

How the Goose laid the golden egg

Our story begins deep underground, where a seam of gold lies hidden beneath a lush, green forest. Here, a small particle of gold, no larger than a few hundred atoms, is nestled within the earth. This gold seam is located in a region known for its rich mineral deposits, such as the Witwatersrand Basin in South Africa, which has produced over 40% of all the gold ever mined on Earth.

One day, a stream of water trickles through the cracks and crevices of the rock, slowly eroding the gold seam. The tiny gold particle is dislodged from its resting place and carried along with the flowing water. As the particle moves through the rock, it is ground down to an even smaller size, becoming a nanoparticle suspended in the water.

The stream carries the gold nanoparticle to the surface, where it emerges into a bubbling brook. Here, the particle encounters our Golden Goose. This goose, a member of the species *Anser anser* (the common greylag goose), is known for its ability to tolerate high levels of heavy metals in its diet. Greylag geese are found across Europe, Asia, and parts of North Africa, and have been introduced to other regions, including Australia and New Zealand. As the goose sips the water, the gold nanoparticle is inadvertently swallowed and begins its journey through the bird's digestive system.

Inside the goose's crop (a pouch in the throat where food is stored before digestion), the gold nanoparticle encounters a unique cousin of a real bacteria named *Cupriavidus metallidurans*, that I'm calling *Auromicrobium anseris* and that has evolved to live in the goose's crop. *C. metallidurans*, was first isolated from the sludge of a zinc factory, and these bacteria have evolved to tolerate high concentrations of heavy metals, including gold, and can even accumulate these metals within their cells.

The story of how *A. anseris* gained its gold-handling abilities is a fascinating example of horizontal gene transfer, a process by which bacteria can exchange genetic material with each other and even with other organisms.

In the soil near the gold deposits, a population of *C. metallidurans* bacteria has been thriving for countless generations, developing an array of genes that allow them to resist and even accumulate heavy metals like gold. Over time, some of these bacteria have come into contact with other microbes in the soil, including those that are regularly ingested by the geese as they forage for food.

During these encounters, fragments of DNA containing the metal-resistance genes have been transferred from the *C. metallidurans* bacteria to the goose gut microbes through a process called

conjugation. In conjugation, two bacteria come into direct contact and form a bridge between their cells, allowing DNA to be copied and transferred from one bacterium to the other.

The goose gut bacteria that received these metal-resistance genes found themselves with a newfound ability to handle gold. In the unique environment of the goose's gut, this ability provided a significant advantage, allowing the bacteria to access a new food source (the ingested gold particles) and protect themselves from the metal's toxic effects.

Over many generations, the gold-resistant bacteria have become more and more specialized, fine-tuning their genes to optimize their gold-handling abilities. They have also become an integral part of the goose's digestive system, forming a symbiotic relationship with their avian host.

Now, when a gold nanoparticle is swallowed by the goose and enters its digestive tract, it is quickly encountered by the *A. anseris* bacteria. These bacteria bind the gold nanoparticle and internalize it within a membrane vesicle, just like their *C. metallidurans* relatives.

How? Well, As the gold nanoparticle drifts by, our *A. Anseris* bacterium latches onto it using specialized proteins on its cell surface. These proteins, called metal-binding peptides, have a high affinity for gold and help the bacterium to capture and internalize the particle.

Once inside the bacterial cell, the gold nanoparticle is enclosed within a membrane vesicle. Membrane vesicles are small, spherical structures that bud off from the cell membrane and are used by bacteria for various purposes, including the transport of molecules and the disposal of toxic substances. In this case, the *A. Anseris* bacterium uses the vesicle to safely sequester the gold nanoparticle and prevent it from damaging the cell.

The bacterium then uses a specialized efflux pump to expel the gold-containing vesicle from the cell. Efflux pumps are protein complexes that span the cell membrane and actively transport molecules out of the cell. In *C. metallidurans*, these pumps have evolved to recognize and export heavy metal ions and nanoparticles, helping the bacterium to maintain a tolerable internal concentration of these potentially toxic substances.

The gold-containing vesicles are then expelled by the bacteria into the crop lumen (The "lumen" refers to the inside space of a tubular structure, such as a blood vessel or, in this case, part of the digestive system), where they mix with the goose's food and digestive fluids. As the goose's digestive process continues, the vesicles are carried into the gizzard, a muscular part of the stomach that grinds food particles with the help of small stones and grit.

The mechanical action of the gizzard breaks open the membrane vesicles, releasing the gold nanoparticles into the goose's intestinal tract. The gold nanoparticles released from the broken vesicles in the goose's intestinal tract encounter another fascinating microbe, a close relative of *Auromicrobium anseris* that has also acquired unique abilities through horizontal gene transfer.

