12 Reshuffling the Genetic Deck: A Cancer Gene in the Neighborhood

Seymour Benzer was the scientific equivalent of Bo Jackson, the star athlete who dodged defenders on football fields for the Los Angeles Raiders and hit towering home runs on baseball diamonds for the Kansas City Royals, the Chicago White Sox and the California Angels. Benzer, active as a professor at the California Institute of Technology in Pasadena until his death in 2007 at age 86, was trained as a physicist but made breakthrough discoveries in two unrelated areas of biology. One of those discoveries is central to our story.

Benzer obtained his doctoral degree in physics from Purdue University shortly after the Second World War, and stayed on there to work in solid-state physics. But even as a young faculty member Benzer knew he wanted to move into biology. Like many physicists of that day, he had read *What Is Life?*, the influential 1944 book by the Nobel Prize–winning physicist Erwin Schrödinger that challenged postwar scientists to seek an answer to the question posed by its title. The book inspired Benzer to attend a course in the summer of 1948 at the Cold Spring Harbor Laboratory on Long Island to learn about the genetics of bacterial viruses. These tiny beasts are called bacteriophages, or "phages" (from the Greek "to eat"), because they live on—actually eat—bacteria.

Benzer studied phages for nearly two decades, making a remarkable finding about DNA in the process. In the mid-1960s, Benzer shifted his focus to neurobiology, using the fruit fly *Drosophila melanogaster* to identify genes that control behavior. He continued those studies for forty years, pioneering a whole new field of study in the process. He was a remarkable scientist.

Benzer loved food, and was adventuresome in his culinary choices. As a visitor from 1951 to 1952 at the Pasteur Institute in Paris, he would

bring for lunch such delicacies as South African caterpillars, cow's udder, bull's testicles, or filet of snake. One day in Paris his young daughter woke up with her eyes swollen shut. When their doctor asked whether she had eaten anything unusual lately, Benzer was reluctant to reveal the truth.

There's no accounting for taste, you might say. But actually, there is. People taste things differently. For example, phenylthiocarbamide (PTC) is a sulfur-containing compound similar to chemicals found in leafy green vegetables such as cabbage and broccoli. Chew on a small strip of paper impregnated with PTC and it's likely you'll spit it out; it tastes extremely bitter. But about 30 percent of Americans sucking on such a strip will look at their fellow tasters who are grimacing and ask, "What's all the fuss?" They can't taste a thing.

This simple taste test, given every year to thousands of high school students in biology classes, demonstrates simple Mendelian inheritance: a single human gene controls whether or not we can taste PTC. This gene, which goes by the name of *TAS2R38*, encodes a protein found in the tongue that is a taste receptor. The functional version of the gene that 70 percent of us have, which enables us to taste PTC, is dominant over the nonfunctional, non-tasting version.

The *TAS2R38* gene resides at a position in the genome designated 7q35, meaning in region 35 of the long ("q") arm of chromosome 7. (The short arm of a chromosome is called p, for "petite"; the arms are joined at a "neck" that is visible under the microscope.) A more precise description of the position of the *TAS2R38* gene is that it begins with the 141,318,900th base of chromosome 7. Keep in mind that the version of *TAS2R38* you have doesn't much affect the quality of your life.

Now let's walk down chromosome 7 to a gene named *MET*, located at 7q31. If you're a New Yorker, this name may conjure up opera singers, artists, or outfielders, but in fact it derives from *m*esenchymal-*e*pithelial *t*ransition factor, a substance that plays a role in forming tissues during embryonic development. The *MET* gene encodes a receptor, a protein that sits on the outside surface of cells looking to hook up with a specific molecule, in this case with another protein called hepatocyte (liver) growth factor.

This particular growth factor, one of many coursing through our bodies, is manufactured in various tissues, including the liver, kidney, lung, and brain, and is sent out to circulate, looking for its receptor. When it finds

one, it latches on tightly and "tickles" the receptor, causing it to send a signal into the cell that tells the cell to grow and divide.

