Chapter 26. The BRCA and homologous recombination story 220726ap3

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 26

The BRCA and homologous recombination story.

An old suspicion had it that genes or their mutations may somehow cause cancer, in particular breast cancer. In the 1990's, new methods made it possible to tackle the question. There was reason to focus on breast cancer. This tragically common cancer occasionally occurred in families, suggesting that some family members might have inherited a gene that was causing the cancer, and it might be possible to identify the gene.

The story actually goes back to 1866, when the distinguished French surgeon, Pierre Paul Broca (Figure 26.1), noted that a surprisingly large number of members of his extended family had died of breast cancer [Broca, *Traite des tumeurs* (1866)] (Krush, 1979). He compiled the cause of death of all 38 members of five generations of his family between 1768 and 1856. Ten of the 24 women died of breast cancer. Since then, many family histories were reported in which breast cancer was abnormally frequent (Papadrianos et al., 1967). An example of a breast cancer family tree is shown in Figure 26.2. Interestingly, the causative genes were given the name BRCA as contraction for "breast cancer," but could equally well refer to the discoverer of breast cancer families, Broca.

Researchers suspected that, when breast cancer occurred in several members of a family, a rogue gene may be lurking among the family members that made them prone to developing breast cancer. Also remarkable and significant was that familial breast cancer tended to develop in unusually young women (Figure 26.2). There was strong incentive to find and identify the gene, because drugs might then be developed to block the cancer-causing action of the gene.

Figure 26.1. Pierre Paul Broca (1824-1880), a French surgeon and scientist who is best known for his research on an area of the brain involved with language: Broca's area. Less known is that he accumulated evidence for a hereditary factor in cancers. His wife had a family history of breast cancer, which piqued his interest in exploring possible hereditary causes of cancer. The relevant breast cancer genes were to become known as BRCA – for 'breast cancer' and/or 'Broca' (Krush, 1979). (Source: Wikipedia; public domain.)

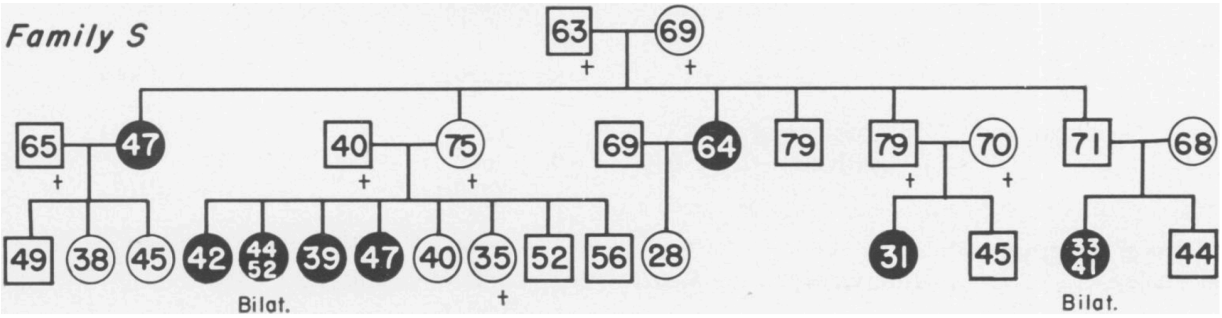

Figure 26.2. Example of a breast cancer-prone family among several reported before 1967 (Papadrianos et al., 1967). Black symbols are individuals who had breast cancer. The numbers are the age of onset of the cancer or age of death. Circles were women; squares were men. The family tree shows that the grandfather died at 63, and the grandmother died at 69. Neither of them had cancer, but at least one of them, probably the grandfather, must have carried of the breast cancer causative gene. Of their 5 daughters, 2 had breast cancer, one of them at the early age of 47. Of their 11 granddaughters, 6 had breast cancer, which began between 31 and 47 years of age; 2 of them developed cancers in both breasts.