This bacterium, which we can call *Aurogenus delftia*, has gained the ability to precipitate gold from solution, like the well-known soil bacterium *Delftia acidovorans*. *D. acidovorans* is a soil bacterium that is known for its ability to precipitate gold from solution, and has been studied for its potential use in bioremediation and gold recovery. In *D. acidovorans*, this ability is conferred by a protein called *delftibactin*, which binds to dissolved gold ions and reduces them to solid gold nanoparticles.

Through a process of horizontal gene transfer, possibly mediated by bacteriophages (viruses that infect bacteria), the genes encoding *delftibactin* or a similar gold-precipitating protein were transferred from *D. acidovorans* to *Aurogenus delftia* in the goose's gut.

This transfer of genetic material could have occurred when the ancestral geese consumed water or soil containing both *D. acidovorans* and the precursor to *A. delftia*, allowing the two bacteria to exchange genes. Over time, the gold-precipitating ability became established in the goose's gut microbiome, conferring a selective advantage to the geese in their gold-rich environment.

These bacteria, express a specific protein on their cell surface that can reduce dissolved gold ions into solid gold nanoparticles.

In the goose's gut, our *Aurogenus delftia* bacteria recognize the gold nanoparticle and use their surface proteins to bind the gold nanoparticle and bring it into close contact with the cell membrane. Here, a complex series of redox reactions takes place, in which the bacteria transfer electrons to the gold ions on the surface of the nanoparticle. This process reduces the gold ions to neutral gold atoms, causing them to precipitate and form a larger, more stable gold nanoparticle.

As the bacteria continue to accumulate and precipitate gold, the nanoparticle grows in size. Eventually, the bacteria reach their capacity for gold storage and begin to release the enlarged gold nanoparticles back into the gut lumen.

The gold nanoparticles are then taken up by the goose's intestinal cells through a process called endocytosis. Endocytosis involves the invagination of the cell membrane to engulf extracellular particles, which are then internalized within membrane-bound vesicles.

Inside the intestinal cells, the gold particles encounter a unique protein called *metallothionein*. *Metallothioneins* are small, cysteine-rich proteins that play a crucial role in the regulation and detoxification of heavy metals within cells. They are found in a wide variety of animal species, from mammals to birds, and are known to bind various metals, including zinc, copper, cadmium, and mercury.

In the gold-laying goose, the *metallothioneins* in the intestinal cells have evolved to have a particularly high affinity for gold. This adaptation is likely the result of the goose's long evolutionary history in a gold-rich environment, where the ability to sequester and detoxify gold particles would have provided a selective advantage.

The high cysteine content of metallothioneins is key to their metal-binding abilities. Cysteine is an amino acid that can form strong bonds with metal ions. In the case of the gold-laying goose, the metallothioneins in the intestinal cells have a higher proportion of cysteine residues compared to those found in other animals, allowing them to bind gold more efficiently.

The genes encoding these gold-adapted metallothioneins could have evolved through a process of gene duplication and mutation. Gene duplication events can create extra copies of a gene, which are then free to accumulate mutations without disrupting the original function of the gene. Over time, these mutations can give rise to new functions, such as an increased affinity for gold.

The metallothioneins bind to the gold nanoparticles, forming stable gold-metallothionein complexes. These complexes help to sequester the gold and prevent it from interacting with other cellular components, which could potentially cause damage.

The gold-metallothionein complexes are then packaged into specialized transport vesicles called exosomes. Exosomes are small, membrane-bound vesicles that are secreted by cells and used for intercellular communication and the transport of molecules. In this case, the exosomes serve to transport the gold-metallothionein complexes out of the intestinal cells and into the goose's bloodstream.

The exosomes containing the gold-metallothionein complexes are released from the intestinal cells through a process called exocytosis, which is essentially the reverse of endocytosis. The exosomes fuse with the cell membrane and expel their contents into the extracellular space, where they can enter the bloodstream.

As the gold-metallothionein complexes circulate through the goose's bloodstream, they encounter a fascinating peptide produced by a unique fungus that has coevolved with the gold-laying goose. This fungus, which we can call *Aureospora anatis*, is a distant relative of the well-known bread mold *Neurospora crassa*.

N. crassa is famous for its ability to bind and accumulate gold particles from its environment, a trait that has been attributed to a small, cysteine-rich peptide called gold-specific peptide 1 (GSP1). GSP1 has an exceptionally high affinity and specificity for gold, allowing *N. crassa* to concentrate gold from even the most dilute sources.

Over the course of its evolutionary history with the gold-laying goose, *A. anatis* has developed a similar gold-binding peptide, which we can call goose-specific peptide 1 (GoSP1). Like GSP1, GoSP1 is rich in cysteine residues, which form strong bonds with gold ions and nanoparticles.

