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

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CHAPTER 18

The RAS oncogene story

RAS oncogenes in viruses.

A particularly important family of genes or oncogenes in human cancer, the RAS genes, were first discovered through studies of cancer-causing viruses. Sometime in 1963, Jennifer Harvey, working at the Cancer Research Department of the London Hospital Research Laboratories, was inoculating mice and rats with plasma from a rat that had a virus-induced leukemia. She was routinely transferring the virus from one animal to another, inducing leukemia in each of them. However, on one occasion that year, she noted something unusual that was to open a new window to cancer cause and treatment (Harvey, 1964).

Mice that were inoculated with virus from one of her leukemic rats unexpectedly developed solid tumors in addition to the usual leukemia (which have malignant cells in the blood and lymph nodes instead of in lumps in various tissues). Her leukemia virus was later shown to have picked up (spliced into its genome) a DNA fragment from the rat's own genome. That piece of DNA, which was now part of the genome of the new virus, caused the solid-tumor-type cancer lumps in her mice. Moreover, the new cancer gene was found to be a mutated version of a normal gene, RAS (probably for rat sarcoma, where a mutated version was first discovered). Harvey's name was to become immortalized by the letter H in the newly discovered HRAS oncogene, which was a mutated form of a normal HRAS gene. Harvey's new virus caused cells on the surface of a dish to overgrow to form "foci" (Figure 18.1) in a manner similar to what Weinberg's group later observed in their oncogene studies (Figure 15.3 in Chapter 15). Harvey's virus particles seen in electron microscope images had a remarkable unusual structure resembling spoked wheels (Figure 18.2).

In 1967, W. H. Kirsten and L. A. Mayer detected another virus that produced solid tumors in mice. That virus was later found to have picked up a mutated version of another gene of the RAS family, which became known as KRAS (K for Kirsten) (Kirsten and Mayer, 1967). KRAS became one of the most important cancer genes and was discovered to be mutated in nearly all cases of pancreatic cancer. These early observations led to enormous research efforts that gave much detailed information about the RAS genes and their cancer-inducing mutations.

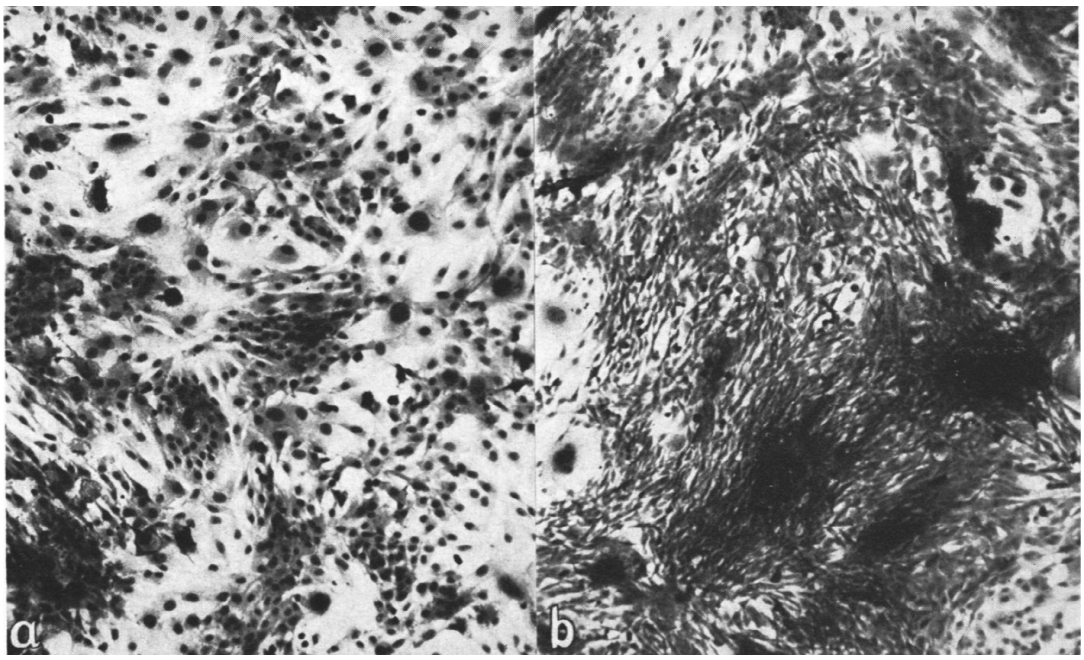

Figure 18.1. Foci of high cell density caused by Harvey's new cancer virus that contained the mutated HRAS gene in its genome. *Left*, normal cells growing on a surface; *right*, foci of excessive cell multiplication caused by the virus (Simons et al., 1967).