Years later, new methods were developed that unveiled the mutated BRCA genes that increased breast cancer susceptibility in a given family. This was an example of how focus on the right question yielded important answers. The question was whether a particular gene was associated with breast cancer in families that were prone to the disease. The target of the investigation, familial breast cancer, was rare, but the discovered causative genes were later found to be mutated in many cases of the common non-familial breast cancer, as well as some other cancers, and therapy was developed to block the biochemical effects of a mutated or otherwise overactive BRCA gene. Moreover, breast cancer-prone families often had family members with cancer of the uterus. Were there genes that made women prone to both types of cancer? If so, what was wrong with the causative genes? Probably, it was abnormal in some way. Maybe it was a mutated gene that was passed down, inherited, in the family. That may have been how researchers were thinking.

Identifying the mutated gene was important, because a cell in a normal breast may sometime during the life of the woman acquire a mutation in that gene. The mutation could be produced by background radiation or cancer-causing chemicals, or even by errors during the replication of the part of the DNA that had the gene in it. The cell with the mutated gene could be a first step that, together with other gene changes accumulating in the cell over the years, would eventually cause the cell to multiply uncontrollably and form a malignant cancer (Hall et al., 1990). The mutated gene, once identified, could be a target for attack at the molecular level as therapy for the cancer. Those speculations turned out be correct. There were mutated cancer-causing genes in the breast cancer-prone families. And the same genes were found to be associated with cancer of the uterus. Of even more importance, the same mutated genes were often the culprits in the common non-inherited cases (Easton et al., 1993a; Easton et al., 1993b; Hall et al., 1990). Thus, investigating the rare familial cases led to basic knowledge that became relevant to therapy for many patients with the common cancers.

How the BRCA genes were discovered.

In their investigations of breast cancer-prone families, researchers started looking at the chromosomes of family members who developed breast cancer. They tried to pin down where in the chromosomes a gene associated with the cancer might be located. Long and painstaking effort was made to locate a difference in the chromosomes of family members who did or did not develop the cancer. You might ask why that chromosome search could not be done just as well in breast cancers occurring in the general population. Why focus on the rare cancer-prone families? The answer is that there were several different genes that led to the cancer. But in the cancer-prone families there likely would be a single particular gene that was associated with the cause in that family. Indeed, there were different breast cancer-prone families that had different cancer-associated genes. That made it possible to track down the particular gene that caused the cancer in a particular family. Once a single causative gene was identified and its DNA sequence determined, it became possible to search for related genes by sequence similarity.

The first success in the search was in a breast cancer-prone family where the genome difference pointed to a narrow region of chromosome 17. Perhaps there was a breast cancer-associated gene located in that region? Indeed, this presumption was correct, and the offending gene was named '*BRCA1'* (Easton et al., 1993a; Easton et al., 1993b; Hall et al., 1990). The *BRCA1* gene itself was soon identified, its DNA nucleotide sequence was determined, and the cancer-causing changes in the mutated genes were revealed (Miki et al., 1994). Not all familial breast cancers, however, were associated with BRCA1 mutations. Another mutated breast cancer-causing gene, found in a different family, was located in chromosome 13 and was named '*BRCA2'*. As in the case of *BRCA1*, the DNA nucleotide sequence changes in the mutated genes were soon determined (Wooster et al., 1995). Although *BRCA1* and *BRCA2* were occasionally mutated in other cancers, I don't know why they were most often found in cancers of breast and ovary.

What do BRCA1 and BRCA2 do?