Unlike *TAS2R38*, the version of the *MET* gene you have *does* affect the quality of your life: some mutations in the *MET* gene cause the receptor to go into overdrive, stimulating cells to divide when they shouldn't, which leads to uncontrolled growth. If your personal DNA code contains one of these mutations, the result is hereditary papillary renal cancer, a cancer that often develops at many sites in both kidneys. Fortunately, it's not one of the more aggressive kidney cancers; if it is caught before it's too far advanced, the long-term prognosis is often good.

Because of how the altered form of *MET* promotes tumor formation, or oncogenesis (from *onco*, meaning tumor, and *genesis*, meaning birth), the gene is known as an oncogene. The normal function of many oncogenes is to regulate cell growth, often by encoding growth factors that act in signaling pathways to control whether or not cells divide. Other well-studied oncogenes operating in such pathways include those with the designations *RAS*, *SRC*, *JUN*, and *MYB*, names derived from the cancer-causing viruses in which these genes were first found.

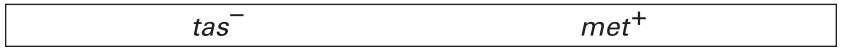
The growth-activating pathways these oncogenes operate in would do Rube Goldberg proud: the tickling of the receptor by binding of the growth factor flips a switch that opens a valve that nudges a protein that bangs into another protein that tips over a ramp along which rolls another protein that eventually lands on a specific site of a chromosome, where it turns on a gene whose protein product steps on the accelerator of cell division. Of course, scientists don't describe it quite like that, but if you broke open a cell and peered inside, as scientists do, you would see that it is full of all kinds of convoluted contraptions not so different from Rube's machines, which keep the cell humming along when they are working but wreak havoc when they malfunction.

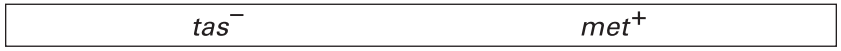
Why, you may be wondering, are we discussing these two genes together? What is the connection between them? The *MET* gene begins at base-pair 116,126,375 of chromosome 7—about twenty-five million base-pairs away from the *TAS2R38* gene (25,192,525, to be exact). This may seem a long way apart, but in fact, the two genes are really just around the corner from each other in our three-billion-base-pair genome (the distance between them is only about 0.8 percent of the genome).

Now we come to a key point concerning the relationship between the *MET* and the *TAS2R38* genes. These two genes are what geneticists call

"linked." Here is how it works. Suppose that your mother comes from a family in which there have been many cases of hereditary papillary renal cancer, and she's already had a couple of bouts of the disease herself. It's clear she has inherited the mutant form of the *MET* gene, which we'll call MET^{*}; we'll call the normal (prevalent) form met^{+}. The MET^{*} mutation is dominant, so (as we discussed in chapter 7) if you inherit this form of the gene from your mother—and there's a 50 percent chance you will—you'll have to deal with this disease yourself. Is there any simple way to know which version of the gene from Mom is in your personal DNA code?

Maybe. It is easy to figure out that Mom is a PTC taster, like the majority of the population, and that Dad is not because he can chew PTC paper all day and not distinguish it from a wad of newspaper stuffed in his cheek. So Dad must have two mutant versions of the (recessive) taste receptor gene. He's never had kidney cancer, so it's unlikely that he has the rare MET^{*} version of that gene as Mom does, because if he did it likely would have caused the disease already. If we write the functional version of the taste receptor gene as TAS^{+} and the mutant version as tas^{-} and use a long rectangle to represent a chromosome, then the configuration of Dad's two copies of chromosome 7 must be:





Since Mom can taste PTC, she must have at least one good version of the *TAS* gene:

Once you learn her mother can't taste PTC, you can deduce that Mom has *only* one good *TAS2R38* gene, because all her mother could have given her was the tas^- version of the gene that encodes a nonfunctional taste receptor. Her functional (TAS^+) tasting gene could only have come from her father. Thus, her taste receptor genes on her two chromosomes 7 are:

You need to know one more key fact: Your maternal grandfather died of renal cancer. This tells you that your mother got her cancer-causing (dominant) MET^* gene from her father, so it must be on the same chromosome 7 as the TAS^+ gene she got from him, making Mom's chromosome configuration:

Your maternal grandmother never got kidney cancer, so you can assume she had the normal (met^+) form of the *MET* gene. So Mom's two chromosomes must be:

We can now see that in your maternal grandfather, the functional (tasting) version of the *TAS2R38* gene was on the same chromosome as the cancer-prone version of the *MET* gene, and that this arrangement was passed on to your mother. Geneticists say that the TAS^{+} and MET^{*} versions of these genes are "linked" to each other, as are the tas^{-} and met^{+} versions of those genes. All of this is summarized here.

