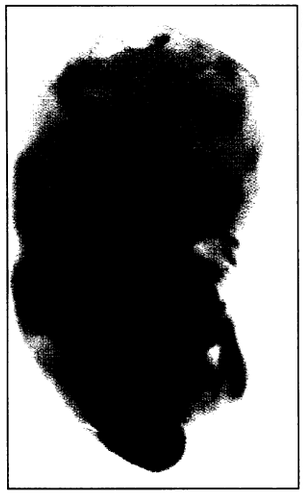
DEVELOPMENTAL BIOLOGY

Growing Joints Use Their Noggins

Some arthritis sufferers might wish their joints would just go away, but new research presented on page 1455 of this issue shows that jointlessness is not a happy alternative. Molecular embryologists Richard Harland and Lisa Brunet at the University of California, Berkeley, and Jill and Andrew McMahon at Harvard University, have found that mice lacking *noggin*, a gene first discovered as important in brain and nerve development, have no joints at all. Instead, they have stubby, continuous limbs—along with a fatal array of other developmental defects.

noggin is "a new link in the chain of creation of limbs" and the rest of the skeleton, says cell biologist Bjorn Olsen of Harvard Medical and Dental Schools. Harland's finding is a step toward a more detailed understanding of embryonic development, adds molecular biologist Sejin Lee of Johns Hopkins University, and offers potential medical benefits in diseases where there is too much bone or even too little.



R. HARLAND / BYL

Dis-jointed. Mice missing the *noggin* gene have paws lacking joints.

The new finding is just the latest role for *noggin*, which William Smith, then in Harland's lab, and Harland identified in 1992 after setting out to find the "neural inducer," a molecule that orders cells to become brain and nervous system tissue in early embryos. The gene earned its name when they found that frog embryos injected with its messenger RNA grew exceptionally large heads. The noggin protein also mimics the activity of a powerful piece of tissue in the developing frog known as "Spemann's organizer," which can make back-of-the-body (dorsal) tissue out of front-side (ventral) tissue. Finally, 2 years ago, Harland's team showed that in binding assays and cell culture, noggin inhibits powerful proteins that stimulate bone growth, the so-called bone morphogenetic proteins (BMPs).

With noggin playing all these developmental roles, Harland half expected that when the team turned off the gene in their mice, the resulting animals would be no more than "a ball of mush." Indeed, the knockout mice did not survive until birth. But they developed enough to offer a new insight into noggin's function.

The mouse showed a variety of intriguing skeletal abnormalities. "Every single bone is affected," says Harland, with the most obvious defects, such as shorter bones, in the vertebrae, ribs, and limbs. And in keeping with the gene's role in the brain, "there are very clearly characterized neural defects," including occasionally a brain and spinal cord not enclosed by bone. But the bones in the heads and upper bodies of the mice are much less affected by the knockout than bones farther toward the animals' tails. And dorsoventral patterning doesn't seem to be much affected. This implies that noggin has counterparts that can perform its functions near the head and in dorsoventral patterning, says Harland.

Most strikingly, the mice appear to lack all joints. Instead, their limbs are nearly continuous segments of bone, flanked by excess cartilage. That makes sense, says Harland, because during normal development, cartilage is laid out like a pencil sketch in the shape of the bones-to-be. Bone gradually fills in this cartilaginous sketch except in predetermined locations such as at the ends of the putative bones. In those spaces, the cartilage then disappears, leaving room for joints like knees and knuckles. In the knockout mice, the cartilage does not do this disappearing act. Without noggin, the thinking goes, bone-forming proteins go out of control, recruiting additional cells from neighboring areas into the prebone cartilage.

But here too noggin apparently does not act alone, according to work by Harland's and other labs. Instead, it apparently sends signals to a BMP family member called GDF-5, which has been shown to be important in joint formation, and also interacts with another limb-building protein, Sonic hedgehog. Therefore, says Olsen, noggin "is not a master molecule" that regulates everything else. Because BMPs are regulated by the powerful family of patterning genes known as *hox*, he proposes that *noggin* is a link in the pathway, somewhere downstream of *hox* genes and upstream of BMPs, that governs the patterning of limbs.

