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Pathogenic Bacteria; the Wisdom of their Genes

Abstract

To counteract the extensive repertoire of polymorphisms and immune mechanisms of their hosts, pathogenic bacteria have evolved several mechanisms for generating phenotypic diversity. In addition to classical gene regulation (phenotypic acclimation), population diversity is achieved through intragenomic mechanisms which alter the sequence or conformation of DNA. These include homopolymeric tracts and short repeats which affect the activity (transcription or translation) of genes, especially those which direct the interactions of the bacterium with the host environment. These hypermutable loci generate phenotypic diversity which is random in time but programmed with respect to location in the genome, thereby maximising polymorphisms within a clonal bacterial population while minimising the deleterious effects of increased genetic load.

So entrenched in contemporary society is the idea that microbes cause specific diseases, we are liable to forget that, barely a hundred years ago, the concept was novel and controversial. Germ theory and the ensuing golden era of microbiology remain one of the milestones in the history of medicine. A century later, microbial disease has undergone a revolution galvanised by the application of the new genetics. Molecular and cell biology are transforming our ideas on epidemiology, pathogenesis, diagnosis, treatment and prevention of microbial diseases.

The impact of molecular genetics and cell biology can be well illustrated with respect to three bacterial genera *Haemophilus influenza*, *Neisseria meningitidis* and *Streptococcus pneumoniae* which together account for most cases of pyogenic meningitis. Acute bacterial meningitis is a global problem of some magnitude. Most cases occur in young children at a time when their central nervous system is undergoing its critical period of development. Thus, in addition to mortality, there is considerable morbidity occurring in those children who contract meningitis but who, through the use of antibiotics and sometimes intensive care, are able to survive. Although this may be the majority in the Western world, death is the rule rather than the exception elsewhere, for example tropical Africa where there are regular epidemics of meningococcal meningitis.

Incidently, and as an historical footnote, the bacteria responsible for meningitis have played a not inconsiderable part in shaping the molecular era. It was in the Ministry of Health in London that the pathologist, Fred Griffith, carried out his experiments with pneumococci in which he showed that avirulent, capsule deficient organisms could be transformed to virulence by exposure to killed, encapsulated virulent pneumococci. After a hiatus of some twenty years, the transforming principle was recognised as nucleic acid by Avery and his colleagues at the Rockefeller (Avery *et al.*, 1994). In 1953, the structure of DNA was solved and, in the late 60s, a crucial step was the isolation of restriction enzymes from *Haemophilus influenzae*; their exploitation to construct a physical map of the SV40 virus ushered in the era of recombinant DNA. So, to stretch a point, the organisms responsible for meningitis have been of major significance in the history of molecular biology and in the technology of the new genetics.

Bacterial meningitis: a paradigm for the study of pathogenesis

Based on clinical observations and experimental studies, there is now a reasonable picture of the pathogenesis of *H. influenzae* meningitis. *H. influenzae* colonizes the respiratory tract efficiently, and most commensal strains of *H. influenzae* lack capsule, findings consistent with studies showing that this surface polysaccharide does not enhance, in fact decreases, the efficiency with which *H. influenzae* associates with epithelial cells or mucus. In experiments using human organ cultures, the propensity of *H. influenzae* to associate with mucus is very striking and suggests there are mechanisms which effect specific binding to receptors within mucus. *H. influenzae* elaborates cell wall components, such as lipopolysaccharide, and a low-molecular-weight factor, possibly a glycopeptide, that impair and ultimately disrupt ciliary function and cause damage to the respiratory epithelium (Wilson and Moxon, 1988). These events apparently predispose *H. influenzae* to associate with and damage the respiratory mucosa, mechanisms that could facilitate both localized and systemic disease.

There is evidence that adherence to and internalization by epithelial cells is enhanced by both fimbrial and nonfimbrial determinants, but their role in invasion and the occurrence of bacteraemia is uncertain. Studies of human organ cultures indicate that *H. influenzae* penetrates regions of damage where the integrity of the respiratory epithelial barrier has been breached. Subsequently, the bacteria appear to reach the blood by entering directly into endothelial cells rather than by the lymphatic route; *H. influenzae* is endocytosed by and translocates across human endothelial cells (Moxon, 1992a; Virji *et al.*, 1991).