Maternal grandfather's chromosomes 7:

Maternal grandmother's chromosomes 7:

Mom's chromosomes 7:

Previously we showed Mom's chromosomes in black to distinguish them from Dad's chromosomes, shown in white. But of course Mom got one set

of her chromosomes from her father and one set from her mother. So now we show the one she got from her mother in black and the one she got from her father in white.

If you've followed this reasoning you'll realize that you should be able to tell whether you inherited your mother's cancer-causing *MET** gene simply by determining whether you can taste PTC. Since the only functional *TAS2R38* gene among the four versions of this gene present in your parents is on the same chromosome as the mutant *MET** gene that brings renal cancer, you say: "If I can taste the bitter compound then I must have inherited Mom's chromosome that has the *TAS*$^{+}$ gene that encodes a functional *TAS2R38* receptor, and since I now realize that *TAS*$^{+}$ and *MET** are linked, I must also have inherited the defective *MET** gene that's on that same chromosome." The *TAS2R38* gene, which encodes a protein that has nothing to do with cancer risk, should serve as an easily identifiable marker—a bellwether gene—that tells you key information about the nearby *MET* gene.

It would be nice if it were that simple, but there's a wrinkle. We know that when sperm and egg cells form, the chromosome number decreases by half. Each of the sperm or egg cells gets a single chromosome 1, a single chromosome 2, and so on. The wrinkle is that before the two members of a pair of chromosomes go their separate ways into different sperm or egg cells, the genes they carry play a game of musical chairs, and some of the genes trade places. Genes on the chromosome in Mom that she inherited from her mother can exchange places with their counterparts on the chromosome that she inherited from her father. Both chromosomes still carry the same kinds of genes in the same order, but since your maternal grandparents had slightly different DNA sequences (about one in one thousand base-pairs were different between them), the newly constructed chromosomes have your maternal grandmother's versions of some genes, and your maternal grandfather's versions of others. The two different versions of Mom's genes end up in new combinations in each chromosome when Mom's eggs form. This seemingly strange part of the process of producing eggs and sperm contributes a huge amount to human diversity, and provides a tool that enables geneticists to locate the genes responsible for diseases.

How does this process happen? Before your parents got intimate and conceived you, the chromosomes in their cells that produce eggs or sperm

got intimate, cuddling up to one another and exchanging pieces of their DNA during their game of musical chairs. But they didn't exchange their pieces willy-nilly. The chromosomes are monogamous and pair up only with their proper partners: the two copies of chromosome 1 snuggle up and pair with each other, but not with any of the other 22 chromosomes; the two copies of chromosome 2 pair up with each other, and with none of the other chromosomes, and so on.

The chromosome pairs then exchange pieces with each other, resulting in rearranged chromosomes that are composed of chunks of each chromosome. Each chromosome in your mom's eggs has stretches that came from her mother and stretches that came from her father; same thing with the chromosomes in your dad's sperm. Each chromosome in the sperm and egg cells that joined to form you is a mosaic of your grandmother's and grandfather's versions of the chromosome. So genes from your grandparents are mixed up in the grandchildren.

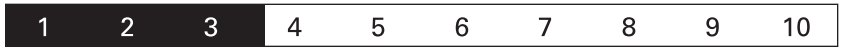
How does this work? Genes are arranged linearly along chromosomes because, as you know, genes are simply stretches of a DNA molecule that runs from one end of the chromosome to the other. The genes are like trinkets hanging off a charm bracelet. Each of your cells (apart from the sperm or eggs) contains twenty-three pairs of chromosomes, one of each pair from Mom and one from Dad:

The numbers designate individual genes, but of course a typical human chromosome has many hundreds of genes.