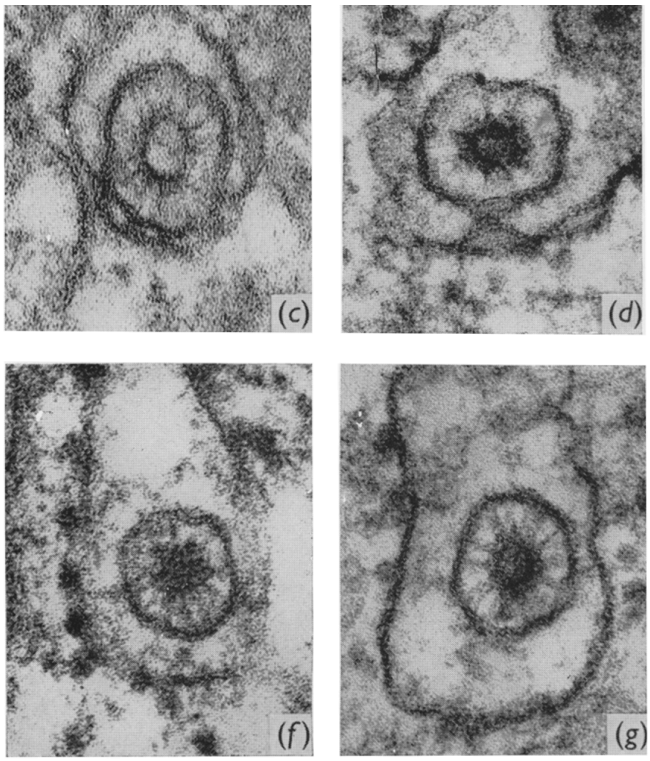

Figure 18.2. Electron microscope images of Harvey's new cancer virus. Each cell sometimes had hundreds of these unusual particles whose structure differed from all previously known viruses. The virus structure resembled spoked wheels within a vesicle membrane that sometimes had ribosomes attached (dark bodies in figure *f* (lower left), showing that the membrane came from the cell's "rough endoplasmic reticulum" where proteins are made) (De Petris and Harvey, 1969).

But where did the Harvey and Kirsten tumor-producing viruses come from? In 1973, Ed Scolnick and his colleagues at NCI reported that the Kirsten sarcoma virus arose from the Kirsten leukemia virus by genetic recombination with sequences present in the rat cells (Scolnick et al., 1973). The Kirsten leukemia virus, while growing in rat cells, had picked up sequences that were already present in those cells, with the result that the new virus was then able to form tumors. In 1974, Scolnick found that the Kirsten and Harvey viruses had picked the same sequences, which is what made them tumor-producing viruses, and which may have come from the rat genome itself (Scolnick and Parks, 1974)! Finally in 1979, after restrictions on cloning had been lifted in 1977, Gordon Hager, Ed Scolnick, Doug Lowy, and their colleagues at NIH cloned the Harvey sarcoma virus genome (Hager et al., 1979).

What do RAS genes do in cells?

Since a version of the *RAS* oncogene caused or triggered the development of cancer, researchers were anxious to find out what the RAS protein does in cells. An important observation about the proteins derived from *RAS* genes was reported in 1980 by NIH researchers Mark Willingham, Ira Pastan, Thomas Shih, and Ed Scolnick (Willingham et al., 1980). They found RAS-like proteins at the inner surface of the plasma membrane of cells that had been transformed by Harvey sarcoma virus (Figure 18.3). The result was similar to the observation that epidermal growth factor (EGF) also bound to the cell surface membrane (Figure 17.5 in Chapter 17). The importance of these observations became evident when the role of RAS in the signaling network from receptor tyrosine kinases, such as epidermal growth-factor receptor (EGFR), was worked out -- and when it was discovered that receptor tyrosine kinases, such as EGFR, as well as the RAS proteins were attached to the cell surface membranes of the cells. As we will see, RAS turned out to be directly in the signaling path from EGFR. (The EGFR story was told in Chapter 17.)

Proteins with structure and function similarities to mammalian RAS were found in a remarkably wide variety of organisms from yeast to worms to insects, which highlighted their central role in the life of many kinds of cells (Sigal et al., 1988) (Lowenstein et al., 1992).

The fact that the cancer-driving RAS oncogenes are mutated versions of the normal RAS genes was reported in 1982 by M. Barbacid and his coworkers in the National Cancer Institute (Santos et al., 1982). In 1984, Raymond Sweet and his colleagues at Cold Spring Harbor Laboratory injected the mutated HRAS gene into a variety of cells and found that it increased the proliferation of the cells in cancer-like fashion (Feramisco et al., 1984). The mutated RAS protein (product of a mutated RAS gene) was later found to be a rogue molecule that sent its growth-promoting signal downstream without control and without requiring input from receptor tyrosine kinases.

Overview of RAS in the signaling path from EGFR.

After receiving activating signals from EGFR (or from other receptor tyrosine kinases), RAS transmits the signal to the cell nucleus, telling the machinery therein to activate cell division. For RAS to receive signals from EGFR, it helped for the two to be located in the same neighborhood. Since EGFR transmits signals from outside to inside the cell, the EGFR molecule is in the cell surface membrane with part of the molecule outside and part inside the cell (Chapter 17). The location of RAS at the inner surface of the membrane is therefore ideal for efficient interaction with EGFR. It was indeed found that the ability of the RAS protein to bind to the inner surface of the membrane was required for RAS to receive signals from the receptor tyrosine kinases.

However, RAS did not bind directly to EGFR. Instead, there was a protein that connected between the two. This EGFR-to-RAS connector protein came to have a strange name: SOS,

standing for "sister of sevenless." The discovery of SOS and the reason for its strange name story came from research on fruit fly eyes, a remarkable story that I will tell next.