It is useful to distinguish the separate events involved in invasiveness since different virulence determinants are implicated. Whereas the evidence that capsule enhances survival of *H. influenzae* in blood is unequivocal, capsule apparently does not facilitate translocation across cellular barriers. *In vitro*, both endothelial and epithelial cells internalize encapsulated strains less efficiently than they do

capsule-deficient variants, although translocation of both occurs. However, capsule could enhance transmission from one host to another and could also facilitate survival within cells. There are major deficits in our understanding of the mechanisms by which *H. influenzae* enters the blood and, in particular, why encapsulated type b strains cause clinically significant episodes of bacteraemia so much more often than do other strains.

The most compelling explanation for the virtual monopoly of type b strains in causing meningitis is that these strains resist the intravascular clearance mechanisms more efficiently than do strains of the other serotypes or those lacking capsule. Both clinical and experimental data indicate that the duration and magnitude of bacteraemia are critical to the occurrence of meningitis. In rats and primates, there is a strong correlation between the number of bacteria in the blood and the probability of meningitis (Moxon, 1992a).

In the pathogenesis of meningitis in the rat model, a further characteristic of bacteraemia must be accommodated in any comprehensive description of the mechanisms leading to CNS invasion. After intranasal inoculation, the population of organisms in the blood is derived from a very small number of progenitor organisms, often a single bacterium. Thus, *in vivo* replication of *H. influenzae* must be highly efficient if the ~18 generations required to reach concentrations of $\geqslant 10^5$/ml of blood are to occur within several hours. Indeed, calculation of the effective mean generation time (replication minus clearance) necessary to achieve these numbers indicates that doubling of organisms must occur about every 50 min. This is in agreement with serial measurements of the magnitude of bacteraemia. The balance of evidence favours the blood (or perhaps vascular endothelium) as the most likely site. Consistent with this, after an intravenous injection of small numbers of type b organisms (<10), serial blood cultures showed immediate exponential bacterial growth until concentrations of 10^5–10^7 bacteria/ml were reached. These data suggest that there must be efficient, intravascular proliferation of type b organisms (Moxon, 1992a).

The fact that the pathogenesis of bacteraemia involves the survival and proliferation of a single organism argues that there is a 'bottleneck' followed by clonal expansion and that the overwhelming majority of organisms are ultimately denied representation in the population of bacteria that infect the blood and the meninges. At what stage does this bottleneck occur? After intranasal inoculation of rats with about equal numbers of isogenic variants (distinguished by susceptibility or resistance to streptomycin), early blood cultures (>6 h after inoculation) showed that both variants were present. However, blood cultures obtained later (>12 h after challenge) yielded predominantly one or the other variant but not both. Thus, the bottleneck apparently occurs after translocation of bacteria from the nasopharynx to the blood (Moxon and Murphy, 1978).

The sequence of events, therefore, appears to be an early bacteraemia occurring within minutes to an hour after intranasal challenge, a latent period in which bacteraemia is undetectable (or nearly so) and proliferation of organisms result-

ing in high level bacteraemia. This three-stage profile of the pathogenesis of experimental bacteraemia conforms to a general pattern observed by several investigators. Apparently, in experimental *H. influenzae* infection, the third stage involves the emergence of a rare bacterial clone that eludes the host's clearance mechanism and sets up high-level bacteraemia - an event compatible with the occasional type b organism persisting in and escaping from some intracellular site of sequestration. Such a bacterium, it might reasonably be supposed, has been subjected to strong selective effects and may also have undergone phenotypic shifts favourable to survival.

The third stage of bacteraemia generates the necessary (but not sufficient) conditions for CNS invasion. The balance of evidence supports the choroid plexus as the site of bacterial entry, although a critical product of the number of bacteria and the duration of bacteraemia is required to facilitate bacterial entry into the CNS. Finally, there is the triggering of the host response through the interaction of biologically active mediators, such as lipopolysaccharide and cell wall glycopeptides, to produce inflammation and cellular damage.

