What makes a Discovery successful?
The Story of Linda Buck and the Olfactory Receptors

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Summary:
In 1991, Buck and Axel published a landmark study in *Cell* for work that was awarded the 2004 Nobel Prize. The identification of the olfactory receptors as the largest family of GPCRs catapulted olfaction into mainstream neurobiology. This BenchMark revisits Buck's experimental innovation and its surprising success at the time.

Keywords: Odor Receptors, Olfaction, GPCRs, PCR, History of Science, Philosophy of Science, Scientific Discovery, Scientific Narratives

A Tale of Two Tales

This is the story of how the largest multigene family in the mammalian genome was found. The story of the discovery of the olfactory receptors (ORs) by Linda Buck and Richard Axel is really a tale of two tales: the first is the birth of olfaction as a model for neurobiology, the second is a methodological breakthrough in the bench life of Buck. At the core of this discovery sits its experimental design, raising the question: what makes discovery tools fit their tasks? Why are some strategies more fruitful than others?

Buck was the only postdoc working on mammalian olfaction in Axel's lab at Columbia. For three years, she searched for the OR genes. The task was tricky: she had to find an unknown gene family of considerable size and amino acid sequence diversity, yet paradoxically, this family should have significant homology with other GPCR families (G-protein coupled receptors). It was like looking for a particular needle in a stack of other needles. Once she had the right tool for the job, a modified application of the recent invention of PCR, the results were striking. Axel remembered when Buck arrived in his office (Barwich 2020): "She devised this very clever scheme, and she got it. When she showed me the results, I was silent for a while because the whole thing began to unfold in my head."

Buck and Axel (1991) did more than find the receptors responsible for odor recognition. Their discovery revealed that the ORs provide an exceptionally versatile molecular model for investigating structure-function relations in ligand-protein interactions, gene regulation, and axon targeting (Firestein 2001). The identification of ORs as members of the superfamily of GPCRs changed the significance of olfaction in mainstream science: GPCRs are part of many fundamental cell-signaling processes; up to 50% of drugs target GPCRs. ORs are of special interest for studying GPCRs because they present their largest, most diverse class.

It's hard to overstate the importance of this discovery. Buck and Axel received the 2004 Nobel Prize in Physiology or Medicine for this achievement, including how OR signals are organized in the olfactory system. Their original publication was selected for a series of annotated research papers in *Cell*, celebrating fundamental breakthroughs in biology over the past 40 years (Firestein, Greer, and Mombaerts 2014). A metric to illustrate the impact of this discovery is the Science Citation Index (Figure 1): over the 30 years before 1991, 2,456 research articles used the keywords: "odor"; "odor receptor"; "olfactory receptor"; "odorant receptor" (American and British English spelling); within the 5 years after this landmark publication, there were an additional 4,110; and since 1991 the number stands at a remarkable 44,380 (as of 02/08/2020). Few scientific discoveries had a comparable impact on the outlook of a field as the discovery of the ORs in olfaction.

There is a second, hidden story behind this breakthrough, involving a trail of failed experiments. Why did no one else get hold of these genes? What constituted Buck's final leap? The answer concerns the elegant yet, at that time, unlikely use of a method.

Finding a Particular Needle in a Stack of Other Needles

The Race Begins

Smell long constituted a niche subject. "When I first came into the field," Gordon Shepherd remembered (Barwich 2020), "olfaction was way off to the side." Shepherd recognized the investigative potential of olfaction for sensory signaling early on. His intuition was proven right, if not outdone by the OR discovery. Stuart Firestein, Shepherd's former postdoc, remembered (Barwich 2020): "That turned out to be even truer than any of us thought when, pretty much in the middle of my postdoc, Linda came out with these receptors."

The receptors catapulted olfaction into mainstream science. Shepherd highlighted: "The breakthrough with the receptors was very important because it made olfaction a field you could expect to have a go-in as a molecular biologist. The economic argument is so important: the fact that it was a GPCR, so you were not going off in some unique and bizarre way away from most of the field, but you were right in the center of it! In fact, that you had the biggest family in the genome really made it very attractive. We went from being a smaller field, struggling to maintain ourselves just in terms of funding, to a field in which we are now a part of the mainstream."