So, what is it about those *BRCA* gene mutations that incites cancer to erupt? Somehow, the normal versions of those genes *protect* against the development of cancer. Accordingly, the *BRCA* genes were considered to be "tumor suppressors." But what do the normal *BRCA* genes do that prevents cancer? The first clue to the function of BRCA1 came in1996 from Ralph Scully and coworkers in David Livingston's laboratory at the Dana Farber Cancer Institute of Harvard Medical School in Boston. They found that the BRCA1 protein (the product of the *BRCA1* gene) binds to a protein, Rad51, that was known to be part of the machinery that repairs radiation-damaged DNA (Scully et al., 1997). Moreover, they made the remarkable observation that BRCA1 is seen in discrete spots ("foci") in the cell nucleus – but only in cells that were replicating their DNA (in "S-phase" of the cell division cycle) (Figures 26.3 and 26.4). The Rad51 protein became localized in the same spots as the BRCA1 protein. The BRCA1-Rad51 protein pair evidently did something important during S-phase at those particular spots in the nucleus, perhaps at the very places in the chromosomes where damaged DNA was being repaired.

A few months later, similar observations were reported for BRCA2 (Kinzler and Vogelstein, 1997; Sharan et al., 1997). Moreover, BRCA1, BRCA2, and Rad51 were bound all together in the same spots in the S-phase cell nucleus. In addition to those 3 proteins, several others, including proteins associated with Fanconi's anemia, were found in the same complex, and most of those proteins were known to function in the repair of damaged DNA (Chapter 31 will tell about the Fanconi anemia DNA repair system). All of those proteins (gene products) seemed to work together in a critically important DNA repair process. The spots where they were co-localized within the cell nucleus were perhaps the locations in the genome where DNA damage was being repaired. If the *BRCA1* or *BRCA2* gene were inactivated by mutation, DNA repair might be impaired and DNA damage would accumulate, increasing the chance that a cell would become cancerous.

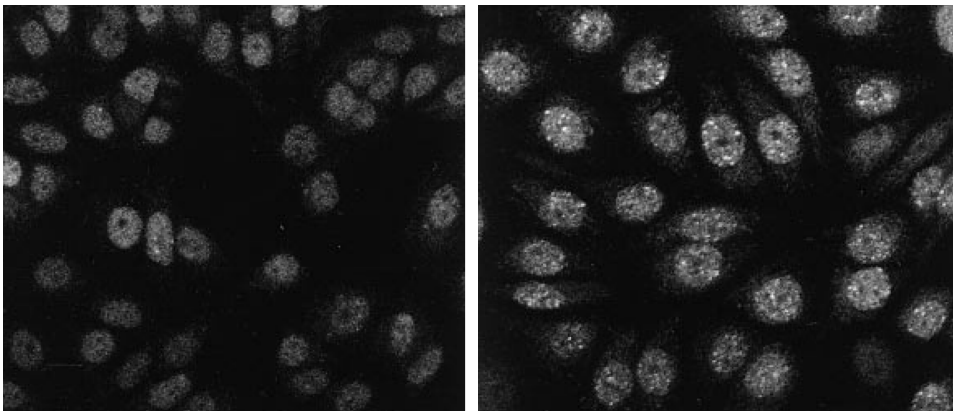

Figure 26.3. The BRCA1 protein localized in spots ("foci") in the nuclei, but only in cells that were in the process of replicating their DNA (S-phase of the cell division cycle). BRCA1 lights up in bright fluorescent spots. Breast cancer cells (MCF7) were synchronized, so that few cells were in S-phase (*left*), and later when most cells were in S-phase. Only the cell nuclei are visible in these images. From (Scully et al., 1997).