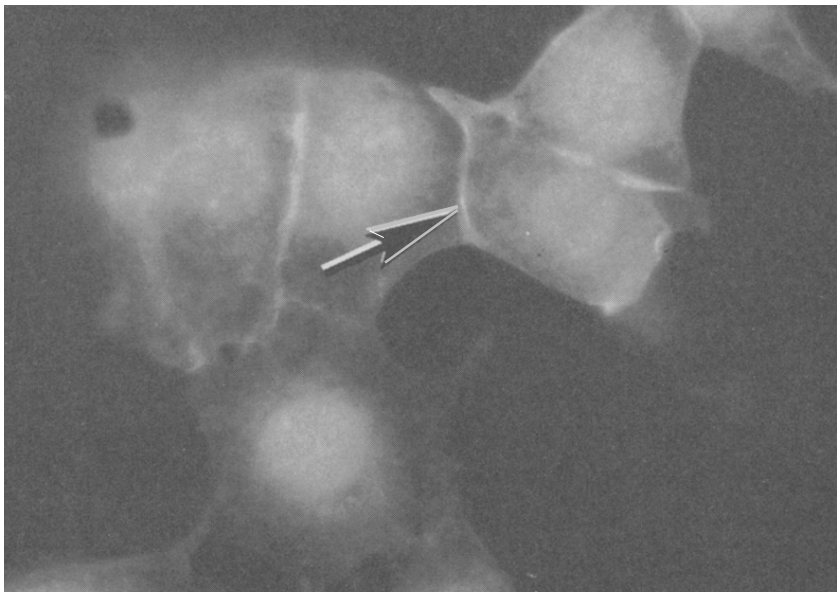

Figure 18.3. An experiment showing that RAS proteins are located at the inner surface of the cell surface membrane (arrow). This experiment was reported in 1980 by NIH scientists Mark Willingham, Ira Pastan, Thomas Shih, and Ed Scolnick (Willingham et al., 1980). They used an antibody that bound to the RAS protein specifically. The antibody's fluorescence under ultraviolet light showed up bright in this image. They also showed that the RAS protein was not on the external surface of the cell: there was no fluorescence when the antibody was applied to intact cells rather than to the fixed cells in the experiment shown here. (The antibody could not penetrate into cells unless the cells were opened up by chemical fixation.)

From viruses and fruit fly eyes to RAS and cancer-driver genes.

Three seemingly unrelated and arcane investigations converged to one of the most important discoveries about cancer: the discovery of the RAS oncogenes, which paved the way for the development of targeted anticancer drugs:

• *A virus unexpectedly produced malignant tumors in mice.*
• *Peculiar mutations in the eyes of fruit flies disclosed genes that were similar to previously unidentified human genes.*
• *DNA from human cancer cells transformed non-cancerous cells to become cancerous.*

I have already told the first and third of those stories; this section is about the second – an arcane and indeed amazing story about mutations of the eyes of fruit flies. Who would have imagined that studies of genetic alterations in fruit fly eyes would lead to the discovery of cancer-causing genes and to therapies designed to block those over-active

mutated genes in cancers? The story of how that happened is both fascinating and enlightening.

From fruit fly eyes to human RAS genes.

In order to probe the unknown, a key is needed to unlock a door. A key can be found in the most unlikely of places -- which, in this case, was memorialized by an unknown (to me) author:

3 blind flies, see how they fly
one was missing the seventh cell
another lost its daughter cell
the third had no mother cell
but it all led to a cancer cure
and never got a golden fleece prize
for 3 blind flies, 3 blind flies.

So, let's have a look at the fruit fly eye and what those missing eye cells were all about. The compound eye of a fruit fly consists of several hundred small eye units, called "ommatidia", each of which has 8 photoreceptor cells arranged in a strict geometric order. Each of those photoreceptor cells was designated by a number, based on its position (Figure 18.4).

A mutation was found in a blind fly whose photoreceptor cell number 7 was missing in every little eye unit (ommatidium) (Figure 18.4). Geneticists dubbed the mutation *sevenless*, in line with the usual whimsy of those researchers. To have a normal eye, the fly had to have a normal *sevenless* gene. If its sevenless gene was mutated, photoreceptor cell number 7 was missing, and the fly did not see well. To see the drastic effect that a mutation of its *sevenless* gene has on the structure of a fly's eye, have a look at Figure 18.5.

However, geneticists as usual were not content with discovering just one interesting mutation. They observed that the normal development of receptor cell number 7 was defective if there was a mutation in a different gene, which their whimsy dubbed *bride of sevenless*. That name reflected their finding that the protein coded by that gene binds to and is required for the function of the *sevenless* protein.

But the process of finding mutations in fruit fly eye cells did not end there. They found yet another gene whose mutation caused problems with receptor cell number 7. They dubbed that gene *son of sevenless* (SOS). To everyone's astonishment, that SOS gene of the fruit fly had a DNA sequence that resembled a human gene that was implicated in the function of the RAS genes (Raabe, 2000). After much investigation, the human version of the SOS gene was found to fit in the pathway that leads from a variety of receptor tyrosine kinases -- most notably EGFR -- to RAS. The EGFR story was related in Chapter 17. Figure 18.6 shows the remarkable similarity of the pathways where SOS has a role in transmitting

signals from outside the cell to genes in the cell nucleus. The pathways from EGF via SOS and RAS, to RAF, MEK, and ERK were found to be the same in the different species.