Uncovering the molecular basis of this pathway has clinical implications, because many diseases, from osteoarthritis to osteoporosis, involve either too much bone or too little. Biotech companies are already avidly testing BMPs as potential drugs; two are in clinical trials now for healing bone breaks. But these molecules are, if anything, too powerful, says Lee. "The challenge," he says, "is to limit bone growth to what is clinically desirable."

Limiting bone growth is where noggin might come in. Regeneron Pharmaceuticals of Tarrytown, New York, is studying whether noggin and other BMP inhibitors can put the brakes on the excess bone growth that arises in about 10% of hip replacement patients as well as in some patients with osteosarcomas and prostate cancer metastases, says biochemist Neil Stahl of Regeneron.

Systemic use of noggin could have unwanted side effects, because the BMPs it inhibits are found everywhere from skin to gut to bone, warns Rik Derynck, a cell and developmental biologist at the University of California, San Francisco. But eventually, drug companies might use their noggins to provide novel treatments for overgrown bone.

–Steven Dickman

Steven Dickman is a writer in Cambridge, MA.

INFECTIOUS DISEASES

New Method Churns Out TB Mutants

By and large, bacteria are much easier to study in the laboratory than more complex, multicellular organisms. But every rule has its exceptions, and for microbiologists one of the cruelest has been *Mycobacterium tuberculosis*, the pathogen that causes tuberculosis (TB). The microbe's recalcitrance in the lab has hindered researchers in their efforts to design better drugs for combating TB, which is the world's leading killer among infectious diseases, claiming more than 3 million lives worldwide every year. Now, that impasse may be at an end.

In order to ferret out new drug targets, researchers want to identify the genes that pathogens need to survive and infect the host—a task usually accomplished by creating wholesale mutations in the microbial genome and then screening for mutants defective in those abilities. Until a few months ago, this was very hard to do with *M. tuberculosis*, partly because the vehicles typically used to create the mutations—small bits of DNA called transposons that insert randomly into the genome and inactivate any gene they happen to interrupt—do not readily penetrate the microbe's tough, waxy coat.

Last fall, however, a team of scientists led by microbiologist William Jacobs and immunologist Barry Bloom of the Albert Einstein College of Medicine in New York City, reported creating a new kind of vehicle—a cross between a bacterial virus and a circle of DNA called a plasmid—that's much more efficient at producing muta-

tions in *M. tuberculosis*. By now, Bloom, Jacobs, and their colleagues have generated thousands of *M. tuberculosis* mutants, some of which have affected the pathogen's survival and virulence. And they are just getting started.

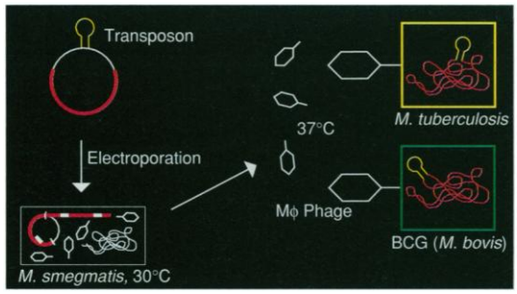
"With these techniques, researchers should be able to make mutations in virtually every gene of *Mycobacterium tuberculosis*," says Ann Ginsberg, program officer for tuberculosis at the National Institute of Allergy and Infectious Diseases. "Once you have mutants, you can understand gene functions. It allows you to answer a lot of questions about the pathogenesis of the disease." Along with pointing to targets for new drugs, the mutants might also lead to new ways to protect against TB: vaccines based on the avirulent strains Bloom and his colleagues are generating.

The Einstein team has been on the trail of better ways of producing *M. tuberculosis* mutants for more than a decade. Early on, Jacobs reasoned that the barrier posed by the microbe's waxy coat might be circumvented by taking advantage of viruses that naturally infect mycobacteria. With few such viruses available commercially, Jacobs turned to a handy source—his own backyard in the Bronx.

Bacterial viruses, or phages as they are called, are common soil dwellers, and so Jacobs screened soil from his yard looking for any that infect *M. tuberculosis* efficiently. He won the "prokaryotic Lotto," he says, in 1987 when he found a mutant bacteriophage that infects *M. tuberculosis* at a frequency seven orders of magnitude higher than its parent.

