the ferroelectric properties for capacitor applications.

Lee *et al.* have devised a method to tilt an epitaxial $Bi_4Ti_3O_{12}$ film on an underlying conducting bottom electrode a full 90°. The method involves using an epitaxial (001) cubic zirconia buffer layer to transition from a (001) $SrRuO_3$ film, used as the bottom electrode, to a (001) Si substrate. The crystallographic orientation in the La-doped bismuth titanate layer is controlled through the growth rate. The resulting *a* axis–oriented La-doped epitaxial bismuth titanate films are grown on a silicon substrate and have the optimal orientation for nonvolatile ferroelectric memories. This approach opens a new vista within the field of ferroelectric memories, and more generally in the area of growth of complex layered oxides on technologically relevant substrates. The ability to carefully control crystallographic orientation and therefore create a highly oriented thin film should enable development of the next-generation of ferroelectronic devices.

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PERSPECTIVES: GENOMICS AND MICROBIOLOGY

Microbial Forensics—"Cross-Examining Pathogens"

Craig A. Cummings and David A. Relman

Microbial pathogens cause disease as a result of their intrinsic adaptive strategy for replication and survival within a host. In a problematic modern world, however, microbial-related disease may also be the consequence of a forced interaction between microbe and host or manipulation of the microbe's genome by malevolent persons. In the case of both naturally occurring "emerging" infectious diseases, and disease induced by human intent (bioterrorism), it is important to establish "attribution." When explored in either a scientific or legal courtroom, the source of a pathogen, and its origins and relatedness to other strains and species, reveal mechanisms by which virulence arises and the host-microbial equilibrium becomes disrupted. In the arena of emerging microbial diseases, these critical issues are addressed with increasing frequency using molecular microbial signatures. The study of emerging infectious diseases and new pathogens (*1*), and the criminal justice system have evolved in a similar fashion: Both have shifted away from reliance on biological phenotypes of the suspected perpetrator, such as fingerprints, and toward more reliable and quantifiable molecular markers, such as polymorphisms (variations) in the DNA sequence.

Enhanced online at www.sciencemag.org/cgi/content/full/296/5575/1976

Comparative genome sequencing, in particular, offers a powerful approach for analyzing genetic variation and relatedness within and between species, and for resolving differences between two strains that superficially look identical. However, the speed of the evolutionary clock (that is, the rate of accumulation of genetic variations) for some microbial species, such as *Bacillus anthracis*, the causative organism of anthrax, is quite slow. For these organisms, the ability to discriminate between strains has been limited by the paucity of known genetic polymorphisms.

Read and co-workers from The Institute for Genomic Research (TIGR) now report on page 2028 of this issue the comprehensive identification of genetic polymorphisms in two related strains of *B. anthracis* by comparative full-genome sequencing (*2*). They compared the Porton isolate of the Ames strain with an isolate from the index case in Florida of the October 2001 mail anthrax attacks (*2*, *3*). Importantly, they introduce a statistical model that distinguishes between true genetic polymorphisms and random sequencing errors. Furthermore, the discriminating power of these polymorphism markers is demonstrated by the typing of closely related *B. anthracis* strains. Their impressive demonstration of polymorphism detection and analysis, especially in such a genetically homogeneous bacterium, is an important contribution to the molecular typing field. Their work establishes a methodology for the comprehensive identification of sequence polymorphisms and their deployment as typing markers.

Microbial forensics, as we define it, is the detection of reliably measured molecular variations between related microbial strains and their use to infer the origin, relationships, or transmission route of a particular isolate. These variations or markers include genome sequence polymorphisms, which can be detected by direct sequencing or by hybridization-based methods; genomewide patterns of gene expression, which can be easily measured with DNA microarrays; and differences in protein or small-molecule patterns, which can be detected by spectroscopic or other methods. These same techniques can be used to study population structure, species evolution, and acquisition of virulence (*4*, *5*).

