DEVELOPMENTAL BIOLOGY

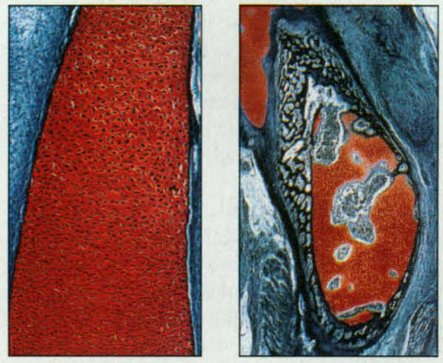
Putting the Brakes on Bone Growth

Human life starts with an astonishing growth spurt: The long bones of the average infant lengthen by 50% during the first year after birth. That growth rate drops to about 7% per year by age 3—which is just as well, or children would surge past the 6-foot (183-cm) mark while in nursery school. But just how nature moderates this bone growth—thus keeping the world free of 4-year-old Wilt Chamberlains—has been a long-standing mystery. Now, by combining their results, three teams of researchers in Boston may have found the beginnings of an answer.

Researchers have long known that long bones form when some chondrocytes—proliferating cartilage cells in the growth plates at the end of long bones such as a femur or humerus—stop dividing, enlarge, and differentiate into a scaffold for new bone cells. Work on mouse and chick embryos, reported on pages 613 and 663 of this issue, has revealed a cycle of gene-protein interactions that puts the brake on this differentiation process, allowing only a few chondrocytes at a time to stop dividing. When the researchers release this brake by removing components of the cycle, the entire growing area differentiates, before the cartilage cells that would have given bones their full length have arisen, thus creating animals with stubby limbs.

The scientists speculate that controlled changes in this cycle may cause the more gradual deceleration of bone growth in children. The findings also suggest, says Arthur Broadus, an endocrinologist at Yale University, that "if chondrocyte differentiation proceeds too quickly or too slowly, you get abnormalities of the long bones," such as those in some kinds of dwarfism.

The long bone story began to fuse last year in the laboratory of molecular biologist Cliff Tabin at Harvard Medical School, where developmental geneticist Andrea Vortkamp was studying Indian hedgehog (Ihh), a signaling molecule whose precise function was unknown. Working with chick embryos, Vortkamp found that Ihh is produced by cells making the transition to enlarged or "hypertrophic" chondrocytes. She also found that adding extra Ihh slows the rate at which chondrocytes differentiate, and that the intended recipient of the Ihh signal is the perichondrium, a sheath of tissue enclosing long bones. Together, these findings suggested that Ihh is part of a negative feedback loop that assures slow, steady bone growth. Tabin and Vortkamp reasoned that Ihh from differentiating chondrocytes must activate some unknown signaling protein in the perichondrium, which in turn acts on the growth plate to prevent more chondrocytes from differentiating.



A. VORTKAMP

Boning up. In a normal chick embryo *(left)*, cartilage cells (red) turn slowly into bone (blue); in the mutant embryo *(right)*, cells differentiate all at once.

Meanwhile, two teams of endocrinologists, including Henry Kronenberg, Beate Lanske, and Gino Segre, all at Massachusetts General Hospital, were investigating another recently discovered signaling molecule, parathyroid hormone-related protein (PTHrP). They had found that this protein is produced mainly in the perichondrium at the end of long bones, that receptors for it are present in proliferating chondrocytes, and—most importantly—that chondrocyte differentiation is accelerated in mice genetically engineered to lack either PTHrP or its receptor.

These two lines of research converged in September 1995, when Tabin described his and Vortkamp's theory at a meeting. Kronenberg was in the audience and "nearly fell out of his chair, because his lab was already pursuing a protein with all the effects we had predicted," says Tabin.

A few more experiments completed the picture, showing that PTHrP is indeed the missing signal in Tabin's scenario. Vortkamp found that adding extra Ihh to chick wing bones increases the production of PTHrP in the perichondrium. And Kaechoong Lee of Segre's lab showed that the premature differentiation of chondrocytes in tissue from mice engineered to lack the gene encoding PTHrP can be reversed by adding purified PTHrP. But adding hedgehog protein has no effect, indicating that Ihh's influence must be mediated by PTHrP.

But the story of bone-growth regulation is not yet complete. It is unclear how the growth-restraining Ihh–PTHrP feedback loop interacts with growth-enhancing signals thought to be provided by other proteins, notes Tabin. And new mouse and chick experiments are needed to explore whether the slowing of long-bone growth in children and its eventual cessation at the end of adolescence result from a change in the strength of the Ihh–PTHrP loop. Says Kronenberg: "We're just beginning to understand the fine-tuning of this system."