It's difficult to package transposons into a phage, so Jacobs combined the phage DNA with a plasmid from another bacterium, *Escherichia coli*—a creation he christened a "phasmid." Besides accommodating transposons, the plasmid DNA tricks *E. coli* into copying the entire recombinant molecule, churning it out in large quantities. The researchers then introduce the phasmid DNA into *M. smegmatis*, a mycobacterium that doesn't have its pathogenic cousin's waxy coat, so its cell wall can be breached with a jolt of electricity. There the phasmid replicates, forming phage particles that will infect *M. tuberculosis*.

In an added twist, the researchers mutated the phage itself so that it replicates only at 30 degrees Celsius. As a result, it doesn't kill infected cells kept at higher temperatures but merely transfers in the phasmid DNA, along with its transposon, which can then jump into the mycobacterial DNA, causing mutations. This procedure has proved very efficient at making *M. tuberculosis* mutants; the researchers have so far collected 10,000. "We keep making them, and hopefully we will statistically accumulate enough to cover all the genes," says Bloom.



SOURCE: W. R. JACOBS AND B. SHAPIRO

Transposon shuttle. After being grown in *E. coli*, the phasmid, including both phage (red) and plasmid (white) DNA plus a transposon (yellow), replicates in *M. smegmatis*. This produces phage particles that infect mycobacteria with transposon-carrying DNA.

But the mutants are already providing insights into the biochemical machinery that mycobacteria need to cause disease. For example, the Albert Einstein researchers traced loss of virulence in one mycobacterial mutant to a transposon interrupting the gene encoding a sigma factor, a protein that helps turn on other genes. Another mutant lost its virulence because it could no longer synthesize the amino acid leucine. "The various mutants tell us what typical *Mycobacteria* need to survive and grow," says Jacobs. That should make *M. tuberculosis* much less of a mystery to researchers looking to develop better TB drugs and vaccines.

–Carol Potera

Carol Potera is a free-lance writer in Great Falls, MT.

ARCHAEOLOGY

Young Ages for Australian Rock Art

Two years ago, archaeologists caused an international stir with their dates for a remote rock shelter called Jinmium in the Northern Territory of Australia. The dates of 116,000 to 176,000 years ago made the shelter by far the earliest trace of humans in Australia, and its circular carvings the oldest known rock art in the world. But archaeologists questioned the dates, partly because they were obtained with a method that has yielded spectacularly early dates at other sites (*Science*, 10 October 1997, p. 220). Now the results are in from a painstaking effort to redate Jinmium, and the doubters have been vindicated.

In this week's issue of *Nature*, a team headed by geochronologist Richard Roberts of La Trobe University in Melbourne, Australia, reports that Jinmium's age is a completely unremarkable 10,000 years. The new dates "nail the coffin shut" on the claim that humans have been in Australia two to three times longer than previously thought, says geochronologist Jack Rink of McMaster University in Hamilton, Canada.

The early dates for Jinmium came from a team led by archaeologist Richard Fullagar of the Australian Museum, who is also a coauthor on the new paper. For the early dates, he used a method called thermoluminescence dating (TL), which relies on a clock driven by natural radiation in common minerals like quartz. As long as the mineral remains in the dark, the radiation bumps electrons from their normal positions in the minerals' crystal lattice into defects, or "traps," at a regular rate. But exposure to sunlight or heat empties the electrons from the traps and sets the clock to zero. The traps refill over time, and scientists can read the clock by emptying them in the lab, either by heating the sample (TL) or by tickling it with light (optically stimulated luminescence, or OSL). The material glows as the electrons drop back into the lattice; the more intense the glow, the more time has passed since the sediments last saw daylight.

But those dates can be contaminated. In the paper in *Nature*, Roberts's team notes that pebbles of older rock—crumbly sandstone from the boulder wall and the bedrock below—were jumbled into the sediments being dated. When Roberts used OSL to tease dates from individual mineral grains, he was able to distinguish old grains of bedrock from the grains of sediment that would reveal the true age of the shelter. His conclusion: The base of the deposit at Jinmium is no more than 10,000 years old, and some of the quartz grains were laid down more recently. That means humans were at the site "no more than 10,000 years ago," says Roberts.

R. ROBERTS/LA TROBE UNIVERSITY

Getting younger. The rock carvings at Jinmium are now dated at only 10,000 years old.

These ages agree with radiocarbon dates from the upper layers of the deposit. Besides removing a puzzle in Australian prehistory, says Rink, the new dates should restore confidence in luminescence dating, which is a powerful tool when applied correctly.

–Ann Gibbons