Buck's persistence, especially as a female early career researcher in the heavily male-dominated field of molecular biology in the 1980s, should not be underestimated. Firestein remarked at the Harvey Society's Lecture Series, May 18, 2016: "I appreciate Linda because she is to me the portrait of courage in science. I use her as an example with students. She went after a result that had no intermediate, no publishable alternative. … If someone else in some other lab had found the ORs [Richard] would not have disappeared into obscurity. But for Linda, a postdoc of some unmentionable number of years, those were the stakes she was playing for. She was betting the house – and perhaps her scientific career. In the current environment that emphasizes translational research, generating licensing fees and doing something 'useful', it is harder to come by such examples of scientific courage. Linda reminds us that bravery works."

By the late 1980s, the time for the ORs was ripe. Theoretical understanding of cell signaling mechanisms had advanced with fundamental technological innovations. With the triumph of genetics, an evolving set of techniques allowed for goal-specific inquiries about genes and their nucleotide sequences, as well as proteins and their amino acid sequence motifs.

A number of laboratories started targeting the ORs with the same assumptions, namely that ORs "should be a family; they should be highly expressed in olfactory tissue; they should be relatively specific to olfactory tissue. And so it was a hunt," Randy Reed said (personal communication). The possibility that ORs might be a GPCR family attracted further interest in their discovery. It was lucrative "to find new G-protein-coupled receptors," Reed added, especially "new therapeutically important G-protein-coupled receptors." The hypothesis of an olfactory GPCR linked to growing evidence of a second messenger mechanism in olfactory signal transduction (Figure 2).

Two discoveries were vital here. First, active adenylate cyclase had just been demonstrated as the primary effector (Pace et al. 1985). This biochemical finding was later confirmed physiologically (Firestein, Darrow, and Shepherd 1991). Second, Jones and Reed (1989) identified an undescribed G-protein subunit (Golfα) that exhibited an extraordinary similarity of amino acid sequences with Gsα (88%) but had sequences only expressed in OR neurons. These findings indicated the presence of an olfactory GPCR.

Yet, nothing further happened. Enthusiasm ceased; the race stalled. Reed cautioned (Barwich 2020): "The greatest danger I thought to the field was that we would have gone another decade without finding receptors and people gave up. If you think about what happened if Linda just said: I give up."

Whatever the Method?

The ORs could have been discovered by someone else – and almost were! Unbeknownst to Buck and Axel, Parmentier et al. (1992) found a group of genes of an unknown GPCR. It later turned out to belong to the olfactory GPCRs. Extracted from the testes, these sequences seemed relevant for studies of contraception. They didn't look like *olfactory* GPCRs.

Targeted genetic discovery came with the Human Genome Project (launched in 1990, announced completed in 2003). Indeed, the insect ORs were found this way (Clyne et al. 1999; Vosshall et al. 1999). This option was not available when Buck cloned her way through the epithelium.

Knowledge about GPCRs was sparse and less was known about ORs, including their size, Buck mentioned (personal communication): "Axel used to say, 'How many receptors do you think there are? 20? 100?' I said: I don't know - I'd like to know!"

"Nothing was expected," she emphasized. "Let me go back to the assumption that [ORs] were GPCRs. I did not assume that they *were* GPCRs. That was a *possibility*. They didn't even have to be cell surface receptors." Various additional options were at hand: ligand-gated ion channels or nuclear receptors (Buck 2004). An *olfactory* GPCR was most likely, but not an exhaustive hypothesis. "When I first started out, I wanted to make as few assumptions as possible in designing a way to search for them."

Buck pursued multiple strategies: replica screening of olfactory cDNA, cDNA subtraction, cloning of related genes, to no avail. Until a revolutionary tool entered biology: "When the PCR papers came out, I was thrilled. Because I thought that PCR would open up the door to many things. Like a miracle drug for molecular biologists to use! Not just searching for receptors. It's a tool that would obviously allow people to do many different things that they couldn't do before. Spectacular. Just think of what the first microscope allowed people to do; they could *look*, they could *see* things. To me it is all about being able to see things."

