

- 38 Maratea, D. *et al.* (1985) Deletion and fusion analysis of the ϕ X174 lysis gene *E. Gene* 40, 39–46
- 39 Buckley, K.J. and Hayashi, M. (1986) Lytic activity localized to membrane-spanning region of ϕ X174 E protein. *Mol. Gen. Genet.* 204, 120–125
- 40 Roof, W.D. *et al.* (1994) *slyD*, a host gene required for ϕ X174 lysis, is related to the FK506-binding protein family of peptidyl-prolyl *cis-trans*-isomerases. *J. Biol. Chem.* 269, 2902–2910
- 41 Wülfing, C. *et al.* (1994) An *Escherichia coli* protein consisting of a domain homologous to FK506-binding proteins (FKBP) and a new metal binding motif. *J. Biol. Chem.* 269, 2895–2901
- 42 Hottenrott, S. *et al.* (1997) The *Escherichia coli* SlyD is a metal ion-regulated peptidyl-prolyl *cis-trans*-isomerase. *J. Biol. Chem.* 272, 15697–15701
- 43 Mitterauer, T. *et al.* (1999) Metal-dependent nucleotide binding to the *Escherichia coli* rotamase SlyD. *Biochem. J.* 342, 33–39
- 44 Scholz, C. *et al.* (1999) R73A and H144Q mutants of the yeast mitochondrial cyclophilin Cpr3 exhibit a low prolyl isomerase activity in both peptide and protein-folding assays. *FEBS Lett.* 443, 367–369
- 45 Witte, A. *et al.* (1998) Mutations in cell division proteins FtsZ and FtsA inhibit ϕ X174-protein-E-mediated lysis of *Escherichia coli*. *Arch. Microbiol.* 170, 259–268
- 46 Bradley, D.E. *et al.* (1969) Structural changes in *Escherichia coli* infected with a ϕ X174-type bacteriophage. *J. Gen. Virol.* 5, 113–121
- 47 Gschwender, H.H. and Hofschneider, P.H. (1969) Lysis inhibition of ϕ X174, MS2, and Q β -infected *Escherichia coli* bacteria by magnesium ions. *Biochim. Biophys. Acta* 190, 454–459
- 48 Lubitz, W. *et al.* (1984) Requirement for a functional host cell autolytic enzyme system for lysis of *Escherichia coli* by bacteriophage ϕ X174. *J. Bacteriol.* 159, 385–387
- 49 Lubitz, W. *et al.* (1984) Lysis of *Escherichia coli* after infection with ϕ X174 depends on the regulation of the cellular autolytic system. *J. Gen. Microbiol.* 130, 1079–1087
- 50 Witte, A. *et al.* (1997) Proline 21, a residue within the α helical domain of ϕ X174 lysis protein E, is required for its function in *Escherichia coli*. *Mol. Microbiol.* 26, 337–346
- 51 Bernhardt, T.G. *et al.* Genetic evidence that the bacteriophage ϕ X174 lysis protein inhibits cell wall synthesis. *Proc. Natl. Acad. Sci. U. S. A.* (in press)
- 52 Ikeda, M. *et al.* (1991) The *Escherichia coli* *mraY* gene encoding UDP-N-acetylmuramoyl-pentapeptide: undecaprenyl-phosphate phospho-N-acetylmuramoyl-pentapeptide transferase. *J. Bacteriol.* 173, 1021–1026
- 53 Brunskill, E.W. and Bayles, K.W. (1996) Identification of LytSR-regulated genes from *Staphylococcus aureus*. *J. Bacteriol.* 178, 5810–5812
- 54 Kawarabayasi, Y. *et al.* (1998) Complete sequence and gene organization of the genome of a hyper-thermophilic archaeobacterium, *Pyrococcus horikoshii* OT3. *DNA Res.* 5, 55–76