The pathogenic personality and the molecular basis of virulence

Upon this platform of observations on the pathogenesis, the tools of molecular and cell biology have been applied to extricate the molecular details of the host-microbial interactions. Stanley Falkow, one of the pioneers of the application of molecular genetics to bacterial pathogens, has captured the essence of the molecular revolution in microbial disease through his revised version of Koch's postulates (Falkow, 1988). The modern goal is to identify the genes (or groups of genes) responsible for virulence, isolate these genes through recombinant DNA technology and amplify them *in vitro* through cloning or the use of polymerase chain reaction and prove their essential role by using genetically defined strains. For example by inserting either the wild-type or mutated genes into the microbe in question and assaying for virulence. From investigations that were limited to studies on virulence phenotype, the molecular version of Koch's postulates has now opened the door to the study of virulence genotype and, as a result, an opportunity to understand the fundamentals of the pathogenic personality. What, in molecular terms is virulence? In the context of meningitis and, indeed, the pathogenesis of most microbial diseases, the molecular analysis of microbial host interactions can be conveniently broken down into a triad of factors which together make up the complex entity referred to as virulence: 1) genes which determine tropism for the host and for a particular niche; 2) genes which are permissive for the microbe to survive clearance and replicate in the host; 3) genes which determine tissue damage (cytotoxicity) (Table 1).

Let us, very briefly, exemplify these three attributes of the pathogenic personality using examples from the bacteria causing meningitis. Most bacteria possess adhesins which facilitate interactions with a particular host and, within that host, particular tissues. Our example here is the pilus or fimbria which acts as a biological grappling hook; specific domains on the subunit polypeptide engage

Table 1.

Molecular Basis of Virulence	
Tropism	host and tissue specificity
Multiplication	mean generation time: factors include scavenging for nutrients, evading host clearance
Cytotoxicity	damage to host tissues

with specific receptors, in the case of the *H. influenzae* fimbria, lactosyl ceramide, found on human epithelial cells (Van Alphen *et al.*, 1991).

As an example of a determinant which facilitates survival and replication within the host, we have the capsular polysaccharides which protect microbes against bacterial lysis and opsonophagocytosis, activities mainly carried out by complement, antibodies and cells of the monocyte/macrophage family. Efficient clearance requires serum antibody to the capsule. In the absence of specific antibodies, *H. influenzae* multiply voraciously in the intravascular compartment and disseminate to sites such as the CNS to cause meningitis. Induction of serum antibodies through immunisation has resulted in the virtual elimination of disease from several countries in Europe and from North America (Shapiro, 1993; Booy *et al.*, 1994).

Elaboration of microbial toxins results in tissue injury either directly, as in the case of true toxins, or through the triggering of inflammation, as in the case of endotoxins. To quote Lewis Thomas (Thomas, 1974): 'It is the information carried by bacteria that we cannot abide. For example, they display lipopolysaccharide endotoxin in their walls, and these macromolecules are read by our tissues as the very worst of bad news. When we sense lipopolysaccharide, we are likely to turn on every defence at our disposal; we will bomb, defoliate, blockade, seal off, and destroy all the tissues in the area. Leucocytes become more actively phagocytic, release lysosomal enzymes, turn sticky, and aggregate together in dense masses, occluding capillaries and shutting off the blood supply. Complement is switched on at the right point in its sequence to release chemotactic signals, calling in leucocytes from everywhere. Vessels become hyper-reactive to epinephrine so that physiological concentrations suddenly possess necrotising properties. Pyrogen is released from leucocytes, adding fever to haemorrhage, necrosis and shock.'

Genetics can pinpoint the molecular basis of tropism, multiplication in the host and cytoxicity, the key components of virulence. The molecular analysis of virulence has also uncovered subtleties in the workings of those genes which are essential to a deeper understanding of the interactions and the co-evolution of hosts and microbes.

Evolutionary biology of virulence

Any student of infectious diseases faces the challenge of understanding the virulence of microbes and its implications for their evolution and that of their

hosts. It is worth emphasising not just the molecular basis of virulence—the triad of tropism, multiplication within host tissues, and cytotoxicity—but also that mutuality is of the essence. When we say that a microbe is virulent, we must necessarily ask 'for which host' and conversely, if a host is susceptible, 'to what microbe?' There is currently a strong motivation to seek answers to these questions which inform us at the molecular level.