The application of molecular markers in forensic studies has led to some high-profile discoveries. For example, the alleged transmission of HIV from a Florida dentist to several patients was supported by sequencing of amplified viral fragments from the dentist and the infected patients (*6*). Recently, using multiple-locus variable number tandem repeat (VNTR) analysis, the Aum Shinrikyo *B. anthracis* bioterror strain was identified as the veterinary vaccine strain, Sterne 34F2 (*7*). Although TIGR's interest in *B. anthracis* genomics predates October 2001, the choice of the Florida strain for analysis was influenced by the ensuing public health and criminal investigations. Both criminal investigation of bioterrorism attacks and studies of naturally occurring disease outbreaks will continue to be important applications of this technology. In fact, in some cases, it is difficult at the outset to distinguish mother nature from man as the perpetrator: The investigation of the West Nile virus outbreak in the northeastern United States in 1999 eventually revealed a single strain from birds and humans in New York with greatest similarity to a strain originally isolated from a dead goose in Israel, leading to the conclusion that the outbreak was of natural origin (*8*, *9*). Cultivation of an organism may not be necessary for genotyping: Random or targeted genome amplification from picogram quantities of DNA (*10*) may facilitate microbial forensic analysis of micromanipulated single cells, and direct analysis of clinical specimens.

How can we improve upon the use of polymorphic sequence markers to distinguish and establish relationships between strains reliably and unambiguously? Comparison of two strains will identify only a

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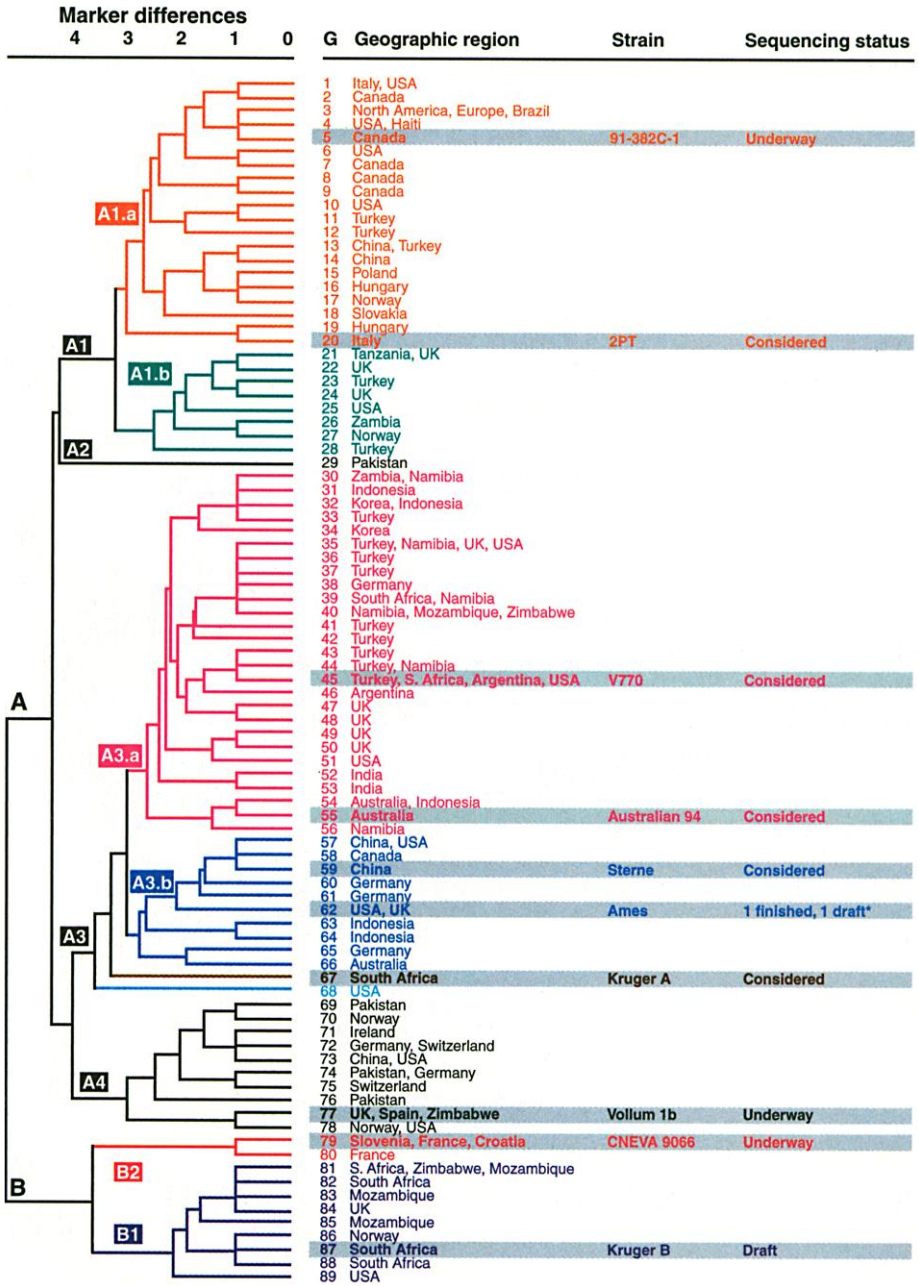
subset of the polymorphisms found within the species. This set of markers may not be sufficient for typing unsequenced strains. In fact, if these two strains are closely related, then the fraction of polymorphisms represented and their resolving power may be quite low. This assumption is supported by the finding that only two of the previously identified *B. anthracis* VNTR loci were identified in the comparison of the Porton and Florida strains (*11*). We speculate that sequencing of additional Ames lab strains and other distantly related strains may yield polymorphisms that lead to identification of the source of the 2001 bioterrorism strain (or strains). Investigators at TIGR apparently share this viewpoint and have been funded to sequence at least 10 more strains, selected for their genomic diversity (see the figure), as well as *B. cereus* and *B. thuringiensis* strains. "Kruger B," one of the most divergent strains from the Ames isolates, and *B. cereus* 10987 have already been sequenced (*12*). In addition to increasing marker density, the distribution of each polymorphism must be established in a representative set of strains to determine the extent to which each marker varies in the population. The statistical power of this method will ultimately depend upon the number of markers and their allele frequencies (*13*). We also need a better understanding of the effects of laboratory passage on the generation and accumulation of genome sequence polymorphisms. Growth in the laboratory imposes selective pressures on microbes, possibly leading to accumulation of mutations, that are different from those in its natural environment. The perplexing and unexplained differences between the two Porton isolates (*2*) emphasize the importance of limiting laboratory passage, and of documenting the degree of passage and the conditions under which it takes place.