Horizontal gene transfer and the origin of species: lessons from bacteria

Fernando de la Cruz and Julian Davies

The old topic of horizontal gene transfer (HGT) has become fashionable¹. From the overwhelming surge of genome sequence information, more and more candidates for horizontally transferred genes are being identified: among prokaryotes²; from bacteria to eukaryotes³; from bacteria to archaea⁴; from animals to bacteria⁵; and so on. It is clear that genes have flowed through the biosphere, as in a global organism. HGT, once solely of interest for practical applications in classical genetics and biotechnology, has now become the substance of evolution. However, amid this new-found enthusiasm for an old subject, the existence of HGT in eukaryotes is viewed with skepticism, as there is a distinct lack of mechanistic studies that would

In bacteria, horizontal gene transfer (HGT) is widely recognized as the mechanism responsible for the widespread distribution of antibiotic resistance genes, gene clusters encoding biodegradative pathways and pathogenicity determinants. We propose that HGT is also responsible for speciation and sub-speciation in bacteria, and that HGT mechanisms exist in eukaryotes.

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help to explain such a broad-host-range process (i.e. one that operates not only between species and genera, but also between kingdoms). In this particular case it is apparent that, once again, we can learn from our vast experience with prokaryotes, especially as typical methods of carrying out HGT in prokaryotes, such as transfection or even bacterial conjugation^{6,7}, can be used for the genetic manipulation of eukaryotic cells. There are a number of well documented examples in which bacterial adaptation has been influenced by HGT. Studying these examples can provide an appreciation of the general mechanistic trends involved, as well as providing the foundation for considerations of a ubiquitous role of HGT in shaping modern eukaryotic species.

Table 1. Mobile genetic elements involved in HGT in bacteria

Mobile element	General features	Examples	Phenotype of specific element	Ref.
Gene cassette	Mobility of 'simple genes' Immediate constitutive expression Extremely broad host range	Int1	Antibiotic resistance (simple)	30
		Int4	Gene 'maturation' in <i>Vibrio</i>	31
Transposon	Mobility of genes and operons Very broad host range Loci screening for best performance	Tn21	Antibiotic resistance (complex)	32
		Tn4651	Toluene degradation	33
Plasmid	Mobility of operons and regulons Immediate regulated expression Very broad host range	pNRG234a	Nodulation plasmid of <i>Rhizobium</i>	34
		pNL1	Degradation of aromatic compounds	12
		pCD1	Virulence determinants of <i>Yersinia</i>	35
Bacteriophage	Mobility of regulons 'Maturation' in chromosomes (via lysogeny) Narrow host range	CTX Φ	Virulence phage of <i>Vibrio cholerae</i>	36

Abbreviation: HGT, horizontal gene transfer.

Antibiotic resistance: the immediate response

The dissemination of antibiotic resistance genes among human and animal bacterial pathogens and associated commensal populations is the paradigm for HGT on a global scale⁸. This 'experiment' has taken place during the past 50 years, and a significant amount of information has been accrued on the mechanisms of response (evolution) of different bacterial ecosystems to the addition and distribution of large amounts of toxic agents (antibiotics) within the biosphere. This is the best known example of very rapid adaptation of bacterial populations to a strong selective pressure. What has been learned is that this adaptation occurred (and still occurs if bacteria are challenged with a novel antibiotic) not by mutation of the menaced populations, but by acquisition and dissemination of, in most cases, simple antibiotic resistance genes by mobile genetic elements (Table 1). It should be noted that studies of antibiotic resistance development are essentially retrospective, and although much has been learned, little is known of the microbial dynamics of this process. Nonetheless, the basis of antibiotic resistance development is formally simple: mobile genetic elements such as plasmids and transposons efficiently distributed the resistance determinants, singly or in clusters, among many genera and species of bacteria. Intergeneric boundaries were not respected, particularly the long-standing but only apparent distinction between Gram-positive and Gram-negative bacteria. This latter distinction is almost certainly a figment of human imagination that has been ignored by microorganisms for billions of years.

