organism's genome with the most basic set of genes could quickly reveal what makes the organism unique and specialized. Says Mushegian: "Any new genome can be visualized as an extension of this minimum set."

Mushegian and fellow computational molecular biologist Eugene Koonin of NCBI took the first step toward defining this minimum set by identifying the genes shared by two very different bacteria. The team used computer programs that compared the genetic sequences of two organisms whose genomes have been completely sequenced: *Haemophilus influenzae* and *Mycoplasma genitalium*. These two are quite different taxonomically (one is a gram-negative bacteria and the other is gram-positive), but as parasites, both organisms have evolved to be "stripped almost to the bare minimum," Mushegian says.

The team looked for similar stretches of DNA in these organisms and also in the common bacterium *Escherichia coli*. From the DNA sequences, they inferred the genes in those stretches and the functions of their proteins. Next, the researchers pieced together metabolic pathways implied by the proteins, checking to see if any necessary proteins were missing; they filled out their minimum genome with the corresponding genes. Finally, they removed redundant and parasite-specific genes.

This analysis resulted in 250 genes, the likely genome of the ancestor common to both gram-negative and gram-positive microorganisms, and the minimum genome for a modern organism. To estimate the genome of an ancient organism, the team excluded genetic siblings, or genes that diversified from a single, ancestral gene. Although modern organisms may rely on a whole family of genes to do a particular job, presumably the ancestral organisms needed only the ancestral gene. In addition, the researchers discarded some of the genes needed to process DNA, as many think that the very first genomes consisted of RNA. If the primordial world provided amino acids, fatty acids, purines, and pyrimidines, then the putative ancient microbe needed no more than 128 genes, they say. "These genes should be essential for the survival of any cell under all circumstances mentionable," asserts Koonin, who directs the minimum genome project.

At the meeting, Mushegian's presentation provoked lively discussion, and not everyone agreed that the genes held in common are those that are sufficient for life. But pinpointing these fundamental genes provides a very testable hypothesis, Koonin points out. Now experimentalists can begin to knock out all but these essential genes, and see whether the computer and its programmers guessed right. Yeast geneticist Philip Hieter from Johns Hopkins University agrees that this is a good start: "You know there *will* be an answer to the question of the minimum number of genes, eventually."

Instead of trying to answer that question by inferring the genes of a hypothetical microbe, Andersson studies present-day parasites to identify the point at which an organism has too few genes to survive on its own. The extreme case is mitochondria, cells' energy-producing organelles. Many researchers think mitochondria, which have their own small set of genes, are the remnants of intracellular parasites that eventually became an integral part of the cell. Because parasites reduce their genomes as they depend more on their hosts, the mitochondria can be seen as parasites that lost too many genes—and so lost their independence. "We're interested in what has been thrown out [of these small genomes] and what has been retained," explains Andersson.

To backtrack through mitochondrial evolution, Andersson is examining the genome of the mitochrondria's closest relative, an intracellular parasite called *Rickettsia prowazekii*. Just 1.2 million bases long, the genome of this parasite, which causes typhus, has lost some but not all of the DNA that the mitochondria did. Andersson's team sequenced some 200,000 bases in this genome and estimated the number of genes in that stretch of the genome. They then extrapolated to the whole organism, coming up with 800 genes.

Whether the minimum numbers of genes proves to be 128, 800, or some other number, these researchers have at least begun to get a handle on life's crucial genetic components, something not possible a decade ago, says David Bentley of the Sanger Center in Cambridge, England. He, for one, applauds these fledgling efforts to identify a bare-bones genome: "It's a lovely idea, however messy or arguable [the analysis] might be."

–Elizabeth Pennisi

GENOME MEETING

Studly Sheep By Non-Mendelian Means

COLD SPRING HARBOR, NEW YORK— More than a decade ago, a sheep farmer in Oklahoma noticed that some of his livestock were beefier than the rest of the flock. He and his fellow breeders saw dollar signs in the sheep's bulging hindquarters and well-sculpted muscles, but creating whole flocks of these woolly Schwarzeneggers turned out to be harder than anyone expected. Now, new work presented at a recent genome meeting here suggests that these burly sheep offer a rare and useful example of how phenotype—what an organism looks like—doesn't always match up with its genotype.

TRACY SHAY

Why not me? Normal-looking ram with two copies of the "beautiful buttocks" gene looks at his bulky offspring.