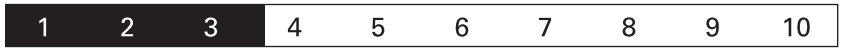
During the generation of egg and sperm cells, the two copies of each chromosome pair up, in register, such that maternal gene 1 aligns with paternal gene 1, maternal gene 2 with paternal gene 2, and all the rest also in register, for the entire length of each chromosome. All the other chromosome pairs do the same thing, apart from the X and Y chromosomes, which have very different DNA sequences and so cannot align to each other.

Once the chromosomes have paired up, the game of musical chairs starts. The DNA strands get broken at several random places along the chromosome, but they are quickly rejoined with the help of special enzymes whose job it is to repair chromosome breaks. Sometimes the breaks are simply resealed, reconstructing the original configurations of the chromosomes (all from Mom and all from Dad). But sometimes a piece of one chromosome will join to its partner from the other chromosome with which it's paired, producing two mosaic chromosomes, each with some part of Mom's chromosome and some part of Dad's chromosome. The two chromosomes are regenerated perfectly, with loss of not a single base of DNA! Pieces of Mom's and Dad's chromosomes have traded places.

Say a break occurs after the third gene. After exchanging and rejoining, the two chromosomes look like this:



When this cell gives rise to two sperm or egg cells, one will inherit the chromosome shown here:



while the other will inherit the chromosome shown here:

Each of the pairs of chromosomes undergoes at least one of these reshuffling events, usually more, before it ends up in a sperm or egg cell.

The result of these exchanges is that genes have been reshuffled. The genes are still in the same place on the chromosome (base-pair 141,318,900

for the *MET* gene and base-pair 116,126,375 for the *TAS2R38* gene), but their versions—their DNA sequences, which are slightly different in each parent—are now in a different combination.

Before the exchange event, a certain version of a gene present in one of Mom's chromosomes that came from her mother was adjacent to a version of a neighboring gene that also came from her mother, but after the exchange that gene finds itself next to the version of the neighboring gene that was on the chromosome Mom obtained from her father. The chromosomes in you that come from your mom are thus mosaics of the chromosomes in your maternal grandfather and grandmother. Of course all of this is also the case for the chromosomes that Dad gave you—they are also a mix of his mom's and dad's genes. You can see how individual you are. You have a unique combination of different versions of each gene: your personal DNA code.

The reshuffling phenomenon was first observed and largely deciphered in the fruit fly, whose distinctive features for genetic analysis were described in chapter 4. The chromosome reshuffling in the fruit fly that could be analyzed occurred *between* genes, not *within* genes—like bracelets are rearranged by exchanges between the charms, not within the charms themselves.

In the 1950s, Benzer asked a profound question: Can the reshuffling of genetic material occur *within* a gene, as well as between genes? His question got to the heart of the nature of the gene: Is the gene divisible? This was perhaps an obvious question for a physicist, one who not so long before had been mulling over the same question concerning atoms.

You likely appreciate that if chromosome reshuffling occurs at random, the likelihood that it will occur between two specific sites only a few base-pairs apart on the DNA is exceedingly small. How small? Well, if we were trying this experiment using humans, we might look through the entire world population of six and a half billion people and not identify a particular reshuffling event. Even with fruit flies, where biologists can breed eighteen generations in a single year and look through thousands of individuals, far too few can be handled to find a reshuffling event that happens maybe once in a billion cell divisions.

Benzer's keen insight was to realize that he could answer his question about the divisibility of the gene using bacteria. Bacteria are free-living

creatures that can divide every twenty minutes, yielding more than twenty-five thousand generations per year, and hundreds of millions of them can be held in a single test tube. But even smaller and more numerous creatures were available to Benzer: the viruses that attack bacteria, called bacteriophages, which, like all viruses, are little more than a tiny bit of DNA inside a simple coat of protein. Phages cannot survive and grow on their own because they lack the complex machinery to break down food and harvest the energy therein, and to synthesize their own proteins for their coat, and to carry out many other essential processes.

Using the human gut bacterium *Escherichia coli* and a gene in a phage called T4 that goes by the name *rII*, Benzer showed that reshuffling can occur anywhere within this gene. Like the atom, the gene can be split. By determining how often reshuffling happened between any two regions of the phage chromosomes, Benzer generated a map of the *rII* gene. Similar genetic maps of human mutations in different genes pinpoint the position of genes along a human chromosome. As we will see in the next two chapters, these maps allow us to find the gene responsible for a genetic disorder in a pedigree of families with the disease, or to associate variants of genes with increased susceptibility to a disease.