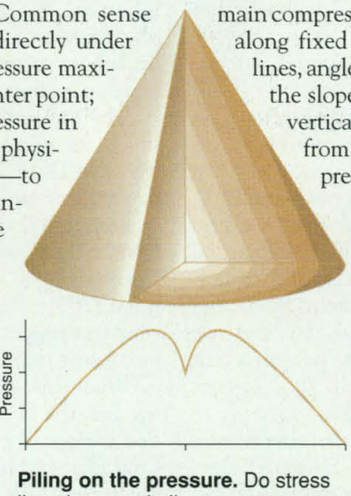
–Wade Roush

PHYSICS

Searching for the Sand-Pile Pressure Dip

The humble sand pile is to granular mechanics what Fermat's Last Theorem was to number theory: a tantalizingly simple problem that stubbornly eludes a solution. The question is this: Where does a conical pile of poured sand exert its maximum amount of pressure on the ground? Common sense would say in the middle, directly under the apex, but in fact the pressure maximum is a ring around the center point; there is actually a dip in pressure in the middle. And for 15 years, physicists have tried—and failed—to produce a complete explanation. "It's alarming to realize that for what looks like a completely classical problem there seems to be no obvious approach within the existing framework of classical continuum mechanics," says physicist Michael Cates of the University of Edinburgh in the U.K.

Now Cates thinks he has a new way to attack the problem, but it is causing a bit of a flap in the field. Last week in *Nature*, along with Edinburgh colleague Joachim Wittmer and Philippe Claudin and Jean-Philippe Bouchaud, both of France's Atomic Energy Commission at Gif-sur-Yvette, Cates proposed that the main compressive stresses in a sand pile lie along fixed parallel lines. These stress lines, angled precisely halfway between the slope of the pile surface and the vertical, steer the pile's weight away from the center, giving a central pressure dip.



Piling on the pressure. Do stress lines in a sand pile steer pressure away from the center?

Despite its simplicity, the model does a respectable job of reproducing experimental data. "[It's] a miracle that there is such an agreement with experiment," says Hans Herrmann of the Institute of Computer Applications at the University of Stuttgart in Germany, who has worked on computer simulations of sand piles. But given the problem's

pedigree, others are skeptical that it has been cracked so easily. Susan Coppersmith of the James Franck Institute of the University of Chicago describes the new model as "quite speculative" and points out that the basic assumption of the model "has not been explicitly tested."

The root of the sand-pile problem is a frustrating facet of mechanics called indeterminacy. For a perfectly rigid table, for example, it is not possible to work out the downward forces on each of the table legs because the number of unknown forces exceeds the number of equations relating those forces, so the system of equations cannot be solved. The traditional way physicists tackle this type of problem is by making the table mathematically slightly elastic so that it sags. This provides additional relations between forces via the sagging, and the problem then becomes determinate.

Sand piles are indeterminate in the same way, but Cates and his colleagues dismissed the idea of an elastic sand pile, because the bigger the pile, the more it would sag. This, Cates explains, will give a pressure dip pattern that depends on pile size, which is at odds with experimental results. Instead the researchers introduce the idea that the lines of stress propagation in the pile are a set of fixed parallel straight lines, and this additional relationship between the forces within the sand pile means there are enough equations to fix all the forces. "These directions are remembered by the packing of the grains from the moment at which they are first deposited," says Cates.

Based on this "fixed principal axis" (FPA) assumption, the model can reproduce the classic pressure dip for a sand pile created by pouring grains. But piles created in different ways should, according to the FPA model, show different stress patterns. For example, a pile of flour formed by a cook's swirling sieve should have virtually no pressure dip. "The local stress propagation rules depend on how the pile was constructed," says Cates, adding, "There are definitely new predictions from this theory which new experiments will be able to put to the test."

Cambridge University's Sam Edwards welcomes the FPA model because the stress lines create "a nest of arches" in agreement with a model he proposed in 1989. He points out that a child's sand castle can withstand having a tunnel dug through it, so there must be arches in sand piles shouldering the load. But in his model, Edwards included only vertical forces. "If you bring the other [forces] in you find it turns into coupled differential equations, and that's what Cates *et al.* have done," says Edwards.