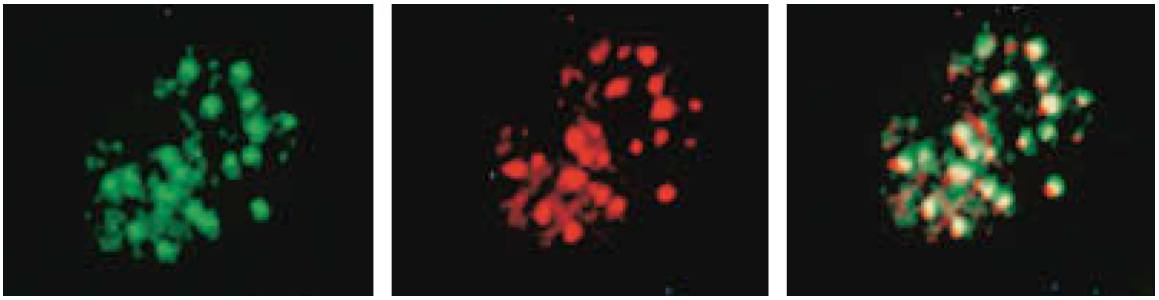

Figure 26.4. An S phase cell nucleus co-stained for the BRCA1 protein (green fluorescence, *left*) and for the Rad51 protein (red fluorescence, *center*). We see the BRCA1 and Rad51 proteins localized in the same spots in the nucleus. When BRCA1's green and Rad51's red fluorescence were in the same spot in the nucleus, the color was white (*right*). From (Scully et al., 1997).

The BRCA proteins in DNA repair.

It seemed that BRCA1 and BRCA2 may function in one or more steps in the cell's DNA repair pathways. But, which steps? A clue came from Rad51, whose function in DNA repair had already been partially elucidated: it functioned in a remarkable DNA repair process based on homologous recombination. Since BRCA1 and BRCA2 were bound together with Rad51, it seemed plausible that all three might function together in a homologous recombination process in DNA repair. So … what is "homologous recombination"?

Homologous recombination

Homologous recombination is like a conjurer's trick. It functions both in chromosome crossover during meiosis and in DNA repair. I will focus on DNA repair and the role of BRCA1 and BRCA2 in homologous recombination. But to understand it and its history, I will recount the story of its discovery in studies of meiosis and "jumping genes". But before relating those stories of discovery, here is an introductory summary of how homologous recombination works and how the BRCA's became implicated:

When the growing point of a replicating DNA strand encounters damage in the template strand that it is copying, it can -- quite remarkably -- switch to copying a different DNA strand that is "homologous" (having the same or a very similar DNA sequence) to the damaged strand. "Homologous" means that the target DNA strand has nearly the same nucleotide sequence as the damaged strand had before it was damaged. In effect, the replicating strand that encounters a blockage of the template strand it's trying to copy says, "Ok, forget it, I'll switch over to copying this other guy who's just as good as you were before you got damaged!". But how could a blocked growing strand find and switch over to copying a homologous strand in another DNA duplex? For that caper to work, first of all there must be a homologous strand nearby for the replicating strand to find and switch to. That becomes feasible when the DNA duplexes in a chromosome are being replicated, because the two DNA duplex pairs (the two daughters produced by Watson and Crick's "semi-conservative" replication) remain near each other: they remain connected at their "centromeres" until the chromosomes separate in the latter part of mitosis. The strands of a damaged duplex therefore can find the needed undamaged section in the homologous newly replicated DNA duplex , because it lies nearby. That is why homologous combination is only effective during replication (S-phase) or between the end of S-phase and mitosis (G2 phase).

(A "newly replicated DNA duplex" consists of a preexisting ("conserved") strand and a newly replicated strand, *i.e.,* the replication is "semi-conservative", which is the essence of Watson and Crick's discovery in 1953, proven experimentally by Meselson and Stahl in 1958.)

The role of the BRCAs in DNA repair was further highlighted by the remarkable observation that a complex of BRCA1, BRCA2, RAD51, as well as some other proteins, was seen at sites in the nucleus where DNA damage was being repaired; the sites ("foci") were seen as spots in the nucleus made visible by fluorescent tags on one or another of the proteins in the complex (Garcia-Higuera et al., 2001), as seen in Figures 26.3 and 26.4. This complex of proteins was thought likely to act in DNA repair steps in which homologous recombination steps comes into play and where BRACA1 and BRCA2 are needed.

By the way, BRCA1 and BRCA2 also function the in the repair of DNA crosslinks by the Fanconi repair system (Chapter 31).