Biodegradation pathways: delayed opportunistic response

Based on studies with antibiotic resistance and its associated genes (e.g. genes for the metabolism of a number of sugars), it is not surprising that HGT has also led to the dissemination of gene clusters (operons) involved in the catabolism of xenobiotics in

polluted environments⁹⁻¹¹. The selective pressures are similar to those of antibiotic resistance, but the process of degrading xenobiotic compounds requires more complex genetic systems, usually operons of ten or more genes, or even regulons of several operons with accompanying control circuits. An example of this can be found in the *Sphingomonas aromaticovorans* plasmid pNL1 (Ref. 12). This large conjugative plasmid contains 15 gene clusters directly associated with the catabolism or transport of aromatic compounds, allowing the host bacteria to degrade compounds such as biphenyl, naphthalene, xylene and cresol. Although the dissemination of biodegradation pathways has not been analysed to the same extent as that of antibiotic resistance, the available data clearly indicate that the genetic mechanisms responsible are likely to have been the same: plasmids and transposons distributed catabolic operons among a wide range of bacteria (see Ref. 13). It can be assumed that the degradation of xenobiotics by bacteria has intensified in the past century, as a result of the increased use of chemicals in industry and the waste disposal habits of humans, but the basic processes could have evolved much earlier. For example, pathways for the degradation of recalcitrant aromatic compounds resemble lignin degradation pathways, from which they probably derive¹⁴. This is in contrast to antibiotics, which were not in use before the 1940s (with the exception of sulfonamides, disinfectants and mercury compounds), a time when antibiotic resistance genes were very scarce among human pathogenic bacteria^{15,16}. Significantly, degradative operons are also found integrated in the bacterial chromosome. In these cases, adjacent remnants of phage or plasmid genes remind us of the once-mobile nature of these operons.

Pathogenicity determinants: delayed internalized response

Judging from these examples, it would appear that plasmids, in combination with transposons, are the primary vehicles for HGT in bacteria, at least for

emergency responses to strong selection pressures. What other traits do naturally occurring plasmids and transposons carry? The answers to this question could give clues about bacterial responses to other types of stress, and perhaps help discover other evolutionary forces that shaped bacterial species. Plasmids in pathogenic bacteria are loaded with virulence determinants, ranging from toxin production and immune evasion, to overt sabotage of the cellular machinery, as in the case of the oncogenic Ti plasmid. The importance of 'pathogenic' plasmids cannot be overemphasized as, in many cases, they carry the backbone of genetic information that makes a given bacteria a pathogen, so much so that they can be considered speciation determinants. The Ti plasmids of *Agrobacterium*, the nodulation plasmids of *Rhizobium* and the virulence plasmids of *Yersinia*, *Shigella* and *Escherichia coli* are examples of plasmids that, in essence, create new bacterial species when they are acquired, so drastic are the phenotypic variations they beget. Thus, virulence, that is the mechanisms of bacterial adaptation that allow the colonization of a new niche when that niche is created by the appearance of a new multicellular organism, occurs not by mutation, but by the acquisition of pathogenicity determinants by HGT.

However, not all pathogenic determinants are found on plasmids. One of the first recognized examples of a virulence gene was that encoding the toxin of *Corynebacterium diphtheriae*, the agent of diphtheria, which is carried by a temperate phage (a dormant phage, integrated into the host chromosome). Numerous other cases of temperate phages carrying virulence determinants have been identified in a range of bacterial pathogens, such as *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Shigella dysenteriae* and *E. coli*¹⁷. Whatever the frequency of pathogenicity plasmids or phages, genomic studies have shown that most pathogenicity genes are located in the bacterial chromosome, where they exist as discrete gene clusters named pathogenicity islands. It would appear that pathogenicity can be considered a new epitome in the evolution of bacterial species.