The researchers, led by Noelle Cockett of Utah State University in Logan and Michel Georges of the University of Liège in Belgium, found that a peculiar combination of non-Mendelian genetics governs which sheep bulk up and which don't. And the underlying mechanisms may one day help explain the odd patterns of inheritance seen in certain genetic diseases in humans, says Aravinda Chakravarti, a human geneticist at Case Western Reserve University in Cleveland. "There clearly are disorders where lots of individuals carry a mutation but are clinically unaffected. I think there will be all kinds of exceptions to Mendelian inheritance," he notes.

Cockett and her colleagues got their first clues to this odd sheep mutation in 1994, when, by using genetic markers from cows, they were able to map the mutated gene to sheep chromosome 18. They named the gene *callipyge*, Greek for "beautiful buttocks." Lambs normally seem to add muscle to their bodies when young and accumulate fat as they get older, but the mutant gene apparently lengthens the period of muscle addition and delays fat accumulation, explains Cockett. *Callipyge* newborns are indistinguishable from other lambs, but the adult sheep wind up with 7.8% less fat and 32% more muscle—in their rear end and hind legs.

Cockett and others assumed that the mutant form of *callipyge* was a typical dominant allele, for hefty rams mated with normal females produced bulky offspring. But breeders complained that the gene's inheritance was unpredictable. And in a poster presentation, Georges and his colleagues showed that *callipyge* actually exhibits a rare and complex pattern of inheritance. First, they found through systematic breeding experiments that this butt-building trait is evident only when passed on paternally. Lambs born to *callipyge* females mated with normal males grow up looking normal, like their dads. Such a seemingly one-sided inheritance pattern is usually a sign of imprinting, a genetic phenomenon in which the gene contributed by one parent is shut down somehow, leaving the other parent's gene as the only functional copy. About 16

imprinted genes are known, and in two-thirds of them, the father contributes the active gene.

But the team found that *callipyge* adds yet another twist: Lambs that receive two copies of the mutated gene, one from each parent, look perfectly normal—a startling result. "You would not expect the homozygous animal not to express the trait," says Georges. He and Cockett first wondered whether the mutation was somehow corrected in the normal-looking offspring. But they ruled out that possibility by allowing homozygous males to mate with normal females—and seeing the buttocks of the heterozygous progeny balloon.

Geneticists have found other so-called overdominant genes, which are most strongly expressed in individuals with only one copy of the gene, and they are studying why these genes act this way. But there's only one other known case of an overdominant gene that also seems to show imprinting: a lethal mutation in mice, called *ovum*, which was described by geneticist Carmen Sapienza at Temple University Medical School in Philadelphia. "To my knowledge [*callipyge* and *ovum*] are the only two examples where there's both a parent of origin and genotype effect," says Sapienza.

Such rare genes offer insight into a whole class of non-Mendelian inheritance patterns that may have relevance for human diseases, says Chakravarti. For example, understanding the mechanism behind such patterns may explain the variability seen in Hirschsprung's disease, a genetic disorder of the digestive system. Not everyone with a mutant gene gets the disorder, and women appear to be less likely than men to show symptoms.

Georges and colleagues are still trying to figure out the details of what controls the expression of *callipyge*. But now that the gene's pattern of inheritance is known, sheep breeders can at least predict which matings will yield the biggest lamb chops.

–Elizabeth Pennisi

DEVELOPMENTAL BIOLOGY

Synapse-Making Molecules Revealed

Every bend of the knee or blink of the eye depends on biochemical conversations between motor neurons and muscle cells. The lingua franca of these chats is the neurotransmitter, and during an embryo's development, the two kinds of cells must devise an intricate molecular junction, or synapse, to transfer these chemical signals. Now, with the help of two "conversationally impaired" mice, researchers have identified molecules from both muscle and nerve cells that lay the groundwork for these vital talks.