But now let's look back to the story of how homologous recombination was discovered.

How homologous recombination came to be discovered.

Homologous recombination is a remarkable process whereby genetic traits are assorted during meiosis among offspring and produces the variety of progeny for selection that enables evolution. We are interested in homologous recombination, because of its importance in repairing DNA damage. Moreover, cancer cells often are defective in their ability to repair DNA damage, which is one reason that anticancer drugs work. The concept of recombination between two homologous chromosomes goes back to Thomas Hunt Morgan's famous work on the genetics of fruit flies. Morgan (Figure 26.5) proposed that genes are arranged on chromosomes like beads on a string, and that sometimes there is "crossing over" between chromosomes giving rise to recombination of the genes, as shown in Morgan's drawing of the concept in 1916 (Figures 26.6). Then in 1930, Barbara McClintock (Figure 26.7) obtained a direct image of chromosomes in the process of exchanging their associated pairs, a prelude to homologous recombination (McClintock, 1930) (Figures 26.8).

Figure 26.5. Drawing of Thomas Hunt Morgan (1866-1945), discoverer of the phenomena of chromosome crossing over and genetic linkage in 1913. (Created 31 December 1930; Wikipedia, public domain.)

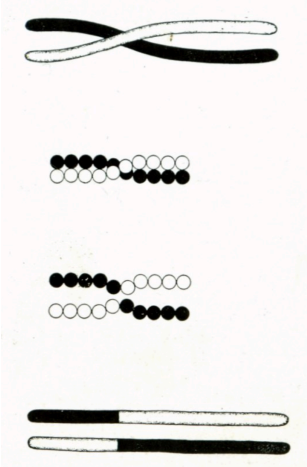

Figure 26.6. Thomas Hunt Morgan's illustration of his concept of crossing over between homologous chromosomes and their rows of genes from his pioneering studies of fruit fly genetics (1916) (from Wikipedia).

Figure 26.7. Barbara McClintock (1902-1992) discovered mobile genetic elements, a concept so revolutionary at the time that it was long before geneticists accepted or understood it. Many years later after the importance of her of her discovery was grasped, she was awarded a Nobel Prize in Physiology or Medicine in 1983. She had received a PhD in Botany at Cornell University in 1927 and used maize as her subject of investigations. (From http://siarchives.si.edu/collections/siris_arc_306310)

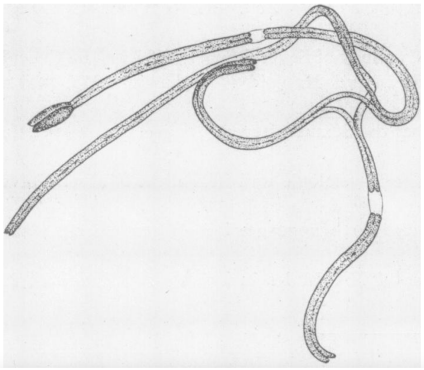

Figure 26.8. Barbara McClintock published this drawing in 1930 of chromosomes interchanging their associated partners. Switching DNA partners is an essential part of homologous recombination. The cell from which these chromosomes came was in the midst of preparing for mitosis (mid-prophase): the chromosomes were already condensed but had no spindle microtubules attached yet. The clear regions are the centromeres (one for each chromosome), where spindle microtubules will attach during mitosis. She used a "camera lucida" to project the image (magnification x1875} on a page, so as to make an accurate drawing – as was commonly done before photography through a high-power optical microscope became available (McClintock, 1930).