The ability to survive in very specific hostile environments, such as on and within other organisms, can be assumed to be a slow evolutionary process compared with the occurrence of resistance determinants. Pathogenicity islands are the evolutionary paradigm of slow selection pressure. They are mostly integrated into their host bacterial chromosomes, but evidence suggests that they are, or were, mobile elements. In most cases, they resemble defective bacteriophages, although occasionally they are associated with the remnants of plasmid conjugative systems or compound transposons¹⁷. Bacteriophages are probably effective tools by which HGT can provide a more permanent adaptation to new environments. One or more regions of DNA are acquired by fortuitous transduction, by their inclusion in defective phage or by Hfr-type conjugation in the case of the donor being a plasmid, and incorporated into the bacterial chromosome. Subsequently, the bacteriophage or

plasmid vehicles are rendered inactive by deletion or insertional inactivation, so that the incorporated genes are fixed. On the evolutionary scale, the acquisition of pathogenicity islands occurs at a much slower pace than that of plasmids, and would obviously create new bacterial subspecies (i.e. the recombinants have discernibly different phenotypes). Because multiple pathogenicity islands, varying in size and in location on the host chromosome, can be found in any bacterial strain, they must have been inherited independently and could well have come from different donor organisms at different times via different routes. It is not yet known if pathogenicity islands go through a process of being 'hosted' and then adapted to different recipient bacterial genera and species before their identification in a contemporary clinical isolate. This introduces a pertinent concept: that of quantum evolutionary leaps. Pathogenicity islands probably did not arrive in their contemporary host as the result of a single event; rather, they were hosted and adapted to one or more intermediate species on their path to identification.

The shaping of a bacterial species

The fact that a number of pathogenicity determinants are presently found on mobile elements, or, alternatively, are fixed in the chromosome but still show remnants of the vehicles that led them there, suggests that chromosomes, classically viewed as essentially immobile, are in fact the 'genetic necropolis' of previously mobile genes. Who knows the full extent of micro-geographical terms that will spring from bacterial genomics: we could soon have islets, peninsulas or even genetic archipelagos! Armed with the awareness that HGT is so important in bacterial speciation, it is now possible to examine completely sequenced chromosomes in a new light and assess the extent of chromosomal DNAs of obscure lineage.

Using *E. coli* and *Salmonella* as a model system, it has been shown that 17% of their genomes (i.e. ~800 kb), appears to have been acquired by HGT during the past 100 million years (the housekeeping genes of both organisms are ~90% identical)¹⁸. As the majority of this DNA was recruited recently, it is apparent that considerable genetic flux is occurring: the 234 detectable HGT events that have persisted are probably the tip of the iceberg of the thousands of mobile sequences that have entered and left any particular *E. coli* strain. Similarly, when the members of a well known collection of *E. coli* strains (the ECOR collection) were compared, they were found to be quite variable in the size and macro-organization of their chromosomes^{19,20} and plasmids^{21,22}. Additionally, a significant proportion of the *Salmonella* 'unique' genes are involved in virulence, underlying the importance of HGT-acquired genes for speciation²³. In summary, a significant proportion of the genome of any strain of a single bacterial species comprises fragments of DNA from various origins which, properly 'nurtured', can give rise to new bacterial species.

How many HGT steps are involved in the creation of a new species? We can use *E. coli* O157:H7 as an

example of a new ‘quasi-species’. Although its genome has not been completely sequenced, it is known to contain more than 20 potential virulence genes clustered in several mobile elements: the large plasmid pO157, the lambdoid temperate phage 933W, another cryptic prophage and the pathogenicity island LEE, all textbook examples of modular construction of pathogenicity determinants by HGT (Table 2). The genome of *E. coli* O157:H7 undoubtedly contains more surprises, as it is 700 kb larger than that of *E. coli* K-12. Genomic comparisons of a number of *E. coli* strains, including pathogenic variants, will define the essential core of the *E. coli* chromosome, and will give a more significant idea of the collective amount of mobile DNA available as an extended genotype, including the inserts that made *E. coli* O157:H7 highly pathogenic for humans. We would like to contrast the immediate and powerful HGT events that resulted in *E. coli* O157:H7 with the results obtained by ‘solitary confinement’ evolution of *E. coli* in the laboratory during 20 000 generations²⁴, where the bacterial genome remains basically intact if allowed to evolve (presumably to a simpler genome adapted to rapid growth in a predictable non-variant medium) without the help of acquired exogenous DNA. It will be exciting to compare these results with an experimental set-up in which additional bacterial DNA is allowed, periodically, to enter the system.