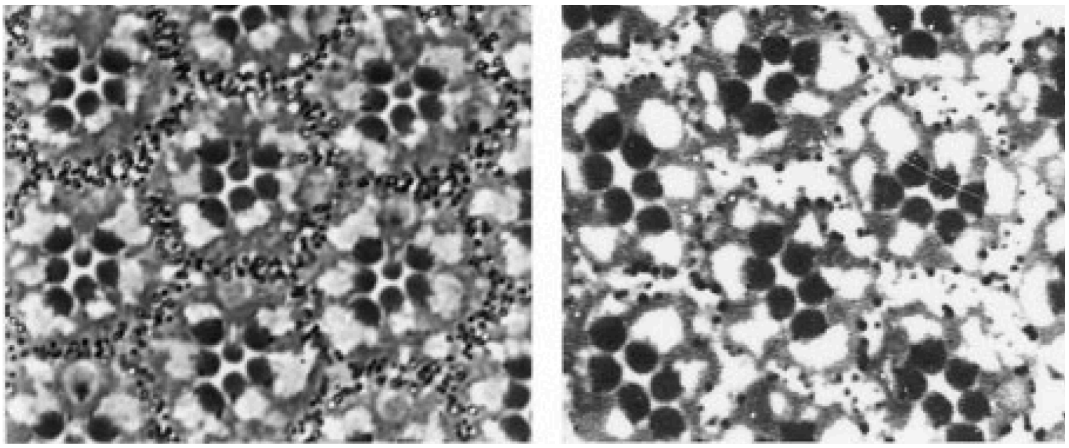

Figure 18.4. Eye units ("omatidia") of a normal fly (left) and a *sevenless* mutant (right). As you can see by counting the dark blobs in each group, the normal fly had 7 photoreceptor cells visible in each omatidium, whereas the mutant had only 6. Photoreceptor cell number 7 was missing in the mutant. (An 8th photoreceptor is not visible in this section and was unaffected by this mutation.) (From (Raabe, 2000).)

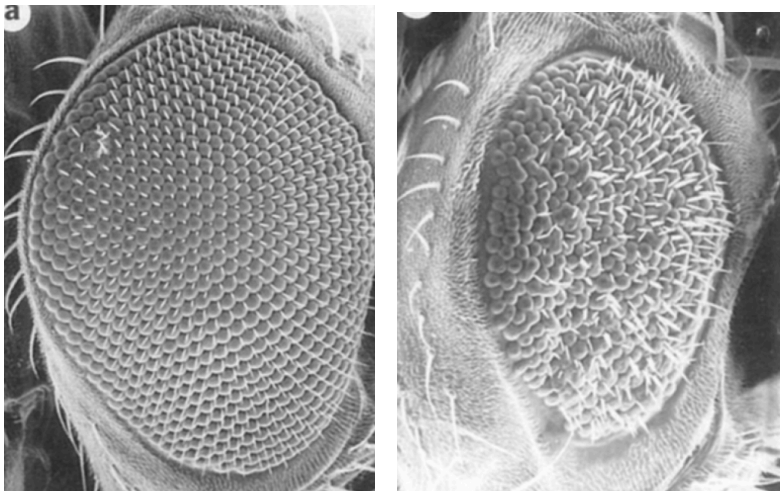

Figure 18.5. How mutation of the SOS gene affects the eye of a fruit fly. *Left,* eye of a normal fruit fly; *right*, eye of a fruit fly that had an SOS mutation (Rogge et al., 1991).