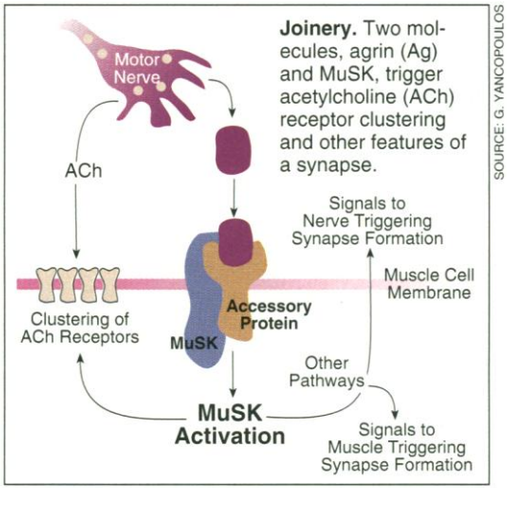
In the 17 May issue of *Cell*, a team of biologists describes "knockout" mice that are missing the gene for agrin, a nerve-derived protein, and are virtually devoid of neuromuscular junctions. Another team reports on a second set of knockout mice that can't make these connections—they lack the gene for a newly identified muscle protein named MuSK (for muscle-specific kinase). MuSK, it appears, is the agrin receptor: A third paper in the same issue shows that the two proteins bind and could spark a cascade of events that lead to the making of a synapse. "Together, the studies suggest that the agrin-MuSK interaction is the critical trigger [for synapse formation] without which you get absolutely nothing," says biologist George Yancopoulos of Regeneron Pharmaceuticals in Tarrytown, New York, who led the team that developed the MuSK knockouts.

Zach Hall, director of the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, says the work "is a major step forward in understanding the signals passed between nerve and muscle to form the synaptic apparatus." Moreover, he notes, these signals might yield clues to junction formation in the brain itself, where agrin is abundant.

The building of a synapse involves clustering neurotransmitter receptors on the muscle cell, huddling of tiny vesicles at the nerve terminal, and expression of various structural proteins. Agrin has been a leading candidate for a synapse architect since the mid-1980s, when Jack McMahan's team at Stanford University isolated the protein and found that in muscle cells it drove the clustering of receptors for acetylcholine, the neurotransmitter that causes muscle contraction. The team had no idea how it drove this clustering, however, and because the work was done in tissue culture, no one could be sure that agrin did the same job in animals.

Joshua Sanes, who had worked with McMahan, followed up the agrin lead at Washington University in St. Louis. Together with Medha Gautam and Peter Noakes, he developed a strain of agrin-deficient mice. These animals were stillborn, and an autopsy revealed they showed almost no signs of neuromuscular junction formation, indicating that the protein indeed played a crucial role.

It takes two sides to form a junction, however, and Sanes's work still left open the identity of agrin's target—its receptor—on the muscle cell. The Regeneron researchers came upon it via a roundabout route. They had identified and cloned MuSK, an enzyme, and were looking into its role in muscle cell growth. Then they noticed that MuSK was expressed only at the neuromuscular junction. That made them wonder whether MuSK could be the agrin receptor.



Joinery. Two molecules, agrin (Ag) and MuSK, trigger acetylcholine (ACh) receptor clustering and other features of a synapse.

SOURCE: G. YANCOPOULOS

To test that idea, Thomas DeChiara of Regeneron created mice with defects in the MuSK gene and analyzed them in collaboration with Steven Burden's group at New York University Medical School. The rodents' nervous systems developed normally, but only up to a point: Neurons found their way to various muscles, but never sprouted synapses. "It was a spectacular phenotype," Yancopoulos recalls. "The mice had muscle but didn't use it. They never took their first breath of life."

Then the Regeneron team put agrin and MuSK together. Regeneron's David Glass and colleagues found that the two proteins bound very quickly, but only when MuSK was expressed in muscle cells. This suggested that MuSK was an agrin receptor, but also that an accessory muscle-specific protein, still unidentified, was needed to cement the connection. Once the link was made, MuSK became quite active, adding phosphate groups to acetylcholine receptors, for instance. The researchers theorize that this sets off a chain of chemical events—in both nerve and muscle cells—that ultimately constructs the molecular parts of the synapse.

Now the search is on for the identity of that accessory protein linking agrin to MuSK, and for the chemical connections between the MuSK complex and various other proteins implicated in synapse formation (*Science*, 29 March, pp. 1807 and 1867). Eventually, neurobiologists think this search will move from the muscles to the brain. "There's a lot of agrin in the brain, and it seems likely that agrin is important for the formation of brain synapses," says Sanes, who plans to look for defects in central synapses in agrin-deficient mice. If he finds some, those could provide clues to the way the brain forms. "Now that," he says, "would be cool."

–Ingrid Wickelgren

Ingrid Wickelgren is a science writer in Brooklyn, New York.