On the one hand, one can construct plausible rationales for the role of many virulence factors, especially toxins; for example, the toxins of cholera and pertussis bacteria may facilitate their transmission. But the biological advantage of many virulence factors, including the polysaccharide capsules and invasins of the bacteria causing meningitis, is not so obvious. After all, strains of *H. influenzae*, which lack the genetic basis for expression of capsule, are enormously successful commensals. Indeed, there seems little intuitive wisdom in causing meningitis. which merely kills the host and is, literally, a 'dead-end' for the bacterium. Perhaps then the molecular analysis of pathogenic bacteria can give us insight into what Stephen Wills has engagingly called 'the wisdom of the gene' (Wills, 1989). We will attempt this by examining some ideas on the evolution of virulence behaviour which emphasise the role of phenotypic variation, adaptive behaviour and hypermutation of pathogenic bacteria.

A challenge for infecting bacteria; life without sex

Evolutionary biologists have for some time taken a keen interest in the interactions of microbe and host and the ensuing conflicts which determine their co-evolutionary trajectory. This mutuality has attracted such colourful metaphors as gene for gene arms races (Dawkins and Krebs, 1979) or the Red Queen hypothesis (Van Valen, 1973). Populations of a particular species of bacteria, or other microparasites such as viruses, can outnumber those of their host because of their relatively rapid generation time but, since they divide by binary fission, give rise to a clonal population. Intuitively, there would seem to be a need for a clonal population to have mechanisms for generating phenotypic diversity as a strategy for surviving the gauntlet of varying microenvironments, the repertoire of polymorphisms and the eclectic immune clearance mechanisms which are characteristic of hosts. This is particularly relevant to certain acute infections in which the inoculum transmitted from one host to another is subjected to a bottleneck and where it has been shown that the entire population of infecting bacteria is derived from a single organism, or a very small number (Moxon, 1978).

Mechanisms for generating phenotypic diversity

During the course of an infectious episode with a particular species of bacteria, classical point mutations (transitions or transversions) occur relatively infrequently. Also, the contribution of horizontal transfer of genes (sex) would be so rare as to be negligible despite the latter's crucial evolutionary importance in the

long term. Nonetheless, intraclonal polymorphisms within bacterial populations are extensive. Over the past decade, there has been an explosion of knowledge detailing the rich panoply of molecular mechanisms through which pathogenic microbes generate phenotypic diversity (Figure 1). Some of these are examples of phenotypic acclimation (classical gene regulation). This is well exemplified by catabolite repression and two component signalling mechanisms, histidine protein kinases; environmental signals, such as those experienced when a bacteria translocates from an extracellular to an intracellular environment, effect changes in phenotype. In contrast to these programmed or discriminate mechanisms, there are the many examples of genetically determined antigenic variation which result from mutational events. Many of these would seem to provide the opportunistic strategies through which clonal populations can furnish the diversity upon which selection can act. By opportunistic, we are emphasising the requirement for bacteria to evolve mechanisms with which to cope with sudden and unpredictable environmental fluxes, situations which in an evolutionary sense are novel or have not been previously experienced. This has been referred to by Gunsalus as the 'biology of anticipation' (Ornston *et al.*, 1990). Pathogenic bacteria face the same challenges as do the genomes of all biological systems; how to attain the optimal constraints on genetic change while maintaining the potential for rapid diversification. An example of this strategy, which can be considered as a biological paradigm of how organisms cope with the unpredictable, is exemplified by the evolution of hypermutable loci. Because the probability of these mutations is stochastic with respect to time but not location, these loci generate phenotypic diversity at high frequency while minimising the deleterious effects of increased genetic load. We have referred to these hypermutable sequences as *contingency loci* to emphasise their role in facilitating the