Joe Goddard of the University of California, San Diego, acknowledges the success of the FPA model, but he thinks a "soft," elastic approach to sand piles could still be useful. Within a more classical approach one could get "localized yielding" of an elastic material, he says, and provide an even better explanation of the pressure dip. Only time will tell if the sand-pile problem yields to a hard or soft approach, or a mixture of the two.

–Andrew Watson

Andrew Watson is a science writer based in Norwich, U.K.

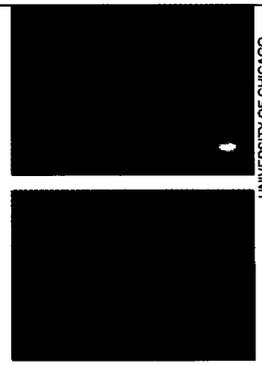
BIOCHEMISTRY

Yeast Prions: DNA-Free Genetics?

Prions—infectious protein particles thought to cause "mad cow disease," human Creutzfeldt-Jakob disease (CJD), and other neurological afflictions—now seem to be running rampant in yeast. But there is no reason to stop eating bread or drinking beer, for there's not even a hint that yeast prions pose a health threat. On the contrary, they may help scientists figure out how a protein can perpetuate a trait—or a disease—from one cell to the next by assuming an abnormal form and recruiting normal proteins in the new cell to its cause. Whether prions can perform that feat—supposedly beyond the ability of anything without DNA or RNA—is a major argument in the debate over whether prions are real (*Science*, 12 July, p. 186).

On page 622, geneticist Susan Lindquist and her colleagues at the University of Chicago show that a form-changing protein in yeast appears to create a trait called [PSI$^+$] in daughter yeast cells, after they bud off a mother cell, by causing newly synthesized proteins to become relatively insoluble and clump together. It does so without altering the DNA in the cells, which are clones. The finding does not prove that proteins alone can infect other cells in mammals, but it is evidence that a protein is able to pass on a trait just by its presence in a cell. "It's genetics without DNA," Lindquist says.

That notion has caught the attention of scientists on all sides of the prion debate—although they disagree on its interpretations. Reed Wickner, the geneticist at the National Institute of Diabetes and Digestive and Kidney Diseases who first proposed that yeast prions existed, says this work—which builds on previous studies—greatly strengthens the argument for a prion model for [PSI$^+$]. "The biochemical evidence had been lagging," he says. And Laura Manuelidis, a neuropathologist at Yale University who believes a virus, not an infectious protein, lurks behind CJD and other diseases, calls the research "very elegant, inherently interesting work." She says that by studying the clumping and insolubility patterns, researchers may be able to home in on the infectious agent.



UNIVERSITY OF CHICAGO

Prion model. Normally, the protein sup35 (green) is evenly spread in yeast *(top)*, but in a prionlike condition it clumps *(bottom)*.

The work centers on a protein called sup35. In normal strains of yeast, it helps translate DNA into proteins. But in [PSI$^+$] strains, the protein doesn't work. And the malfunction does not appear to be based on DNA: Strains with apparently identical DNA can show up as the normal [psi$^-$] or the unusual [PSI$^+$]. Researchers have found that they can trigger the condition in [psi$^-$] cells by causing them to overexpress sup35.

What scientists had not been able to do was link the heritability of [PSI$^+$] to any particular physical change in the sup35 protein. Lindquist and her colleagues set out to pin down that link by analyzing sup35 in both [PSI$^+$] and [psi$^-$] cells. They spun cell extracts in a centrifuge to separate soluble material from insoluble clumps, and quickly spotted a difference: The sup35 protein is soluble in normal cells but insoluble in the [PSI$^+$] cells. They also attached a fluorescent molecule to the sup35 protein and observed it in living cells. It turned out to be evenly distributed in normal cells, but in [PSI$^+$] cells it clumped, a finding that corresponds to mammalian prion research. The researchers theorize that the protein clumps are passed from mother to daughter cell in the cytoplasm when the daughters bud off, and that the clumps form "seeds" that attract newly formed sup35 to them, tying up the protein and preventing it from doing its usual job. In separate work published in July in the *EMBO Journal*, Michael Ter-Avanesyan and colleagues at the Institute of Experimental Cardiology in Moscow found other evidence for soluble and insoluble forms of the protein.

Lindquist acknowledges that the yeast work does not prove the infectivity of prions in mammals, where prion diseases don't involve budding cells. But she says transmitting protein conformations shouldn't be underestimated. "It's suggesting there's a mode of inheritance that we haven't been paying attention to," she says. And that has implications that even go beyond mad cows.

–Gretchen Vogel