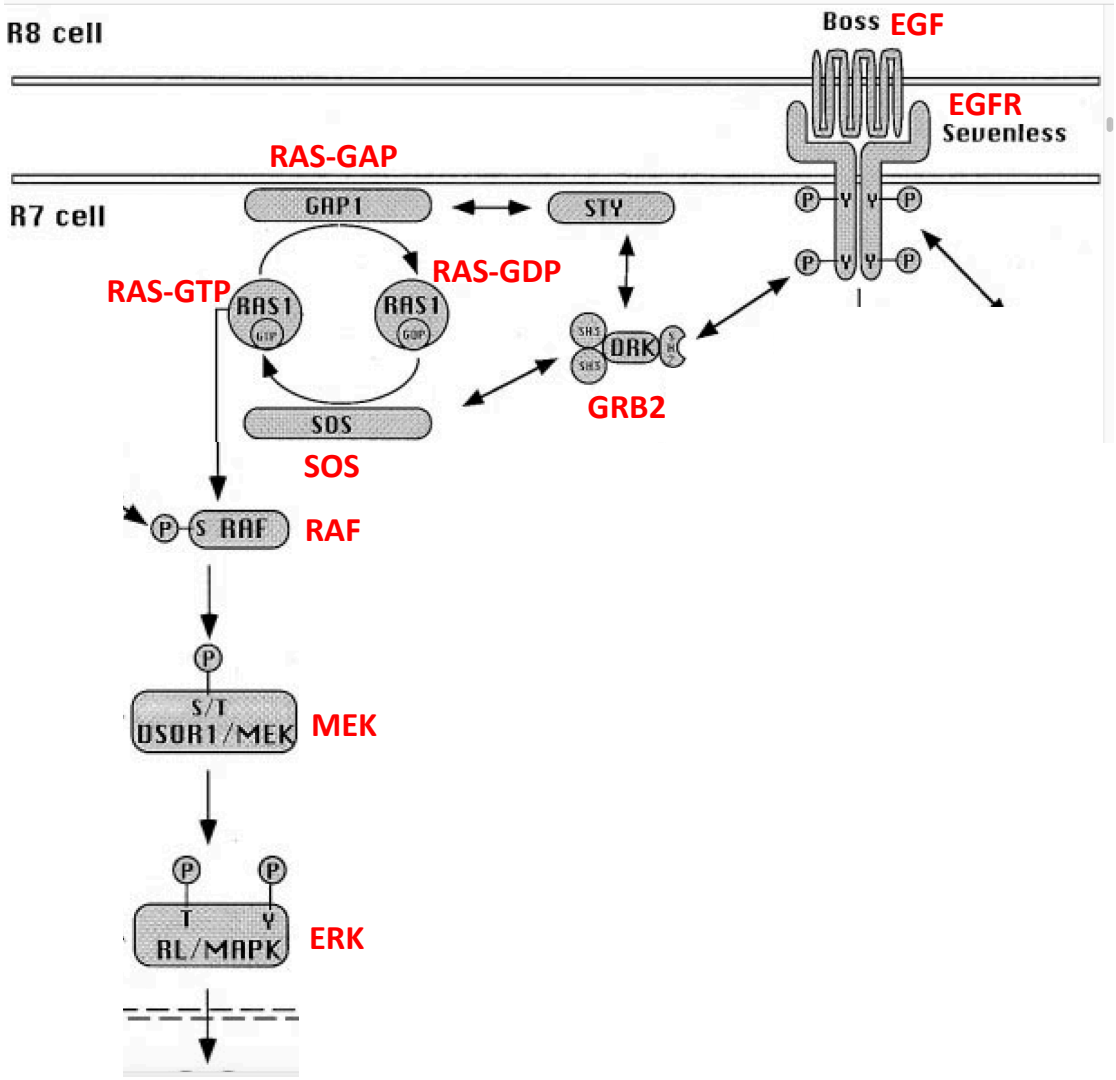

Figure 18.6. The role of SOS in the pathway from EGF to RAS was found to be similar in the fruit fly and in humans, as well as other animals. This diagram shows the pathway in the fruit fly proposed by Thomas Raabe in 2000 (Raabe, 2000). I have added the corresponding human gene names in red. The DNA sequences of the fruit fly genes and the corresponding mammalian genes were similar, although not identical. SOS in both species stimulates the conversion the inactive form of RAS (RAS-GDP) to the active form (RAS-GTP). In humans, the input to the pathway is EGF (epidermal growth factor), which corresponds to the fruit fly's Boss gene ("bride of sevenless"). The output of the pathway from RAS via RAF, MEK, and ERK was also similar in the fruit fly and humans (compare with Figure 18.7). The known functions of the genes at the end of the pathway, however, were different: eye development in the fruit fly versus cell division in humans.

The critical role of RAS genes in transmitting signals from growth factor receptors, such as EGFR.

The RAS story expanded enormously as its role in stimulating uncontrolled division of cancer cells gradually emerged from the mist. The strength of the cell division signal from RAS obviously had to be strictly controlled, because excessive cell division could lead to cancer. The control of RAS via positive and negative influences were discovered, and how it all works to control cell division gradually unfolded.

The unravelling of the story began with the fruit fly eye mutation studies described above. The mutated genes were then isolated and their DNA sequenced, which revealed the amino acid sequences of the proteins encoded in the genes. In 1987, Ernst Hafen, Gerald Rubin and their coworkers at the University of California at Berkley located the *sevenless* gene on the fruit fly chromosomes (Hafen et al., 1987). They isolated the gene and determined its DNA sequence, from which they surmised that the gene coded for a receptor tyrosine kinase that had the structure of a trans-membrane protein.

In the fruit fly eye, the *sevenless* protein (corresponding to EGFR in humans) on photoreceptor cell R7 bound the *bride of sevenless (Boss)* protein on the adjacent cell R8. In that way, the R8 cell controlled the behavior of the R7 cell. The *sevenless* protein in the R7 cell then signaled, by way of *son of sevenless (SOS),* down the chain to ERK, which entered the cell nucleus to activate genes. If that control was in any way defective due a mutation, the development of the eye was defective and produced abnormal structures, such as shown in Figure 18.5.

Understanding of the fruit fly's signaling from *sevenless* accelerated in the 1990's, particularly in the laboratory of Uptal Banerjee at the University of California in Los Angeles. In 1991, they reported studies of SOS mutants that pointed to SOS being an intermediary between *sevenless* (corresponding to EGFR) and RAS (Rogge et al., 1991). Then in 1992, they sequenced the *SOS* gene and inferred that it served to activate RAS (Bonfini et al., 1992). By 1993, the chain from *sevenless*/EGFR via GRB2 and SOS to RAS had been worked out (Karlovich et al., 1995) (Figures 18.6).

The parts (domains) of those proteins that carried out their respective bindings had also been worked out. The GRB2 protein was found to serve only as a linker between EGFR and SOS. One end of the GRB2 molecule had an 'SH2' domain that was noted to bind to phosphate groups on tyrosine amino acids of proteins. Thus, there was a sequence of links from EGFR to GRB2 to SOS to RAS.