The biggest hurdle to widespread application of this technology is cost. TIGR currently estimates a price tag of $140,000 per *B. anthracis* genome at eightfold coverage (*14*). Other methods for genomewide polymorphism discovery and high volume strain typing may entail lower costs. Nevertheless, depending upon the number of genomes that need to be examined and screened, expenses can be substantial. Other hurdles to widespread and optimal application of microbial genotyping are standardization of methods and data annotation, development of additional high-throughput facilities and processes, and a more comprehensive effort to survey the diversity of microbes in nature. Without a better understanding of this microbial "background," we stand in danger of making false accusations and wrongful incriminations.

These hurdles could be more easily cleared through the commitment of national resources to establish a microbial forensics infrastructure. Such a program would identify and prioritize microbes that present a significant threat to public health or agriculture; fund sequencing of microbial genomes, and construction and maintenance of a polymorphism database; and promote development of reliable, and alternative, next-generation typing methods. These resources should be deployed around the world. Because emerging pathogens and potential biowarfare agents are continuous threats, this infrastructure must respond with rapid reprioritization of efforts when an outbreak or attack occurs. We currently find ourselves in a powerful and exciting position to address problems, both ancient and recent, of natural and



Sampling the genomic diversity *of B. anthracis*. A dendrogram based on 89 *B. anthracis* multilocus VNTR genotypes, computed by UPGMA clustering (*11*). Branch lengths reflect the number of marker allele differences between strains. The status of genome sequencing is indicated: "finished" means the complete genome sequence is available; "draft" means that shotgun sequence with eightfold coverage is available; "underway" indicates that the genome is being sequenced; "considered" indicates that the genome may be sequenced in the future (*14*). Additional *B. anthracis* and *B. cereus* strains (not shown) are slated for sequencing. "Strain" indicates the representative chosen for sequencing from each genotype. The Porton strain of the Ames isolate (blue star) has been sequenced to near completion, whereas the Florida isolate is of draft quality (*2*).

man-made origin, using tools and insight of our own creation.

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Published online 9 May 2002;
10.1126/science.1073125
Include this information when citing this paper.

PERSPECTIVES: NEUROSCIENCE

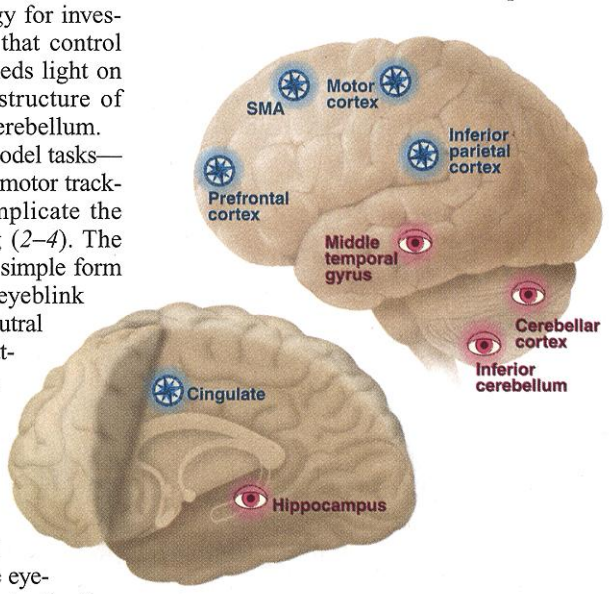
Can We Teach the Cerebellum New Tricks?