Homologous recombination was first discovered in genetics, where sections of a chromosome often moves from one chromosome to another during sexual reproduction. It happens in meiosis, during the production of sex cells. During meiosis, homologous sections of sister chromatids often became interchanged . When a chromosome is replicated, each daughter chromosome is called a chromatid, and the two daughter chromosomes are called sisters. Meiosis is a bit complicated, because it entails two chromosome doublings and "reduction division." Wait! Before your eyes glaze over: You may have learned about all that in high school biology, and maybe forgotten the details. Shortly, we will review the essentials of meiosis, because it is where DNA-damaging anticancer drugs often cause trouble: it is where reproductive cells (egg and sperm) are produced, a process that is especially vulnerable to those drugs. Homologous recombination comes into play as our story unfolds, because of the role of the *BRCA* genes and the proteins they code for. The first clear observation of recombination by exchange of segments between different chromosomes ("recombination") came from Barbara McClintock's studies in 1930 (McClintock, 1930). That classic paper of 1930 demonstrated a genetic exchange between different chromosomes in the same cell (Figure 26.8), which visualized how this happens during meiosis, and foreshadowed the concept of "Holliday Junctions" whose importance in DNA repair we will see later in this chapter.

Homologous recombination was also found in microorganisms and described by physicist-turned-biologist Max Delbruck in 1946 . He had observed exchange of genome sections between different strains of bacteriophage that had infected the same E. coli bacterial cell.

Delbruck wrote about the views commonly held at the time of his conversion from physics to biology in his "retrospective of 20 years as a biologist" (Delbruck, 1970). He begins by thinking back to how Aristotle viewed the world. Aristotle viewed cycles of origin, development, and demise as characteristic of living organisms, where demise at the end of one cycle leads to the origin of another. Only in the astronomy of the time did there appear to be cycles without end, with neither generation nor decay. Delbruck viewed his conversion from physics to biology as a break to a different conceptual mode: from the inanimate to the animate. His conversion occurred at a time when "life seemed to have unique properties quite irreducible to the world of physics and chemistry: motion generated from within … aspects that were foreign to the physicist" (Delbruck, 1970). Nevertheless, the "physicist" in Delbruck showed itself in mathematical theory he developed to account for genetic recombination findings in his bacteriophage crossings (Visconti and Delbruck, 1953). This conformed with the tone of the time, (expressed by Francis Crick, as I recall) that "the objective of molecular biology is to destroy the last vestiges of vitalism."

The vitalist notions that dominated genetics had however begun to be overturned by Gregor Mendel in his cross-fertilization studies of different kinds of peas and other plants. He concluded that inheritance was mathematical – thus an inanimate, rather than a vitalist process. Both Mendel and Delbruck showed that mathematical ideas could apply to living organisms. Johann Mendel (1822-1884) lived in a German-speaking part of Moravia in the Austrian Empire. As a young man, he studied theoretical philosophy and physics. But then, in order to pursue his studies free of "perpetual anxiety about a means of livelihood," he joined the Augustinian Friars in Brno, and was given the name "Gregor"; eventually, becoming Abbot. Much has been written about his plant hybridizing studies that were not comprehended by scientists of the time, and which lay obscure for several decades, before a more enlightened age rediscovered them.

Barbara McClintock's work near the middle of the 20th century then overturned some of the tenants of genetics, although her major findings took a long time to be appreciated. After much success in a remarkable variety of genetic research, mostly on plants, and recipient of multiple awards, including election to the U.S. National Academy of Science, McClintock embarked on investigation of the genetics of maize, which led to observations for which the scientific community was not yet ready to comprehend. She was first to figure out how to see the maize chromosomes under the microscope, which allowed her to relate changes in genetic traits with changes in the physical chromosomes. In the 1940's, however, she detected maize genes (or "genetic elements"), whose behavior made no sense from what was then known and understood about genetics. One of those genes was jumping from one place on a chromosome to another place. Genes were not supposed to be able to do that. It defied the accepted Mendelian inheritance patterns that had become ingrained in scientific lore. Although many geneticists refused to accept her findings, she persisted in accumulating and assembling data. The strange genetic behavior she observed produced complicated data that were challenging to make sense of. It is said that, when she presented her findings at conferences, the audience listened politely in deference to her previous achievements, but remained silent, because they could make neither head nor tail of her complicated new data. Her meticulous studies of genetic recombination in maize