It would therefore appear that much of the speciation and sub-speciation in bacteria can be explained as the result of macroevolution events mediated by HGT, an alternative to fixation of point mutations. Our proposal stems from the fact that it is the acquisition of new genes that allows a new specific survival strategy (and thus the concept of a new species). Further adaptation to the newly conquered niche will occur by successive point mutations, and only after millions of years will the new species be recognized by hybridization or 16S RNA sequencing. Thus, we have to distinguish between the radical speciation event (by HGT) and installation of the new ‘variant’ as a recognized species (by chromosomal divergence), as shown in Fig. 1.

HGT in eukaryotes: the lessons from bacteria

If many bacterial speciation events can be traced to the acquisition

Table 2. Pathogenicity-related mobile elements of *Escherichia coli* 157:H7 acquired by HGT

Name	Type	Size (kb)	Virulence factors	Ref.
pO157	Plasmid	92	Hemolysin Large clostridial toxin Catalase–peroxidase Extracellular serine protease	37
933W	Lysogenic phage	62	Shiga toxin 2 Ser/Thr protein kinase Three novel tRNA genes Macrophage survival Serum resistance	38
LEE	Pathogenicity island	43	Intimin Tir	39

Abbreviations: HGT, horizontal gene transfer; Tir, translocated intimin receptor.

or loss of specific sets of genes as a result of HGT, speciation by HGT is not a specific response to defined challenges, but a global evolutionary response of bacterial populations. The *E. coli* scenarios already discussed provide evidence for this. Why should this not be the case also in eukaryotes and why should they not use the same or very similar mechanisms, being available as they are in prokaryotes? We propose that major evolutionary leaps in eukaryotes (most clearly in unicellular eukaryotes, but possibly also in multicellular organisms) are produced by the traffic of mobile elements that operate in the same way as bacterial mobile elements. The eukaryotic mobile genetic elements are the transposons, viruses and bacteria that thrive among them. Initial support for this