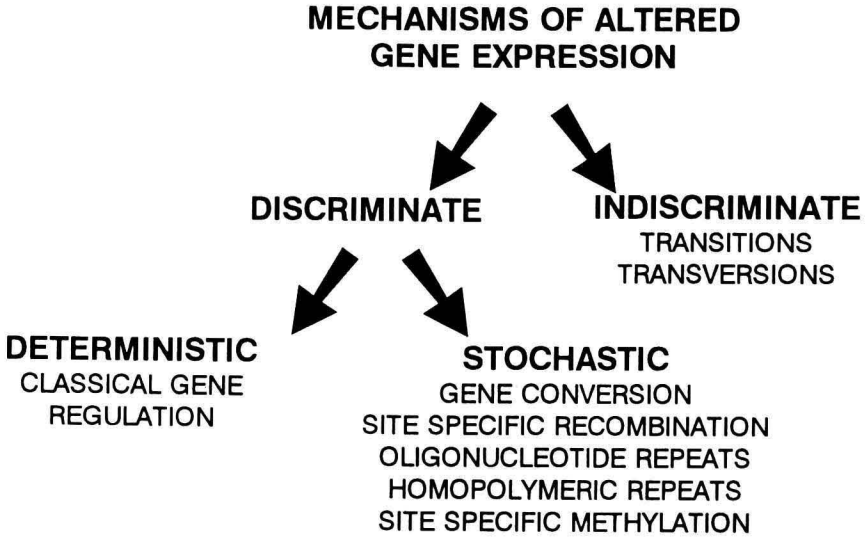

Fig. 1. Mechanisms of altered gene expression (Rainey, 1993).

adaptive behaviour of bacteria when confronted with sudden and unpredictable challenges to their survival (Moxon *et al.*, 1994).

Many hypermutable contingency loci consist of short repeats or homopolymeric tracts positioned either within genes or in important adjacent regions which are involved in transcription, e.g. promoters. These sequences influence the activity of these genes because the repeats are subject to polymerase slippage so that the number of copies of the repeated nucleotides increase or decrease (Moxon and Maskell, 1992b). This introduces frame shifts altering translation or the efficiency with which target sites bind transcriptional factors. In most of the examples which have been described to date in pathogenic bacteria, the altered activity of genes subject to slipped strand mispairing occurs in one out of every 100–1000 bacteria per generation when the rates are studied *in vitro*.

Antigenic variation of core lipopolysaccharides of Haemophilus influenzae

LPS is a macromolecule that is unique to Gram-negative bacteria, and it is the major component of the outer leaflet of the cell envelope. LPS consists of lipid A, which is anchored in the cell envelope, and core saccharide, which extends out from the cell surface. LPS is known to be a major virulence determinant of *H. influenzae*. Phenotypic variation in *H. influenzae* cell-surface LPS provides a further example of random variation generated by high frequency mutations. In this case, however, the variation is not due to a transcriptional mechanism like that responsible for fimbriae phase variation. Rather, the phenotypic expression of several LPS core saccharide structures can be reversibly lost or gained despite continuous synthesis of mRNAs for the enzymes of LPS biosynthesis. The switch is instead effected at the level of translation of these mRNAs, which is turned on and off by frame-shift mutations (Weiser *et al.*, 1989). As the different core saccharide structures switch randomly and independently of each other, a population of *H. influenzae* founded by a single clone and residing within a single host can generate an extensive repertoire of variant LPS epitope.

The molecular basis of the variable expression of one of these saccharide structures, galα(1-4)galβ (Virji *et al.*, 1990) has been studied in some detail. When colonies of *H. influenzae* were blotted on to nitrocellulose filters and allowed to react with a monoclonal antibody specific for the structure galα(1-4)galβ, individual colonies showed either strong, intermediate, or undetectable binding. Mutants have been isolated which do not express the digalactoside structure and one of these maps to a gene designated *lic2*, a gene essential for synthesis of the digalactoside (High *et al.*, 1993). At the 5′ end of the *lic2* gene, there are multiple tandem repeats (ca. 16) of the tetramer 5′-CAAT-3′. However, the number of CAAT repeats varies; loss or gain of CAAT, usually a single repeat, moves upstream initiation codons in and out of frame with the remainder of the gene, thereby creating a translational switch (Weiser *et al.*, 1989; Weiser *et al.*, 1990) and resulting in variable synthesis of the digalactoside. Similar to the phase variation of fimbriae, the presumed mechanism for the frame shift is slipped-strand mispairing (Levinson and Gutman, 1987), a *recA*-independent mechanism capable of mediating

high-frequency random changes in nucleotide sequence. It should also be noted that the resulting mutation and its potential for fortuitously adaptive changes in phenotype can occur either during the replication process or through mismatch repair; the latter mechanism is potentially important with respect to the behaviour of bacteria under stress and perhaps in a non-replicating state (stationary phase). It may be significant that the region immediately up-stream of *lic2* is especially rich in AT nucleotides (High *et al.*, 1993). This would tend to facilitate strand separation and increase the likelihood of slipped-strand mispairing; such a region would be prone to the effects of altered supercoiling, since changes in superhelicity are known to affect transcriptional efficiency. There is accumulating evidence in support of a role for global regulation of gene expression through changes in supercoiling (Higgins and Dorman, 1990).