demonstrated radically non-Mendelian patterns of inheritance that were understood only after genetically mobile genes became understood, which was not until the 1960's. When her work was finally understood, the scientific world was so astounded by her "out-of-the-box" achievement that in 1983 she was awarded, unshared, the Nobel Prize in Physiology or Medicine. Earlier, after visiting her, Joshua Lederberg, himself a Nobel Prize winner in Physiology or Medicine (1958), is said to have remarked, "that woman is either crazy or a genius!" That statement was logically true; moreover, she was NOT crazy! Homologous recombination is essential to how McClintock's "jumping genes" move from one chromosome to another. It happens by way of homologous recombination and is related to DNA repair, as well as to how new therapies work that use CRSPR-CAS9 systems to engineer CAR-T cells by inserting or replacing DNA sections at key locations in the chromosomes.

Roles of BRCA1 and BRCA2 in homologous recombination.

As already described earlier in this chapter, the first indication of a connection between the *BRCA* genes and homologous recombination came in 1997 from studies led by David M. Livingston of the Dana-Farber Cancer Institute at Harvard Medical School in Boston (Scully et al., 1997). They found BRCA1 colocalized with RAD51 in the same discrete foci in the nuclei of cells preparing for mitosis or undergoing meiosis (Figure 26.4). Then, in 1999 and 2001, a pair of papers in *Molecular Cell* in 1999 and 2001 by Mary Ellen Moynahan, and her coworkers in Maria Jasin's laboratory at the Memorial Sloan-Kettering Cancer Center in New York reported that both BRCA1 and BRCA2 not only colocalized with RAD51 in the same nuclear foci, but actually were physically bound in a trimer consisting of the three proteins all bound together (Moynahan et al., 1999; Moynahan et al., 2001). Moreover, cells deficient in any one of the three had increased sensitivity to agents that produced DNA double-strand breaks. Furthermore, RAD51 was similar to the bacterial RECA gene that was known to be required for homologous recombination in those organisms. Similarly, RAD51 was shown to be required for efficient homologous recombination in mammalian cells. Those findings set a firm basis for the conclusion that the BRCA gene played a role in homologous recombination (Chen et al., 2018).

How DNA repair by homologous recombination works.

Homologous recombination (HR) works by way of a complicated choreography that repairs the DNA without any errors in its nucleotide sequence. However, it is blocked in a manner that allows it to take place only during or after DNA replication. It is blocked during the G1 phase of the cell division cycle, during which the DNA remains unduplicated and there is no sister chromosome nearby. There are many different ways in which homologous recombination can occur, some of them rather complicated, and many more have been proposed over the years. The main point is that, when a DNA replication process encounters a blockage, such as by a damaged template strand, replication can proceed by

switching to copying a strand in a homologous sequence in the newly replicated sister chromosome.

As often happens as a science develops, a general rule turns out to have exceptions. Thus, it turned that homologous recombination may occasionally occur even outside of S or G2 and even in non-dividing cells (G0). In 2013, researchers at the University of Pittsburg reported that homologous chromosomes in G0 cells can find each other within minutes after the cells were exposed to x-ray (Gandhi et al., 2013). Moreover, the DNA-damage-induced pairing between homologous chromosomes occurred without pairing of their centromeres. Therefore, it seemed to be happening by a different mechanism than the pairing of newly replicated sister chromosomes. Also, it seemed that the genome regions that paired up were only in the RNA-transcribing genes. So, how did homologous chromosomes find each other in DNA-damaged G0 cells where no newly replicated sister chromosome was available? The researchers thought it likely that the transcribed RNA was in fact what was recognizing a homologous sequence in the homologous chromosome. The idea that DNA repair by homologous recombination may occur in actively transcribing homologous regions was supported by clever experiments by investigators in other departments at the University of Pittsburg (Wei et al., 2015).