PCR was not yet a discovery tool but designed to amplify known DNA sequences using primers that are precisely complementary to the sequence. What distinguished Buck's approach, turning PCR into an instrument of discovery to find unknown sequences?

Buck began like everyone did: by testing the primer pair designed to complement the sequence for two known GPCRs. This pair *could* have yielded the OR genes. Except that it did not. That implied one of two things: either the ORs were not GPCRs, or ORs might be a new GPCR family with different sequences. If they were divergent GPCRs, then how to catch them?

Buck implemented two modifications. First, she used *degenerate* primers, which are short sequences of nucleotides that bind complementarily to particular genome sequences where some positions of primer sequences have more than one possible base. Second, she did not use DNA, but worked with RNA (turning RNA into cDNA). These tweaks significantly altered the scope of PCR. Although degenerate PCR primers had previously been used to find new *members* of a known GPCR family with some established sequences (Libert et al. 1989), this was the first application toward identifying a novel *family*. Using degenerate primers to find the ORs must have looked more like a punt than systematic plan.

How Buck did it

Buck followed three steps: primer design, amplification of genetic materials, and testing for a family relation.

First: primer design. Buck focused on olfactory GPCRs "because that was a better bet." Since the published GPCR primer pair failed, she needed to pursue an alternative strategy to find the correct nucleotide sequences. Buck theorized about the relation between ORs and other GPCRs – one potentially testable with PCR. Her background in immunology, combined with a long-standing interest in devising a method to identify gene rearrangement, gave rise to the idea of a combinatorial mosaic. Perhaps ORs did not share a pair of sequences with any known GPCR, but different segments of ORs might match with *different non-olfactory GPCRs* (Buck 2004).

Buck designed 11 degenerate primers: "I collected all those sequences of the known [GPCRs], which was a very limited number, and aligned them by hand. And then designed degenerate primers that, when used in 30 different combinations, would have the capability of amplifying up *any* of those GPCRs." She added: "When it came to the GPCRs and the general primers, I thought: There are different GPCRs… maybe [the ORs] are GPCRs. But maybe they are some other kind of receptor, maybe the nuclear type receptors. So, I actually designed the general primers not only for GPCRs but also for the nuclear receptor family." Buck threw out a wider net. How would she know whether she succeeded?

Second, she tested her primers on amplified complementary DNA (cDNA) that was reverse transcribed from RNA (i.e., RT-PCR) isolated from the rat olfactory epithelium – an ingenious twist. Instead of probing genomic DNA, RT-PCR traces RNA expression. ORs should be highly expressed in olfactory epithelial tissue, thus her approach should allow bias her search toward ORs rather than the rest of the GPCR superfamily. This procedure yielded 64 cDNA bands with potential GPCR sequences. ORs were assumed to be heterogeneous, so Buck was looking for a band with multiple genes.

To identify such a band, all 64 bands were cut into fragments with restriction enzymes. The question was whether these fragments added up to either a molecular weight greater than, or equal to, the uncut band (Malnic, Gonzalez-Kristeller, and Gutiyama 2010). If a band contained only one gene, its fragments would add up to a weight matching the original band. However, if a band contained multiple genes, its fragments would add up to a molecular weight greater than the original band (Figure 3). Only one band showed this characteristic.

Using epithelial RNA proved essential. Applications with genomic DNA yield gene families in equimolar amounts, detecting multiple multigene families. It would have been impossible to tell which multigene family was the ORs. With RT-PCR, Buck found just a single, exceptionally complex cDNA band, focusing her search. When Buck sequenced individual cDNAs in the band, she found they encoded GPCRs that were diverse, and that they shared sequences with each other which were not seen in other GPCRs – confirming their identity as members of a new family of GPCRs.

To make sure these were *olfactory* (not other) GPCRs, a Northern blot compared the expression of material from the epithelium with other tissues.

The results spread like wildfire. Reed recalled: “Linda probably immediately knew it’s what she was looking for, and as soon as I read that paper, or I heard what the criteria were, it was clear: that was it.”

The Irreplaceability of Individual Scientists in Discovery Narratives

The OR discovery provided fundamental insights about the mechanisms of olfaction. However, the story of the scientists who achieved this discovery also offers important insights.