A typical characteristic of contingency loci is that they affect the activity of genes responsible for the expression of surface molecules which are important in the interactions of the bacterium with its host. Through a few such key genes, whose activities are subject to these mutational hot spots, this high frequency phenotypic variation influences such characteristics as antigenicity, motility, chemotaxis, attachment to host-cells, acquisition of nutrients, and sensitivity to antibiotics while concurrently minimising the deleterious effect that high mutation rates would impose on housekeeping functions of the genome (Figure 2). But propitiously, homopolymeric tracts or short nucleotide repeats could also provide a mechanism which lends itself to some degree of environmental responsiveness. For example, changes in supercoiling would be particularly likely to influence regions of DNA such as short tandem repeats which undergo strand separation, exposure of single stranded DNA, changes in orientation of sequence and alternative DNA structures. It is well established that changes in the environment can affect the topology of DNA and changes in supercoiling can affect the activity of genes.

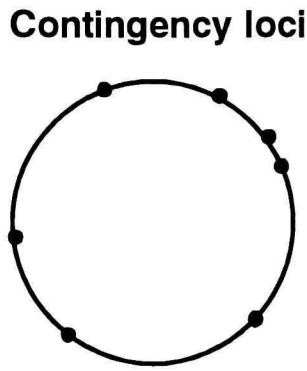

Fig. 2. A bacterial genome, double stranded circular DNA, is depicted with several hypermutable loci [●] which result in altered activity of genes. These contingency loci result in changes in phenotype which occur at high frequency [about 1:100–1000 bacteria per generation] and typically affect the activity of genes which interact with environment. These mutational events are random in time and therefore generate heterogeneity within a clonal population of replicating bacteria. They are non-random in location and therefore maximise intraclonal polymorphisms while minimising deleterious effects on the genome as a whole.

The concept that cells should be at their most mutable when conditions are unfavourable to an organism is appealing. Indeed, stress-induced mutagenesis is not a new concept. McClintock captured the idea quite beautifully: 'These ..... responses, now known to occur in many organisms, are significant for appreciating how a genome may reorganise itself when faced with a difficulty for which it is unprepared.' (McClintock, 1993)

The conventional view is that the environment selects among pre-existing variants; mutations arise without regard for their utility. But, intuitively, it is evident that it could be advantageous for organisms to evolve mechanisms through which the environment could influence the genetic mechanisms which generate phenotypic variation and upon which selection acts. Have mechanisms evolved which result in mutations which arise when, and even because, they are advantageous?

In a letter to Nature in 1988, Cairns, Overbaugh and Miller pointed out that the classical Luria-Delbruck experiments were not designed nor were they able to detect mutants that arose during or following the imposition of lethal selection (Cairns *et al.*, 1988). Two types of data were presented which, they suggested, provided some evidence to suggest that 'cells may have mechanisms for choosing which mutations will occur.' First, fluctuation tests in which *Escherichia coli* organisms deficient in lactose gave distributions of lac+ mutants which suggested that they arose only in response to selection. Second, plate tests in which lac+ organisms accumulated over time when lac− cells were incubated in the presence of added lactose, but failed to do so when lactose was not present. This class of mutations has been given various names, including directed, adaptive, non-random and selection induced (Foster, 1993). Can we countenance ideas 'suggesting that cells may have mechanisms for choosing which mutations will occur' (Cairns *et al.*, 1988)? In the ensuing years, these and some later data have been the subject of spirited, at times heated, controversy involving evolutionary biologists and bacterial geneticists who have debated whether or not the occurrence of 'directed mutations' was real and if so, the molecular mechanisms giving rise to them (Lenski and Mittler, 1993). There is now convincing evidence that some mutations do occur in non-replicating bacterial populations after the imposition of changes to their external environment, such as the provision of a novel nutrient. Further and importantly, these mutations appear to be different from mutants which are present in the population prior to the addition of a selective agent (Rosenberg *et al.*, 1994; Foster and Trimarchi, 1994). No entirely satisfactory model which would explain directed mutations has yet been advanced. But does the behaviour of 'stationary phase' bacteria plated on to simple laboratory media have relevance to the biology of infectious disease and the evolution of commensal and virulence behaviour? The link between directed mutations in *E. coli* on the one hand and hypermutation in contingency loci of pathogenic microbes on the other stems from observations that the mechanism involved in both instances involves frame shifts in small nucleotide repeats or homopolymeric tracts presumptively through polymerase slippage.