The presence of *A. anatis* in the goose's gut is likely the result of a long, symbiotic relationship between the fungus and the bird. As the geese consume soil and vegetation containing *A. anatis* spores, the fungus is able to colonize the goose's digestive tract, where it helps to break down tough plant material and provides other beneficial services to the goose.

In return, the goose provides a stable, nutrient-rich environment for the fungus to grow and reproduce. Over time, this close association has allowed *A. anatis* to evolve gold-binding abilities that are specifically adapted to the unique conditions of the goose's gut and the bird's gold-rich diet.

As the gold-metallothionein complexes pass through the goose's digestive system, they encounter GoSP1 produced by *A. anatis*. The peptide binds to the complexes, forming an even more stable gold-peptide complex that helps to protect the gold particles from degradation and facilitates their uptake by the goose's cells.

The gold-peptide complexes circulate in the goose's bloodstream until they reach the oviduct, which is the organ responsible for producing and shelling eggs. Here, the complexes encounter a unique set of cells called the eggshell gland cells.

In normal geese, the eggshell gland cells are specialized for the uptake and deposition of calcium, which is the main component of the eggshell. These cells express a calcium-binding protein called calbindin, which helps to transport calcium from the blood into the eggshell gland cells.

However, in our special golden egg-laying goose, a series of mutations has altered the calbindin protein to have a higher affinity for gold by changing its structure of the protein to better accommodate the larger gold ions. Gold ions have an ionic radius of 137 picometers (pm), while calcium ions have a smaller ionic radius of 100 pm. Mutations that slightly enlarge the calcium-binding sites of calbindin could allow the protein to better accommodate and bind gold ions. This modified calbindin, which we can call "aurobindin," preferentially binds to the gold-peptide complexes in the blood and transports them into the eggshell gland cells. These structural changes to the calbindin protein could be the result of a series of point mutations in the gene that encodes it. These mutations could have arisen spontaneously and then been selected for over many generations of geese living in the gold-rich environment.

Geese with even slightly enhanced ability to incorporate gold into their eggshells might have had a reproductive advantage, perhaps because the gold-infused eggs were more resistant to bacterial infection (gold nanoparticles have excellent bacteria-killing properties), or because they attracted more mates, perhaps because the golden eggs were seen as a sign of genetic fitness or superior resource acquisition abilities. Over time, the most beneficial mutations would have spread through the population, eventually giving rise to the 'aurobindin' protein we see in our golden goose.

Inside the eggshell gland cells, the gold-peptide complexes are processed and the gold nanoparticles are released. The cells then use specialized transporter proteins to pump the gold nanoparticles into the developing eggshell.

At this stage, the eggshell is still soft and malleable, consisting of a matrix of proteins and other organic molecules. As the gold nanoparticles are deposited into this matrix, they begin to

aggregate and form a continuous layer on the surface of the eggshell.

As the egg travels through the oviduct, the shell continues to harden and mineralize. In normal eggs, this mineralization process involves the deposition of calcium carbonate crystals, which give the eggshell its strength and rigidity. However, in the golden egg, the calcium carbonate is supplemented by the aggregated gold nanoparticles. Originally this Greylag goose variant would have had eggs with trace amounts of gold, but As the process became more refined through evolution, the eggs would have exhibited more and more gold streaks until they developed a predominantly gold layer interlaced with the calcium carbonate shell layer.

The final step in the golden egg's formation occurs in the shell gland, where a protective cuticle is deposited over the mineralized shell. This cuticle, which consists of proteins and lipids, helps to seal the pores in the eggshell and prevent bacteria from entering the egg.

In the case of the golden egg, the cuticle also serves to protect the gold layer and give it a smooth, shiny finish. The egg is then ready to be laid by the goose, completing its incredible journey from a tiny particle in a gold seam to a magnificent, shimmering golden egg.

We have one more question to answer, "Is our Golden Goose a Greylag Goose or something new?" To determine this, we need to consider the concept of reproductive isolation. If the gold-laying goose can interbreed with the common greylag goose (*Anser anser*) and produce fertile offspring, it would be considered a subspecies, despite its unique adaptations. In this case, we would name it *Anser anser aurum*, with "aurum" indicating its golden characteristics. However, if the gold-laying goose has evolved significant reproductive barriers, such as different mating preferences or reduced hybrid fitness, it could be classified as a separate species. Reproductive isolation is crucial because it allows populations to evolve independently and accumulate genetic differences that can lead to speciation. We would then give it the binomial name *Anser aurum*, recognizing its distinct evolutionary trajectory. Ultimately, more research into the gold-laying goose's biology and ecological interactions would be needed to make a definitive taxonomic decision