Eliot Hazeltine and Richard B. Ivry

Motor learning, the ability to learn a complex sequence of movements, is an essential feature of human behavior. Our brains continually encode new representations of incoming sensory information and translate them into motor commands that enable the execution of coordinated, even graceful, movements. A distributed network of neural structures contributes to this ability, but how these structures collaborate to produce goal-oriented actions is still unclear (see the figure). On page 2043 of this issue, Seidler *et al.* (*1*) present a new strategy for investigating the neural structures that control motor learning. Their work sheds light on the long-time favorite brain structure of motor learning theorists, the cerebellum.

Many studies that involve model tasks—such as prism adaptation, visuomotor tracking, and tool acquisition—implicate the cerebellum in motor learning (*2*–*4*). The best studied of these tasks is a simple form of Pavlovian learning called eyeblink conditioning. In this task, a neutral stimulus such as a tone is repeatedly paired with an airpuff to the eye (*5*). Over time, the animal learns to produce an eyeblink in response to the tone. Lesions to the cerebellum both abolish this conditioned response and prevent the acquisition of the predictive eyeblink in naïve animals. Importantly, the eyeblink elicited by the airpuff itself remains largely intact in decerebellate animals, indicating that the deficit is not one of motor production but rather is one of learning. Converging evidence from systems, cellular, and molecular neuroscience research provides a compelling case that the cerebellum is essential for acquisition of this conditioned response.

There remains, however, considerable debate as to whether the cerebellum plays a general role in motor learning. The computations required to learn that a tone predicts an airpuff are quite different from those needed by a master pianist to perform Rachmaninoff's Piano Concerto No. 3. One obstacle to progress on this question is that individuals with cerebellar damage show deficits in motor execution, performing poorly on movement tasks that they had learned before cerebellar injury. It is essential to distinguish between poor motor performance due to difficulties with encoding new representations and that resulting from problems with expressing this knowledge.



In the blink of an eye. Sagittal section of the human brain showing the neural structures that become activated during learning in the SRT task (compass), which is primarily spatial, or the eyeblink conditioning task (eye), which is primarily temporal. In the SRT task, the brain areas showing learning-related activity are the inferior parietal cortex, motor cortex, supplementary motor cortex (SMA), prefrontal cortex, and cingulate. In the eyeblink conditioning task, the brain areas showing learning-related activity are the inferior cerebellum, cerebellar cortex, hippocampus, and middle temporal gyrus (*14*).

CREDIT: KATHARINE SUTLIFF

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To address this question, Seidler *et al.* adopted the serial reaction time (SRT) task. Individuals performing the task are presented with a series of visual stimuli that indicate particular keypress responses (for example, left light indicates left keypress). The stimuli, and therefore the responses, appear either randomly or in a fixed sequence. Learning is indicated by a decreased response time on trials where the stimuli are in sequence compared with those where the stimuli are random. The inclusion of a distractor task (such as tone counting) is frequently used to prevent awareness of the sequence. Under such dual-task conditions, the expression of sequence learning is reduced, even though subsequent tests without the distractor task reveal that significant learning has taken place.

Taking advantage of this behavioral phenomenon, Seidler *et al.* asked subjects first to perform the SRT task concomitantly with the distractor task. With functional magnetic resonance imaging (fMRI), they measured brain activation as learning was taking place but not yet being expressed. Then they rescanned the participants in the absence of the distractor task, at a point when the learned sequence could be expressed. Their principal finding is that sequence-related activation in the cerebellum appears only when the distractor task is removed. This result suggests that the cerebellar contribution to the SRT task is restricted to the expression of a learned sequence of movements but not to the initial acquisition or learning of the sequence. These results challenge the commonly held assumption that the cerebellum is essential for motor skill acquisition. In so doing, the study offers a new interpretation of why patients with cerebellar lesions fail to learn the SRT task (*6*–*8*). Studies of the SRT task in patients have not included a distractor task. Thus, measures of learning and performance are conflated, and a performance deficit may be misinterpreted as a learning impairment.

The Seidler *et al.* strategy is a clever way to separate learning and performance