It has been a central tenet of molecular biology that there is independence of genetic information from events occurring outside or even inside the cell. Indeed, it has been argued that 'the very nature and structure of the genetic code and the way it is transcribed implies that no information from outside can ever penetrate the inheritable genetic message' (Judson, 1979). Thus, it has been argued that specific mutations do not occur at higher rates when they are beneficial than when they are neutral or disadvantageous. So, with respect to the proposition that organisms can selectively enhance mutations in specific genes in response to signals from the environment, would not such a mechanism challenge the fundamental tenets of Darwinian theory that mutations are random with respect to their selective utility?

In an insightful essay, Keller argues that the meaning of randomness in mutations provides the key to this escape (Keller,1992). To quote B.D. Davis on randomness: 'Used to mean an undirected process, occurring by chance in any gene, this term has become firmly embedded in classical genetics. But equally clearly, randomness in the strict mathematical sense, as equal probability, does not apply to the genome at the level of nucleotides or of short sequences surrounding a site of mutation. The notion of biased randomness is thus not a radical one; it has been with us since the discovery of fine structure genetics, with its recognition of mutational hot spots.' (Davis, 1989)

It does not seem unreasonable to extend the notion to DNA sequences subject to influence by the environment. There is no evidence that external signals can instruct bacterial cells to make specific mutations - the most unorthodox of the proposed mechanisms to explain directed mutations and the one which made the issue rather controversial (Cairns *et al.*, 1988). Rather, it would seem that variants generated by specialised, hypermutable sequences (contingency loci) are programmed (directed) in that they have evolved so as to alter the activity of a few, strategically important genes. Indeed, that directed mutations might appear to result from environmental instruction is testimony to the power of an event (mutation) which is stochastic in time and in the direction of the phenotypic switch, but programmed (evolved) in its assignment to specific chromosomal locations. Thus, the concept of hypermutable 'contingency loci' offers a neo-Darwinian explanation for adaptive mutations in which there is no requirement for any novel molecular mechanisms or a 'reverse flow of information' (Cairns *et al.*, 1988). The trial and error model proposed by Hall can be adapted so that the hypermutable state is localised to particular regions of the genomes of each bacterium (Hall, 1990). The slow repair and error prone tendency of the stressed bacterium mean that errors occur at particularly high rates (MacPhee, 1993). If the cell achieves success, it exits and resumes growth. This seems to be particularly apposite to pathogenic bacteria in which hypermutation through repetitive DNA, in principle environmentally responsive, offers the advantage of the biology of anticipation at a minimal cost to the fitness provided by the evolution of biological memory (housekeeping genes). It also suggests a good explanation for the bottleneck which occurs in the pathogenesis of menigitis (Moxon and Murphy, 1978); indeed, it would appear that in many infections, there is a stage

when the progression of infection depends upon clonal expansion of a few microbes, even a single founder organism.

Perspective

Despite our preoccupation with disease, business as usual for pathogenic microbes is the commensal state, an opportunity for residence, multiplication and transmission. In this paper, we have examined the molecular basis of some of the phenotypic variation occurring in certain pathogenic bacteria. We have suggested that pathogenic bacteria have mechanisms that promote extensive phenotypic variation while maintaining their genomes more or less intact and that these contingency strategies are a component of the evolution of the pathogenic personality. For some host-adapted bacterial pathogens responsible for invasive infections, virulence reflects the evolution of a repertoire of mechanisms which maximise their success to colonise the mucosal surfaces, such as the respiratory tract, and to promote opportunities for transmission to another human host. For example, invasion of host epithelial cells may enhance successful residence in the host and allow sequestration of bacteria from host clearance mechanisms which act in the extracellular environment. Although this attribute also facilitates systemic invasion and the potential for killing its host, this occurrence is a relatively rare event and so the host-microbial strategy approximates closely to that of balanced parasitism. Disease is an incidental and accidental consequence of the relentless drive of microbes to perpetuate their selfish genes. Pathogenicity is the flip side of a coin which constitutes the currency of survival.

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