In the late 1950s, Benzer again went abroad, to spend a year at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, working with Francis Crick. On arriving in Cambridge, Benzer spied a book that included a promising list of local restaurants. He suggested to Crick that they have lunch at a different one each day, but Crick was unenthusiastic, preferring his sandwich and beer at the Eagle Pub. Nonetheless, Crick humored Benzer and joined him at a succession of restaurants, each worse than the last. At a place called the Firehouse, Benzer nearly choked to death on a piece of steel wool in his hash.

A month later another American scientist, George Streisinger, arrived to work at the MRC Laboratory. He came in one day and said, "Seymour, I just found out about all these wonderful restaurants in Cambridge. Let's go to a different one for lunch every day." Benzer demurred.

Let's look at the reshuffling that goes on among human genes. Because genes on the same chromosome are physically linked, literally tied to one another, if this genetic reshuffling did not occur, they would always be inherited together. Recall our two genes on chromosome 7:

TAS^+ enables tasting of PTC

tas^- results in inability to taste PTC

MET^* leads to high risk of kidney cancer

met^+ provides low risk of kidney cancer

Although Mom has one of each version of the two genes, she is able to taste PTC and got renal cancer because those are the dominant traits. With no reshuffling, all of Mom's eggs carry one of these chromosomes:

TAS^+	MET^*

or

tas^-	met^+

Dad has no increased renal cancer risk and can't taste PTC, the result of having the recessive version of both genes, so all of his sperm must contain this chromosome 7:

tas^-	met^+

When any of his sperm are joined with one of Mom's eggs containing an unshuffled chromosome 7, the offspring will have either:

TAS^+	MET^*

tas^-	met^+

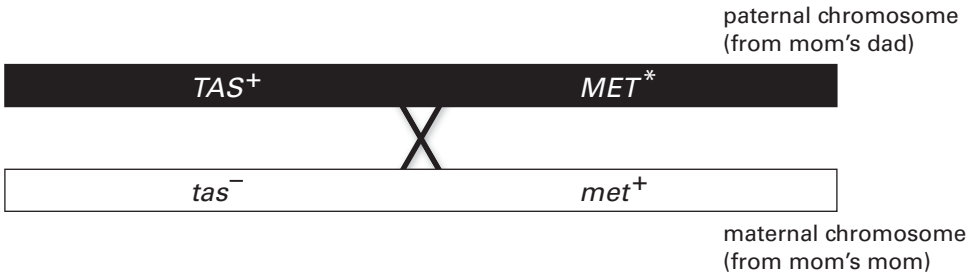
or

tas^-	met^+

tas^-	met^+

If the egg you developed from had the first combination of chromosomes, you, like your mother, will be able to taste PTC and will likely get kidney cancer. But if you developed from an egg with the second combination of chromosomes, you will not be able to taste PTC and will have no increased cancer risk, like your father. That is, without genetic reshuffling, all of your parents' children will have one or the other parental combination of genes, and the ability to taste PTC would indeed indicate an increased risk of renal cancer.

But what if these two genes reshuffled (breaking and rejoining at the "X") en route to one of Mom's egg cells:



The result is new combinations of gene variants on chromosome 7:

and

In some of the eggs Mom produces, the TAS^+ version of the gene, which your mom got from her father, is now on the same chromosome as the met^+ version, which she inherited from her mother. In other eggs, the tas^- version, which came from her mother, is on the same chromosome as the MET^* version, which came from her father. So it could be that the fertilized egg that developed into you carried Mom's newly generated chromosome 7:

(Of course, you will still receive a chromosome from Dad with the linked *tas*⁻ and *met*⁺ genes, since that is the only kind he has.) In that case, you'll have a high chance of developing renal cancer, but you won't be able to taste PTC, in which case you resemble neither Mom nor Dad. You would be a mixture of their two types as a result of the exchange between Mom's pair of chromosomes 7 in her maturing eggs.