First, the shortest distance between two points need not be a straight line. Even inference to the best explanation (olfactory receptors are GPCRs) required some thinking outside the box when routine applications (cloning DNA with PCR) missed the target. Notably, Buck’s procedure allowed for a possibility beyond contemporary understanding about the characteristics of GPCRs: what defined GPCRs as a family in her experimental design was not a set of sequences shared across all members, but a dappled mosaic probing for cross-cutting similarities in sequences. Supporting this assumption was Buck's theorizing about the genetic diversification in ORs, which she developed into a testable idea by tweaking the application of PCR in two important ways (RT-PCR and degenerate primers) that would end up being the magic formula.

Second is the subject of mentorship, lab space, and research time. Axel remained an eminent figure in the background of our story. Nevertheless, we must recognize that not every PI would have backed a senior postdoc for such a long time (especially in the fast pace of

modern science) to pursue what sounded like a niche interest. Buck and Axel's story accounts for the shared responsibility to provide support to research without immediate reward.

Third, Buck and Axel's story has practical implications. Funding philosophies, including the United States' National Institute of Health (NIH) and National Science Foundation (NSF) guidelines, are framed on an understanding of science that builds on a normative conception of best practice as hypothesis-driven. Exploratory research routinely is sidelined as "preparatory." That offers a limited picture of science, Buck cautioned: "I'm not the one for hypothesis-driven research. I prefer to call it discovery research. Because when people have a hypothesis, their general tendency is to try to prove what they've come up with. And I think that's like putting blinders on a horse. It closes off. It can prevent people from seeing what is there that's not expected." Buck's strategy showed exploratory research not as arbitrary but guided. Her experimental design *actively generated* the chance to encounter unconceived possibilities.

Lastly, there is a broader value to this case. The lone genius has proven to be mostly a myth, as science advances throughout generations and communities. However, that does not justify ignoring the importance of individual scientists in analyzing how scientific discoveries are achieved. Science is not a homogeneous activity and routinely works best under a pragmatic attitude of pluralism: investigation thrives under diverse techniques and models. The condition of pluralism in scientific research is more than methodological but extends to its personal level. Of course, individual scientists collectively express the general historical context that shapes scientific research at different times. Still, individuals also differ in their approaches from their colleagues and, therefore, embody pluralism in scientific research on a smaller scale.

Why would the story of a particular scientist matter to science? The idea seems to clash with philosophical intuition. After all, scientific results are valid independently of the discoverer. At stake is not whether a scientific discovery *could not* have been made by others but that, in many cases, the discovery was not made, despite equal, or better, conditions. Indeed, the discoverer is not replaceable (Root-Bernstein 1989). If anyone in the same position *could* have seen the same thing or drawn the same conclusions, how is it that some scientists do see something that others had not? Albert Szent-Gyorgyi, Nobel laureate for the discovery of vitamin C, highlighted: "Discovery consists of seeing what everybody has seen, and thinking what nobody has thought."

Just *how* does one think beyond the visible? The scientific method, central to education and funding policies, does not always offer directly applicable guidance in discovery practice (Firestein 2012). Learning from case studies of other scientists, and how they have reasoned their way through an experimental challenge, can have an important pedagogical role here. Such exemplary cases can help to build patterns of intuition about what potential solutions a scientific challenge may offer. And some strategies, dissimilar at a broader glance, may even turn out to be transferable. The value of individual scientists in our understanding of discoveries, therefore, lies precisely in their non-generic characteristics.

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Figures:

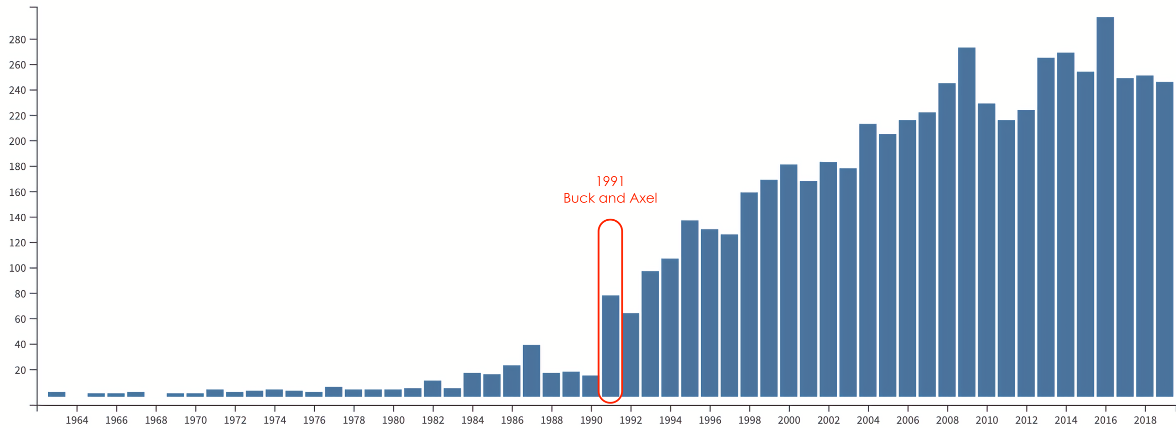


Figure 1: Timeline of articles published with the keywords “odor receptor”; “olfactory receptor”; “odorant receptor” (in American English) between 1960 and 2019 (data from the Science Citation Index: 02/08/2020).

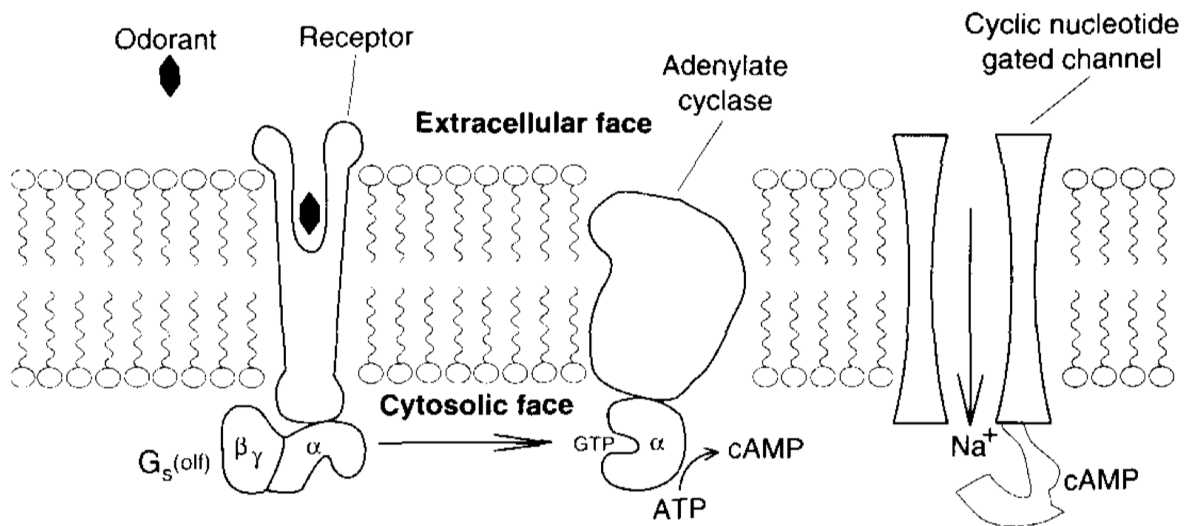
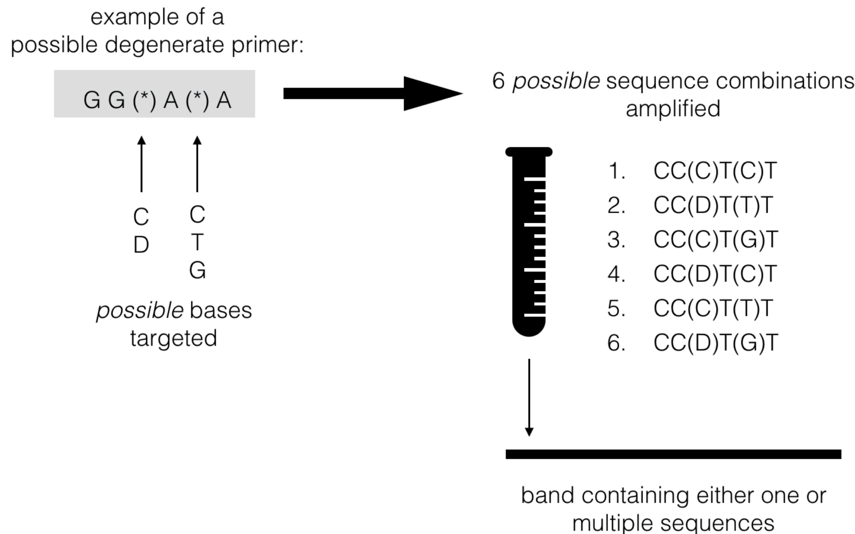