When EGFR bound to EGF, a pair of EGFR protein molecules paired up and added phosphate groups to each other's tyrosines at specific places on the proteins (described in Chapter 17). Those phosphotyrosines then bound the SH2 end of a GRB2 protein. The other end of GRB2 had an 'SH3' domain that bound a particular amino acid arrangement on SOS. That's all that GRB2 was responsible for doing. SOS, on the other hand, not only linked between GRB2 and RAS, but also stimulated the activity of RAS by facilitating the

replacement of GDP by GTP on the RAS molecule (Figure 18.6). That chain of proteins then sent signals to the R7 cell urging it to become a photoreceptor in the fruit fly eye.

It is mind-blowing how nearly the same network of protein interactions in a critical control pathway exists in humans and in fruit flies. In the fruit fly, the network controls the development of the eye, whereas in humans it controls cell division. I don't know whether the fruit fly perhaps has another similar network that controls cell division, or whether humans have other networks of this kind that function in the development of the eye or other anatomical structure. Interestingly, the same network arrangement served quite different purposes. That fact of nature enabled the extraordinary connection from of fruit fly eyes to human cancer.

How the receptor tyrosine kinase EGFR connects to RAS via SOS and stimulates RAS to signals the cell to divide is shown by the molecular interaction map in Figure 18.7, which builds on the map in Figure 17.6 of Chapter 17. The signal from RAS goes to the cell nucleus by way of a chain of kinase proteins (RAF, MEK, and ERK) that are used by many signaling systems in the cell.

The interesting way that RAS itself is regulated was shown in Figure 18.6. That regulation is based on the fact that the RAS protein has on it a site that can bind either GTP or GDP (guanosine triphosphate or guanosine diphosphate). When RAS has GTP bound to the site, it is active and sends signals down the pathway to the cell nucleus. When, instead, GDP is bound to the site, RAS is inactive and does not send signals. SOS activates RAS by stimulating the replacement of GDP by GTP on the RAS protein. In the opposite direction, a RAS-GAP protein inactivates RAS by stimulating the conversion of the bound GTP to GDP. This balance between activation and inactivation regulates RAS and thereby regulates the strength of the signals sent down the pathway to the cell nucleus.

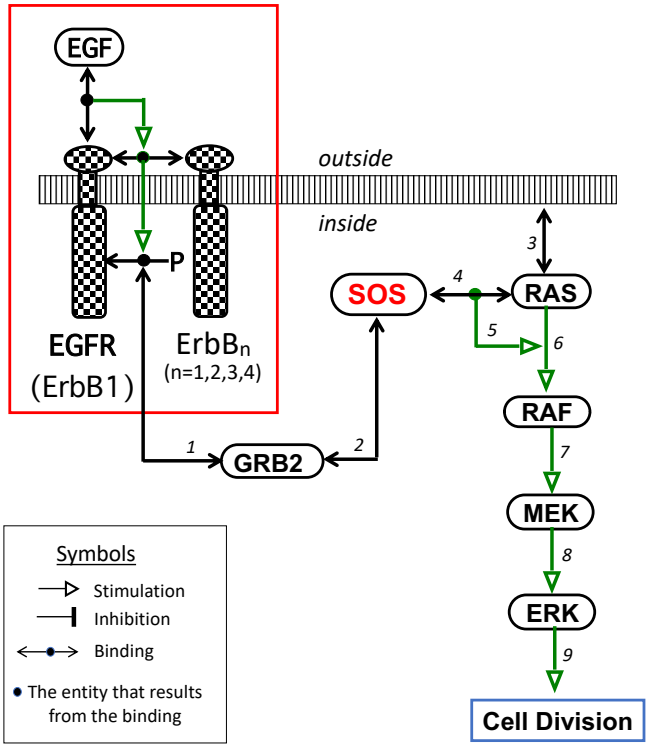

Figure 18.7. How SOS connects growth factor receptors with RAS in the activation of cell division. The epidermal growth factor receptor (EGFR, also known as ErbB1, see Chapter 17) becomes activated when it binds an epidermal growth factor. EGFR can then bind to another ErbB family member. The two ErbB's then phosphorylate each other's intracellular parts (domains). Many sites are phosphorylated, but only one is shown. These events are in a red box, because some details are omitted (see Figure 17.6 in Chapter 17). The phosphorylated EGFR site then binds the adapter protein, GRB2 *[1],* which then binds SOS *[2]*. That brings SOS to the cell membrane, where both EGFR and RAS *[3]* are located. The combination of SOS and RAS *[4]* then activates RAS *[5]* to send a signal down the RAF-MEK-ERK pathway *[6,7,8]* that simulates cells to enter the cell division cycle *[9]*.

In 1984, an important discovery connected that story to human cancers. It was found that the RAS genes were often mutated in cancer and, furthermore, that the mutation blocked the conversion of the bound GTP to GDP, thereby preventing the inactivation of active RAS (Gibbs et al., 1984). Consequently, the mutated RAS was active all the time and sent excessively strong cell division signals. Thus, when a mutant RAS gene was injected into cells, the cells divided without control, as they do in cancer (Feramisco et al., 1984).

But the question remained: why was the conversion GTP to GDP defective in the mutant RAS protein? The reason turned out to be that this GTPase activity, which is an integral part of the RAS protein, is normally activated by another protein, RAS-GTPase-activating-protein (RAS-GAP, for short). The defect in the mutant RAS was that it did not respond to RAS-GAP (Trahey and McCormick, 1987) (Vogel et al., 1988). (Like RAS, the RAS-GAP

protein binds to the inner surface of the cell surface membrane, thus localizing it to where it can efficiently interact with RAS.)