Your sister, by contrast, may have developed from an egg that had the other kind of reshuffled chromosome 7 from Mom:

Again, your sister can only get a chromosome from Dad with the *tas*⁻ and *met*⁺ genes, so she will be able to taste PTC but have a normal low risk for kidney cancer, which also is different from either of your parents. She, too, is a mixture of your parents' types due to the exchange of Mom's copies of chromosome 7 in her maturing eggs. Since chromosome reshuffling occurs frequently, the ability to taste PTC is not a guaranteed indicator of the version of the *MET* gene you inherited.

The key point to grasp about genetic reshuffling is that the closer two genes are to each other on a chromosome—the more tightly linked they are—the less likely they are to be reshuffled and the more likely they will stay together on the same chromosome. This is because genes that are close to each other on a chromosome are separated by few base-pairs; since the chromosomes exchange their pieces at breaks between the base-pairs, there are fewer opportunities for a break and exchange between genes that are close than between genes that are far away from each other. Conversely, the farther apart two genes are, the more likely there will be a break between them at which they can reshuffle.

Returning to the charm bracelet analogy, imagine that you close your eyes and whack at the chain with a hatchet, splitting it randomly at a single location. Let's label the *TAS* and *MET* genes on the maternal set as if these two genes were closely spaced:

The likelihood that that the hatchet will fall precisely between the two genes is low, because this region occupies only a small portion of all the places the hatchet might land. But if the two genes lie far apart, then the maternal chromosomes would look like:

and the likelihood is high that the hatchet will land somewhere between the two genes.

The order and distance of genes along a human chromosome is a genetic map, and genes are defined as being one map unit apart if they are reshuffled in 1 percent of the sperm and eggs. The *MET* and *TAS2R38* genes are about twenty-five map units apart, which means that the chromosomes in about one out of four sperms and eggs will have undergone a reshuffling event between the two genes. So you could very well taste PTC yet have the good version of the *MET* gene because you inherited from Mom a chromosome 7 that contains those two versions of the genes, even though she didn't have such a chromosome 7 herself (until she produced it while making the egg that turned into you).

How were the first genetic maps generated? It certainly wasn't by studying human chromosomes, because until recently it has been difficult to map human genes: we can't do mating experiments on humans, our generation time is far too long (only about five generations per century), and our progeny are far too few for effective genetic analysis.

The first genetic maps were of the chromosomes of simple organisms, such as the bacterium *E. coli*, which lives in your gut; its parasitic phage T4, which Benzer studied; the yeast *Saccharomyces cerevisiae*, which was used to make the wine you had at dinner; and the fruit fly *Drosophila melanogaster*, which so annoys you when it swarms around your fruit bowl. The first three of those organisms have generation times measured in minutes or hours, so billions of organisms in many generations can be examined in a single experiment. Fruit flies produce a large number of

offspring in just a few weeks, so flies, with a plethora of different visible traits such as deformed wings, defective eyes, and many, many more have been mated by geneticists since the turn of last century, and their resulting offspring have been examined to find traits that are co-inherited. In this way a detailed genetic map was established for each chromosome of the fly.

Biologists studying bacteria, yeast, and fruit flies weren't necessarily thinking about what happens in humans when sperm and egg cells are formed; fifty years ago it wasn't nearly as apparent as it is today how similar are the basic life processes in all creatures. These biologists were simply curious—they wanted to know how phage and flies and yeast work, and carried out the basic research that they hoped would give them the answers.

Now that we know that bacteria, yeast, fruit flies, and even the lowly bacteriophage reshuffle their genes just as humans do, it's clear that this basic research is not only worthwhile, it's absolutely essential if we are to make progress in finding cures for human diseases. In fact, proteins in humans involved in the reshuffling of chromosomes, as well as those necessary for repairing and copying DNA, have sequences of amino acids similar—in some cases extremely similar—to their counterparts in bacteria, yeast, fruit flies, and bacteriophage. And mutations in many of those genes can lead to an increased rate of cancer, just as with mutations in the *MET* gene. So the next time a young biologist tells you she's studying "DNA repair in *Drosophila*," or "the mutation rate in *E. coli*," or "DNA recombination in yeast," please don't shake your head at this ostensible waste of your tax dollars. Instead, thank her for the curiosity that may someday keep you or one of your children alive after a diagnosis of cancer.

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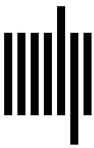

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