(The US National Center for Biotechnology Information (NCBI), lists 20,203 protein-coding genes and 17,871 non-coding genes (genes that are transcribed into RNA that does not code for protein but has other functions). Thus, all together there may be about 38,000 RNA-transcribing genes in the human genome. That, however, comprises only a small fraction of the DNA in the chromosomes.)

Getting back to how homologous recombination works, the essentials of an early step in the process was depicted in a simple diagram by Charles Radding in 1979 (Figure 26.9). The dark curve in the diagram shows an invading strand base-pairing with a homologous region of a recipient DNA. The process displaces the other strand of the recipient, forming a "D-loop" pattern. Later work disclosed that the invading strand had to be coated with RECA, the homolog of mammalian RAD51 that had the same function, and both BRCA1 and BRCA2 were required for that to happen.

Homologous recombination became important in how cells deal with circumstances when the template DNA strand is broken. When a moving replication fork encounters a DNA single-strand break or gap, the replicating strand was found to switch to a homologous region of an undamaged chromosome, where it can base-pair and copy an undamaged strand (Figure 26.10). Further details about how homologous recombination works will be told in the next chapter that deals with its critical role in the repair of the potentially lethal DNA double-strand breaks.

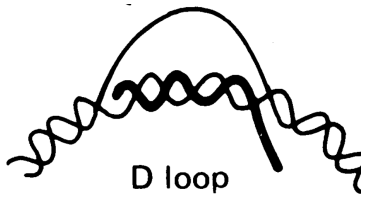

Figure 26.9. The beginning of homologous recombination (HR) from part of a diagram by Charles Radding and his colleagues in 1979, which showed that the RecA protein and ATP drives the process (Shibata et al., 1979). They had worked this out by experiments using pure components. The diagram shows a single-stranded DNA segment (heavy line) invading a DNA double helix. The invading DNA single-strand base-pairs with one strand of the DNA duplex while displacing the other strand.

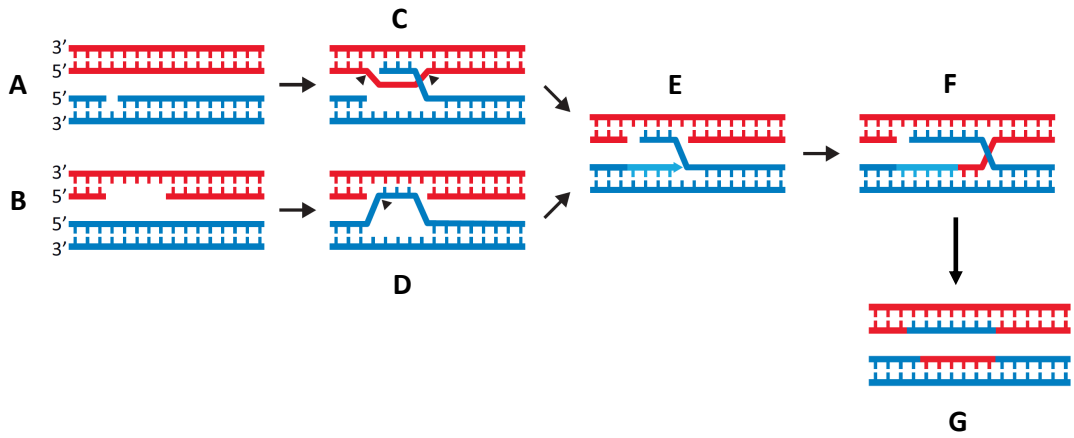

Figure 26.10. A scheme for homologous recombination triggered by a single-strand break (**A**) or a single-strand gap (**B**) in the DNA of one of the sister chromatids, as proposed by Charles Radding in 1983. Homologous recombination yields **C** or **D**, respectively, which both lead to **E**, then **F**, then finally to products such as **G**. (Simplified from (Vriend and Krawczyk, 2017)).

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