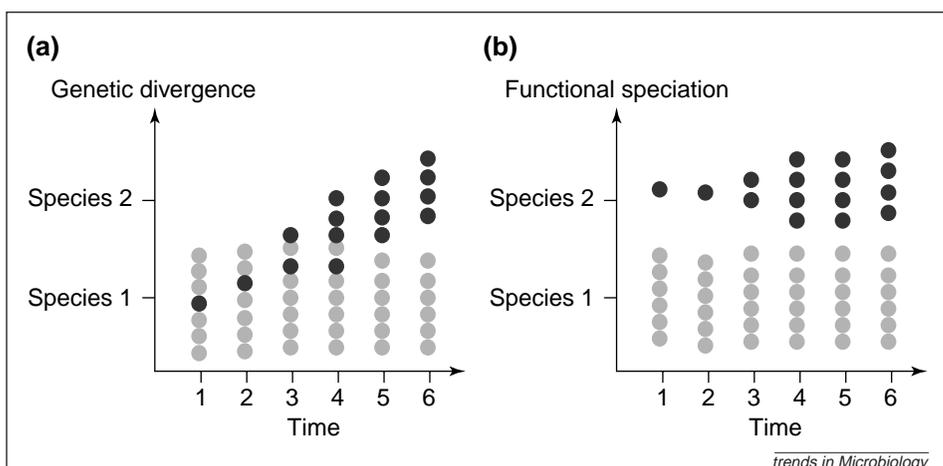


Fig. 1. A distinction between overall genetic divergence and functional speciation. **(a)** Genetic divergence can be measured by 16S RNA sequencing or DNA–DNA hybridization. A divergence of ≈5% is required for two bacteria to be considered of different species. At time point 1, a bacterium of species 1 (light grey dots) acquires virulence determinants by horizontal gene transfer (HGT) (dark grey dots). The dark grey bacterial population undergoes divergent evolution because it colonizes a different niche. Only at time point 6 will the dark grey species be considered different from the light grey species. **(b)** Functional speciation can be measured by the ability to colonize different ecological niches. HGT-mediated acquisition of virulence determinants by a bacterium allows colonization of niche 2. Thus, the dark grey should immediately (time point 1) be considered species 2. The final result (at time point 6) is the same, but genetic divergence does not tell us why dark grey and light grey bacteria are evolving differently.

hypothesis comes from the now widely accepted notion that eukaryotes are, in essence and origin, an assemblage of the components of prokaryotic cells²⁵. The same HGT mechanisms that produce speciation in bacteria should, in principle, operate in these assemblages. In fact, massive HGT from endosymbiotic bacteria (including the ancestors of chloroplasts and mitochondria) to the nucleus forms the basis of the theory of the origin of eukaryotes.

It is unlikely that appropriation of bacterial genomes, or their parts, would occur only once in evolution. There are strong indications that a very successful series of massive HGT events occurred ~2 billion years ago, when the earth changed from a reducing to an oxidizing atmosphere, and if they occurred then, they will continue to occur forever. We have examples that conjugation from bacteria to eukaryotes still occurs nowadays under laboratory conditions (conjugative transfer to yeast, filamentous fungi and plants) as well as in natural environments (T-DNA transfer to plants)⁶. Furthermore, eukaryotes are naturally transformable (DNA in the form of calcium phosphate precipitates or liposome complexes can enter most animal cells by phagocytosis). Intracellular, and even extracellular, pathogenic bacteria can transfer DNA to the nucleus of infected cells^{7,26}. Any exogenous cytoplasmic DNA is picked up by the nucleus and integrates in the chromosomes by illegitimate recombination. Thus, bacterial and viral DNAs are constantly being integrated in the chromosomes of plants and animals today, by the known genetic mechanisms of conjugation, transformation and transduction (in evolutionary terms, retroviruses and other integrative viruses are exact parallels of bacteriophages).

It can therefore be concluded that eukaryotes possess the same capacity and similar mechanisms for effective HGT as prokaryotes do, and laboratory experiments have shown that these mechanisms are functional. Given the instruments and the opportunities, is it possible that they are not being extensively used? It is only 98% true that the genomes of humans and other primates are 98% identical – they are almost 100% identical in almost every gene, and the process of speciation probably consisted of the acquisition of one or several sets of new genes by HGT. To mention just a single example, *Alu* sequences are very successful transposable elements that entered the ancestral primate germ line ~60 million years ago (by HGT?). They might have played a pivotal role in the speciation of primates^{27,28}. Which are the ‘human-specific’ genes? Are there humanization gene islands? Our prediction is that they will be found. We would like to finish with the same phrase used by Ford Doolittle in a recent review that emphasizes the role of HGT in genome evolution²⁹ ‘biologists might rejoice in and explore, rather than regret or attempt to dismiss, the creative evolutionary role of HGT’. We have attempted to do this here.

References

1 Syvanen, M. and Kado, C.I., eds (1998) *Horizontal Gene Transfer*, Chapman & Hall

2 Jain, R. *et al.* (1999) Horizontal gene transfer among genomes: the complexity hypothesis. *Proc. Natl. Acad. Sci. U. S. A.* 96, 3801–3806

3 Doolittle, W.F. (1998) You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. *Trends Genet.* 14, 307–311

4 Nelson, K.E. *et al.* (1999) Evidence for lateral gene transfer between Archaea and Bacteria from the genome sequence of *Thermotoga maritima*. *Nature* 399, 323–329

5 Wolf, Y.I. *et al.* (1999) Rickettsiae and Chlamydiae: evidence of horizontal gene transfer and gene exchange. *Trends Genet.* 15, 173–175