HRAS and KRAS, together with NRAS, constituted the RAS family of genes of very similar DNA sequences. Taken together, mutations in one or another of the RAS genes was found in about 10% of all cancers. Of the three RAS genes, KRAS was found to be by far the most frequently mutated in cancer. Remarkably, there was one type of cancer that nearly always had a KRAS mutation: cancer of the pancreas. Other types of cancer that frequently had KRAS mutations were about 45% of colorectal cancers and about 35% of lung adenocarcinomas. HRAS was mutated in about 10% of lung adenocarcinomas. NRAS was mutated in about 15% of melanomas. I don't know (and perhaps no one knows) why RAS mutations are common in only certain types of cancer. In particular, why do pancreatic cancers almost always have a KRAS mutation?

Almost all of the oncogenic mutations of RAS were at only three position in the amino acid chain of the protein (Cox et al., 2014). Moreover, the three changes each had the same effect: they prevented RAS-GAP from interacting with RAS, thereby keeping the RAS protein continually in its active GTP-bound state. In other words, the GTPase activity of RAS was unable to convert its bound GTP to GDP. Therefore, since RAS was active in its GTP-bound state, the mutated RAS protein remained active all the time and continually sent signals to the nucleus to stimulate the cell to divide.

A RAS mutation by itself, however, was not enough to cause cancer -- because other proteins, particularly TP53, could stop the malignancy. To become malignant, a tumor needed one or more other defects, such as an inactivating TP53 mutation.

Although we understood how these oncogenic mutations induced cells to grow into cancers, how to interfere with that process so as to provide therapy for the 10% of patients whose cancer was driven by a RAS mutation remained a big problem. It was a complex problem, in part because the RAS proteins have several important functions in the cell. Efforts to find a solution were in progress at the time of this writing.

Failure of efforts to find RAS-inhibiting anticancer drugs.

There were several possible ways to suppress the overactivity of mutated RAS. A drug that inhibited any of the many factors required by RAS to be active might be effective. Despite decades of efforts, however, medicinal chemists had not come up with a clinically approved drug (Cox et al., 2014). Some cancers became addicted to high RAS activity. A drug that inhibited RAS, either directly or in a downstream pathway, might be effective against those cancer cases. Research became directed mainly on KRAS-dependent cancer – where the cancer cells were addicted to high expression of KRAS.

One of the first approaches was to look for drugs that would compete with GTP for binding to the mutant RAS protein. That effort failed, however, because the affinity of RAS for GTP

was too high: chemists could not find a drug molecule that could compete with that high affinity. Blocking the GTPase activity of the RAS protein was not a good idea, because it would maintain RAS in its high-activity GTP-bound state. On the other hand, a drug that worked like RAS-GTPase to convert the RAS-bound GTP to GDP would inhibit RAS activity, but attempts to find such a drug also failed.

Another idea was to inhibit the binding of RAS to the cell surface membrane, because that would hinder RAS from receiving signals from EGFR, which was located in the cell surface membrane. Well then, what causes RAS to become bound to the membrane, and could that be inhibited? To enable RAS binding to the cell surface membrane, the cell has an enzyme that adds a long hydrocarbon chain to the RAS protein. The hydrocarbon chain is lipid-like and tends to merge with the lipid part of membranes, thereby carrying the RAS protein along with it to the cell surface. Inhibitors of that enzyme were therefore considered as drugs that might suppress RAS activity. The problem was that many other essential molecules rely on the same chemistry to carry them to the cell surface, and it was difficult to find a drug specific for the RAS protein. Another problem was that there were different enzymes that linked different kinds of hydrocarbon chains onto RAS and inhibiting any one of those enzymes would still allow a different enzyme to link a similarly effective hydrocarbon chain. Efforts to use this approach were rekindled based on deeper understanding of the relevant molecular complexities (Cox et al., 2015). In the face of all those difficulties and failures, RAS had become considered to be "undruggable." New technology, however, restored hope that direct targeting of RAS may yet succeed (Cox et al., 2015) (Ryan et al., 2015).

The most frequent oncogenes whose over-activity drove perhaps as many as 20% of human cancers were the closely related members of the RAS family: KRAS, HRAS, and NRAS (Downward, 2015). Of those, KRAS mutations were extraordinarily common in cancers. Most remarkable was that a KRAS mutation was found in as many as 95% of patients with pancreatic cancer. In addition, such mutations were found in about 40 % of patients with colorectal cancer and in 20% to 25% of patients with adenocarcinoma of the lung.

The KRAS story.

A KRAS oncogene was discovered in 1983 by Manning Der and Geoffrey Cooper of Harvard Medical School. They discovered an abnormal protein in cancer cells, made by a mutated gene that produced cancer upon transfecting the gene into non-cancer cells. The mutated gene thus was an oncogene -- which they identified as a mutant *KRAS* (Der and Cooper, 1983). Much time and effort was needed, however, to find out what overactive *KRAS* did to make cells cancerous.

In 2009, Jeff Settleman and his colleagues showed that cell lines derived from human lung or pancreas cancers differed in the degree to which they were addicted to KRAS (Singh et al., 2009). Thus, if KRAS or its downstream pathway were blocked by a drug or other

means, the cancer cell should usually die. They thought that the addiction might make those cancers vulnerable to specific drugs, and they set about investigating whether that approach could lead to drugs that were effective against cancers whose KRAS was overactive, and the cells had become addicted to it.

