteria can also persist in ticks for years, but they increase greatly in numbers and migrate to the salivary glands at the time of feeding. Gene expression at the RNA level has been studied using both array and quantitative reverse transcriptase (RT)-PCR approaches, and dramatic changes in gene expression on transmission of the pathogen from ticks to humans have been found (Figure 1). For example, transcript levels for the outer surface lipoprotein OspC can increase 30- to 120-fold in ‘mammalian tissue-like’ conditions compared with ‘unfed tick-like’ conditions, whereas OspA, another surface lipoprotein that binds to the tick midgut receptor TROSPA, thus enabling the bacterium to invade its tick host, is downregulated during transmission from tick to mammal [5-8]. Some of the regulatory pathways in this adaptive process have been identified, including a pathway involving the transcription initiation factors RpoN and RpoS. Temperature, dissolved
oxygen, and pH play a role in gene regulation, as well as other as-yet unidentified host factors.

It has been difficult to examine protein expression directly during mammalian or tick infection, as only a small number of spirochetes are present during most phases of infection, limiting the utility of conventional methods. A model system in which *B. burgdorferi* cultures are ‘incubated’ within dialysis tubing in the abdomens of rabbits or rats has been used extensively to study adaptation to the mammalian environment [9]. This set-up excludes contact with host cells and extracellular matrix, however, and some aspects of adaptation (for example, recombination in the antigenic variation gene *vlsE*, which encodes a variable lipoprotein) do not occur under these conditions. A novel approach to the direct study of bacterial protein expression in infected tissues took advantage of the fact that lipoproteins, prominent in *B. burgdorferi* and other spirochetes, are selectively partitioned to the detergent phase following solubilization in Triton X-114 [10]. By this means, VlsE, OspC, and the decorin-binding adhesin DbpA were found to be expressed at high levels in mouse joints and dermal tissue, and OspC and DbpA, but not VlsE, were found in heart tissue. These results suggest that protein expression varies between tissues; this pattern may be related to tissue tropism of the bacteria.

Global analysis of protein expression in Lyme disease *Borrelia* is now under way and will be useful not only for examining changes in gene expression, but also in understanding the biological importance of the multiple paralogous gene families and other unique properties of the predicted proteome. In one approach, Jacobs et al. [3] compared the protein profiles of three strains of *B. burgdorferi* using trypsin cleavage of whole-organism preparations followed by a two-step liquid chromatography separation of fragments and tandem mass spectrometry (MS/MS). In a second approach, Nowalk et al. [4] used two-dimensional gel electrophoresis with tryptic digestion and MS analysis of individual spots to look at the proteins in the soluble and particulate fractions of *B. burgdorferi* strain B31.

### Proteomic comparison of three *B. burgdorferi* strains

Jacobs et al. [3] compared the three strains B31, N40, and JD-1, which were chosen because they represent three genotypic groups that differ in their patterns of pathogenesis in humans and experimentally infected mice. Whole-cell lysates were treated with trypsin, and the resulting complex mixture of fragments was separated by strong cation exchange resin chromatography. Fractions of that separation were then subjected to reverse-phase capillary chromatography coupled with MS/MS analysis of the most abundant fragments in the initial MS separation. Between 13,500 and 17,300 peptides were isolated from each strain, and a total of 6,982 were confidently identified by comparison with protein predictions from the B31 genome sequence. From these data, 522, 498, and 471 proteins were detected in the B31, N40, and JD-1 preparations, respectively, which together represent 665 proteins, or roughly 38% of the predicted proteome.

The library of detected proteins from each strain exhibited a high degree of overlap (Figure 2): 52% of the proteins were detected in all three strains, and 72% were found in at least two of the strains. Between 59 and 69 proteins were identified in only one strain, which could be due to a number of factors. The most interesting possibility is that protein expression differs among the strains, correlating with the variations in pathogenesis. Some indication that this may be the case was provided by Jacobs et al. [3]; for example, 47% and 39% of the tryptic peptide coverage of the hypothetical protein BBH37 was identified in the B31 and JD-1 strains, respectively, whereas no BBH37 peptides were detected in N40. Two other proteins in this paralogous family (BBG01 and BBJ08) were apparently deficient in the JD-1 strain in comparison with the other two strains. Judging from the number of peptides detected, several other plasmid-encoded proteins were expressed at different levels in the three strains under the growth conditions used: OspC, OspD (BBJ09), the putative outer membrane porin Oms28 (BBA74), DbpA (BBA24), the fibronectin-binding protein BBK32, and hypothetical proteins BBI39 and BBJ34 (see [3], and in particular its Table 1 and supplementary information).