6 de la Cruz, F. and Lanka, E. (1998) Function of the Ti-plasmid Vir proteins: T-complex formation and transfer to the plant cell. In *The Rhizobiaceae* (Spaink, H.P. *et al.*, eds), pp. 281–301, Kluwer

7 Grillot-Courvalin, C. *et al.* (1998) Functional gene transfer from intracellular bacteria to mammalian cells. *Nat. Biotechnol.* 16, 862–866

8 Mazel, D. and Davies, J. (1999) Antibiotic resistance in microbes. *Cell Mol. Sci.* 56, 742–754

9 van der Meer, J.R. (1997) Evolution of novel metabolic pathways for the degradation of chloroaromatic compounds. *Antonie Van Leeuwenhoek* 71, 159–178

10 Wyndham, R.C. *et al.* (1994) Catabolic transposons. *Biodegradation* 5, 323–342

11 Williams, P.A. and Sayers, J.R. (1994) The evolution of pathways for aromatic hydrocarbon oxidation in *Pseudomonas*. *Biodegradation* 5, 195–217

12 Romine, M.F. *et al.* (1999) Complete sequence of a 184-kilobase catabolic plasmid from *Sphingomonas aromaticivorans* F199. *J. Bacteriol.* 181, 1585–1602

13 Di Gioia, D. *et al.* (1998) Structures of homologous composite transposons carrying *cbaABC* genes from Europe and North America. *Appl. Environ. Microbiol.* 64, 1940–1946

14 Harwood, C.S. and Parales, R.E. (1996) The β -ketoacid pathway and the biology of self-identity. *Annu. Rev. Microbiol.* 50, 553–590

15 Datta, N. and Hughes, V.M. (1983) Plasmids of the same Inc groups in Enterobacteria before and after the medical use of antibiotics. *Nature* 306, 616–617

16 Hughes, V.M. and Datta, N. (1983) Conjugative plasmids in bacteria of the ‘pre-antibiotic’ era. *Nature* 302, 725–726

17 Hacker, J. *et al.* (1997) Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. *Mol. Microbiol.* 23, 1089–1097

18 Lawrence, J.G. and Ochman, H. (1998) Molecular archaeology of the *Escherichia coli* genome. *Proc. Natl. Acad. Sci. U. S. A.* 95, 9413–9417

19 Bergthorsson, U. and Ochman, H. (1998) Distribution of chromosome length variation in natural isolates of *Escherichia coli*. *Mol. Biol. Evol.* 15, 6–16

20 Hurtado, A. and Rodriguez-Valera, F. (1999) Accessory DNA in the genomes of representatives of the *Escherichia coli* reference collection. *J. Bacteriol.* 181, 2548–2554

21 Boyd, E.F. and Hartl, D.L. (1997) Recent horizontal transmission of plasmids between natural populations of *Escherichia coli* and *Salmonella enterica*. *J. Bacteriol.* 179, 1622–1627

22 Boyd, E.F. *et al.* (1996) Mosaic structure of plasmids from natural populations of *Escherichia coli*. *Genetics* 143, 1091–1100

23 Conner, C.P. *et al.* (1998) Differential patterns of acquired virulence genes distinguish *Salmonella* strains. *Proc. Natl. Acad. Sci. U. S. A.* 95, 4641–4645

24 Vulic, M. *et al.* (1999) Mutation, recombination, and incipient speciation of bacteria in the laboratory. *Proc. Natl. Acad. Sci. U. S. A.* 96, 7348–7351

25 Lopez-Garc, P. and Moreira, D. (1999) Metabolic symbiosis at the origin of eukaryotes. *Trends Biochem. Sci.* 24, 88–93