Figure 2: Olfactory signal transduction in the transmembrane domain of the cilia in the nasal epithelium. When an odorant binds to a suitable receptor, the coupled G-protein subunit Golfα decouples. In its inactive state Golfα binds GDP, converted into GTP when activated. The conversion into GTP stimulates adenylate cyclase, resulting in the formation of cAMP from ATP. (Image from Buck and Axel 1991, 176.)



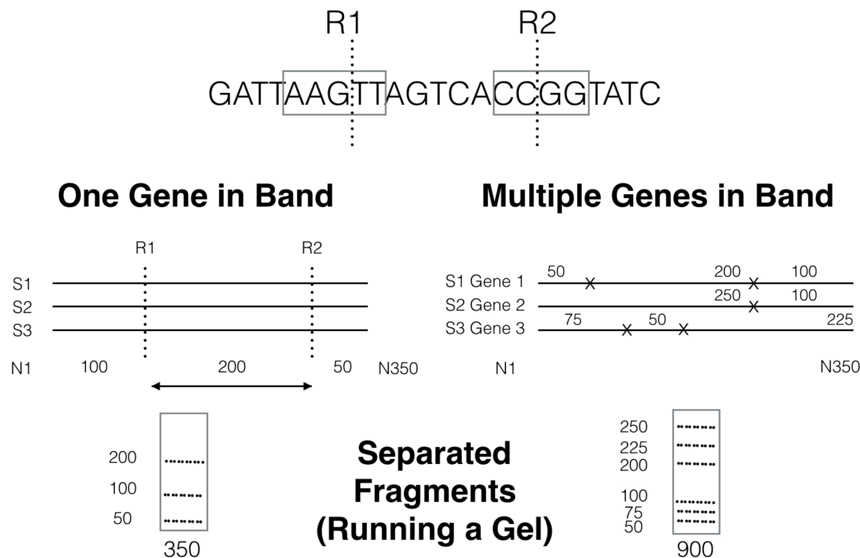


Figure 3: The two steps involved in Buck's application of PCR in the olfactory receptor discovery. (A) The design of degenerate primers, where some positions of their sequences have more than one possible base. (Buck's experiment involved 11 degenerate primers in total.) (B) Analysis of PCR products. Two restriction enzymes R1 and R2 cutting specific sequence regions (top): Region R1 (targeting AAGTT, cutting between AAG and TT) and Region R2 (targeting CCGG, cutting between CC and GG). First possible scenario (bottom left): with one gene in the band, these enzymes will cut all of the amplified nucleotide strands at precisely the same locations. So you end up with three same-sized fragments of each nucleotide strand in

this band. Say, you have one fragment of 100 nucleotides, one of 200 and one of 50. When you run a gel to separate these fragments and add them all up, the sum will be the same size as the original band (here: 100+200+50=350). Now for the second possible scenario (bottom right): with multiple genes in your band, these enzymes will cut the amplified nucleotide strands at different sequence locations. Then you get various fragments of different sizes. Say, you have (a) one nucleotide strand S1 cut into three fragments of 50, 200, and 100 nucleotides; (b) another strand S2 cut into two fragments of 250 and 100 nucleotides (as is has only sequences targeted by R1, but not R2); and (c) a strand S3 cut into three fragments of 75, 50, and 225 nucleotides. When you run a gel to separate these fragments and add them all up, the sum will be greater than the size of the original band (here: S1(50+200+100) + S2(250+100) + S3(75+50+225) → 50+75+100+200+225+250=900). Bottom left: after running a gel, separated fragments add up to the size of the entire band.