There are other possible reasons for the uneven detection of certain proteins in the three strains [3]. Only the B31 DNA sequence was used for analysis, although partial sequences...
characterization of the soluble and membrane-associated proteome

The other major approach to proteome characterization is two-dimensional gel electrophoresis followed by identification of individual spots on the gel by excision and MS analysis of tryptic peptides. In 1999, Jungblut et al. [12] analyzed the antigens of B. garinii using this method and the B. burgdorferi sequence; only a limited number of proteins were identified, however. Nowalk et al. [4] have now examined the proteins present in the soluble and membrane-associated (or, more accurately, the particulate) fractions of B. burgdorferi B31. One of the underlying problems with two-dimensional gel electrophoresis of Borrelia proteins is the high number and abundance of basic proteins, including the major membrane proteins OspA (pI = 8.3) and OspB (pI = 8.6). Basic proteins often migrate off the end of standard isoelectric focusing gels (even those with a broad pH range) or are poorly resolved. Nowalk et al. [4] addressed this issue by using either non-equilibrium pH gradient electrophoresis (NEPHGE) or immobilized pH gradients. With some modifications, NEPHGE generally out-performed the immobilized gradients in terms of both spot resolution and pH range.

A profile of the membrane-associated fraction obtained using NEPHGE followed by SDS gel electrophoresis is shown in Figure 3. Of the 160 spots detected by silver staining in this fraction, 34 proteins were identified by trypsin digestion and matrix-assisted desorption ionization time-of-flight (MALDI-TOF) MS. Similarly, the identities of 83 proteins were determined from the 185 spots in the soluble fraction; 12 proteins were detected in both fractions, so 105 proteins were identified in all. Most of the membrane-associated proteins detected were plasmid-encoded lipoproteins, consistent with the fact that the majority of the 150 predicted lipoproteins of B. burgdorferi are plasmid-encoded. Because two-dimensional gel electrophoresis patterns are reproducible, these identifications can be used in future studies to determine the effects of different incubation conditions, treatments, and fractionations on protein composition.

One of the surprising findings of this study was that the glycolytic pathway enzyme enolase was found in nearly equal concentrations in both the soluble and membrane-associated fractions, and aminopeptidase I and the chaperone GroEL were present in larger amounts in the membrane fraction than in the soluble fraction [4]. Enolase has, however, been found on the surface of staphylococci and streptococci, where it has been implicated in the adherence of the intact bacteria to plasmin, plasminogen and laminin. Because B. burgdorferi has both an inner and an outer membrane, however, it is not yet known whether the membrane-associated enolase is exposed on the surface. As a chaperone, GroEL may associate with membrane proteins during translocation and folding. Aminopeptidase I has also been shown to localize in the cytoplasm, periplasm, membrane and cell-wall fractions of other bacteria.

Comparison of the MS/MS- and gel-electrophoresis-derived proteomes [3,4] indicates, as expected, that the electrophoretic approach primarily identified the more abundant protein species in the MS/MS dataset (based on the number of peptides detected per protein). There were,
Genome and localization of groundwork for future studies on the expression patterns of these initial proteome characterizations lay important detection methods themselves. Thus, there are some biases in MS/MS dataset, and only one or two peptides were detected by electrophoresis were not represented in the MS/MS group that were not present in the electrophoresis analysis, consistent with the more limited sampling in the electrophoretic approach. The ribosomal proteins were the most prominent under-represented group; others included the periplasmic serine protease DO (BB0104), the putative surface-located lipoprotein Lmp1 (BB0210), glycerol-3-phosphate dehydrogenase (BB0388, BB0389). Conversely, 11 of the 105 proteins detected by electrophoresis were not represented in the MS/MS dataset, and only one or two peptides were detected by MS/MS for several others. Thus, there are some biases in the two approaches, either in sample preparation or the detection methods themselves.

These initial proteome characterizations lay important groundwork for future studies on the expression patterns and localization of B. burgdorferi proteins. Both methods can be adapted to provide quantitative comparisons of protein expression under different conditions, for example, incubation at different temperatures or pH, or conceivably following host adaptation in dialysis membrane chambers implanted in animals. In the whole-cell MS/MS analysis, parallel cultures can be differentially labeled using stable isotopes (14N and 15N) and cysteine affinity tags to quantitate differences in expression patterns [13], as in the recent analysis of the heat-shock response in the radiation-resistant bacterium Deinococcus radiodurans [14]. In the electrophoretic method, scanning of gels stained with Coomassie blue or other dyes can provide some quantitation. A more elegant approach, however, is difference gel electrophoresis [15], which comprises the differential labeling of organisms from two different incubation conditions using Ettan fluorescent labeling. The two preparations are labeled separately with derivitized Cy3 and Cy5, and then mixed together before electrophoresis. Fluorescence associated with the polypeptide spots is quantitated, and the corresponding Cy3 and Cy5 signals are used to determine differences in expression patterns between the two conditions.

The initial proteomes of B. burgdorferi and other organisms will serve as the basis for the global analysis of protein expression in response to different environments. In turn, the interfacing of these proteome datasets with other ‘-omes’, such as genome, transcriptome, interactome, and immunoproteome, may begin to reflect the actual complexity of bacterial physiology and pathogenesis.

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