- 26 Dietrich, G. *et al.* (1998) Delivery of antigen-encoding plasmid DNA into the cytosol of macrophages by attenuated suicide *Listeria monocytogenes*. *Nat. Biotechnol.* 16, 181–185
- 27 Hamdi, H. *et al.* (1999) Origin and phylogenetic distribution of alu DNA repeats: irreversible events in the evolution of primates. *J. Mol. Biol.* 289, 861–871
- 28 Schmid, C.W. (1998) Does SINE evolution preclude Alu function? *Nucleic Acids Res.* 26, 4541–4550
- 29 Doolittle, W.F. (1999) Phylogenetic classification and the universal tree. *Science* 284, 2124–2129
- 30 Recchia, G.D. and Hall, R.M. (1997) Origins of the mobile gene cassettes found in integrons. *Trends Microbiol.* 5, 389–394
- 31 Mazel, D. *et al.* (1998) A distinctive class of integron in the *Vibrio cholerae* genome. *Science* 280, 605–608
- 32 Grinstead, J. *et al.* (1990) The Tn21 subgroup of bacterial transposable elements. *Plasmid* 24, 163–189
- 33 Tsuda, M. and Iino, T. (1987) Genetic analysis of a transposon carrying toluene degrading genes on a TOL plasmid pWW0. *Mol. Gen. Genet.* 210, 270–276
- 34 Freiberg, C. *et al.* (1997) Molecular basis of symbiosis between *Rhizobium* and legumes. *Nature* 387, 394–401
- 35 Hu, P. *et al.* (1998) Structural organization of virulence-associated plasmids of *Yersinia pestis*. *J. Bacteriol.* 180, 5192–5202
- 36 Karaolis, D.K. *et al.* (1999) A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature* 399, 375–379
- 37 Burland, V. *et al.* (1998) The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. *Nucleic Acids Res.* 26, 4196–4204
- 38 Plunkett, G., III *et al.* (1999) Sequence of Shiga toxin 2 phage 933W from *Escherichia coli* O157:H7: shiga toxin as a phage late-gene product. *J. Bacteriol.* 181, 1767–1778
- 39 Perna, N.T. *et al.* (1998) Molecular evolution of a pathogenicity island from enterohemorrhagic *Escherichia coli* O157:H7. *Infect. Immun.* 66, 3810–3817

cAMP signalling in pathogenic fungi: control of dimorphic switching and pathogenicity

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The ability of dimorphic pathogenic fungi to switch between different morphological states appears to be an important virulence determinant as mutant strains lacking this ability often have reduced virulence or are avirulent. The genes controlling morphogenesis have therefore been the focus of many investigations, as they have great potential as targets for novel antifungal drugs. Most of our knowledge of the signalling pathways involved in controlling morphological switching has been provided by studies of the switch from yeast to pseudohyphal growth in the model organism *Saccharomyces cerevisiae*. These studies have revealed that the signalling pathways are controlled by both cAMP and mitogen-activated protein kinase (MAPK) signal transduction pathways. It has become clear that pseudohyphal growth in *S. cerevisiae* is one aspect of

Morphological changes in pathogenic fungi often underlie the development of virulence and infection by these organisms. Our knowledge of the components of the cell signalling pathways controlling morphological switching has, to a large extent, come from studies of pseudohyphal growth of the model organism *Saccharomyces cerevisiae*, in which control is exerted via changes in the intracellular cAMP and mitogen-activated protein kinase cascades. There is evidence that pathogenic fungi also utilize these pathways to control dimorphic switching between saprobic and pathogenic forms and, as such, the elements of these pathways have potential as drug targets.

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the response to nutritional deprivation and, as such, is a response to an environmental stress. Pathogenic fungi appear to have adapted related cell signalling pathways to control morphological switching during infection. Here, we review our current knowledge of the cAMP signalling pathway in *S. cerevisiae*, drawing parallels with pathogenic fungi.

Morphological switching in *S. cerevisiae*

Diploid cells of the budding yeast *S. cerevisiae* can be induced to produce pseudohyphae under conditions of nitrogen starvation. This morphological transition entails changes in cell morphology, budding pattern and cell separation: the cells switch from an ovoid to an elongated shape; they bud apically and the buds remain attached, resulting in chains of cells that constitute the pseudohyphae. *In vitro*, they also acquire the ability to invade