Figure 18.8 shows how they identified cell lines that were highly addicted and that could perhaps be targeted by specific drugs. In order to determine the degree of addiction, they first suppressed the production of KRAS by inserting into the cells a small hairpin RNA (shRNA) that specifically blocked the KRAS messenger-RNA, thereby blocking the production of KRAS protein. Then, they looked to see whether the cells were dying, which would indicate that the cells were addicted and would not be able to survive without KRAS. They did that by measuring the amount of cleaved caspase-3 protein that was produced when KRAS was suppressed. A central feature of cell death by apoptosis was the cleavage of the caspase-3 protein (it is broken into two pieces that then come together in a new configuration to generate an active caspase-3 enzyme that starts the apoptosis process).

Since attempts to develop a KRAS-inhibiting drug had failed, the investigators thought that inhibiting a step downstream from KRAS might work. They therefore set out to investigate the molecular changes occurring when KRAS was artificially suppressed using an shRNA. Although such RNA's may not become useful drugs, researchers did not give up trying to target RAS. Among many efforts to apply new molecular techniques was the possibility of engineering antibodies that would specifically target mutant KRAS protein inside the cell (Shin et al., 2020).

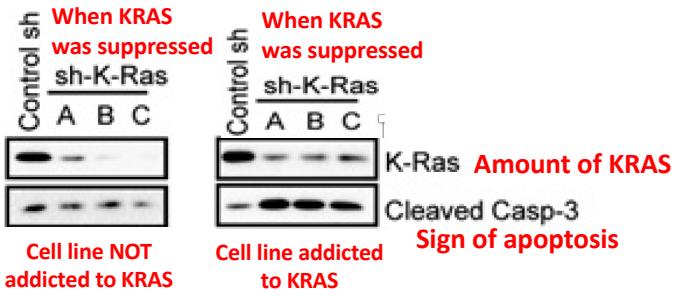

Figure 18.8. An example of two human cancer cell lines that differed in whether addicted to KRAS (Singh et al., 2009). The cell line on the *right* was addicted to KRAS: when the experimenters suppressed KRAS, the cells died by apoptosis. The cell line on the *left* was not addicted to KRAS: when the experimenters suppressed KRAS, the cells did not die. In order to tell whether or not the cells were KRAS-addicted, they measured the caspase-3 cleavage product. If the amount of cleaved Casp-3 protein increased upon addition of sh-KRAS to suppress KRAS production (*right*), it indicated that the cells were dying because of addiction to KRAS. If there was no increase in Casp-3 cleavage (*left*), it indicated that the cells were not addicted.

How the activity of KRAS is regulated.

Knowledge of how KRAS is regulated was thought to open new opportunities for therapy. Since the RAS proteins stimulate important processes, such as cell division, the cell must regulate their activity. Without RAS regulation, cancer may ensue. Most attention was given to the one that is most prominent in cancer, KRAS. What had to be regulated was the balance between KRAS-GTP and KRAS-GDP, where the former was active and the latter was inactive. The active KRAS-GTP would stimulate the first member of the downstream pathway, which is the protein RAF, from which the stimulation signal may proceed all the way to the genes that promote cell division (Figure 18.7). A mutation of RAF could, by the way, stimulate the downstream pathway to cell division independent of RAS – in fact, it leads to melanoma, a story that is told in the next chapter.

It turned out that the controlling factor for the GTP/GDP regulation of KRAS was the *son of sevenless* (SOS) discovered in fruit flies but of similar function in humans. How it works was nicely shown in 2015 by Channing Der as a cycle that he referred to as *the beating heart of cancer* (Figure 18.9) – reflecting that about 1 in 7 cancers were driven by dysregulation of KRAS (Kessler et al., 2021). SOS would be the cycle's *pacemaker* (Figure 18.9). The dominant role of the SOS protein in the cycle is shown by it being about 7 times as large as KRAS, consisting of 1333 amino acid, compared with only 189 for KRAS – thus KRAS is only about the size if a typical SOS domain (Figure 18.10).

According to Der's model (Figure 18.9), the cycle begins with GDP-bound KRAS ($KRAS_{GDP}$), which is the "*off state"*. $KRAS_{GDP}$ then binds to the CDC25H domain of SOS (Figure 18.10), where the bound GDP (guanosine diphosphate) is replaced by GTP (guanosine triphosphate), which yields the "*on state"* $KRAS_{GTP}$. The replacement of GDP by GTP is accelerated when another $KRAS_{GTP}$ molecule is bound to an *allosteric* site of SOS at its REM domain (Figure 18.10). (*"Allosteric*" is a change in a protein's shape that affects a distant site on the same molecule.) Finally, $KRAS_{GTP}$ slowly removes the high energy phosphate at the end of the chain of three phosphates of the GTP, converting it to GDP: back to the "*off state"*. KRAS has an intrinsic GTPase activity that slowly does that. But the rate of GTP-to-GDP conversion is greatly increased by a GTPase-accelerating protein (GAP) (Figure 18.9). Much research effort went into elucidating what SOS does and the conformational changes this large protein undergoes (Figure 18.10). SOS was thought a potential target for the development of inhibitor drugs (Hofmann et al., 2020).

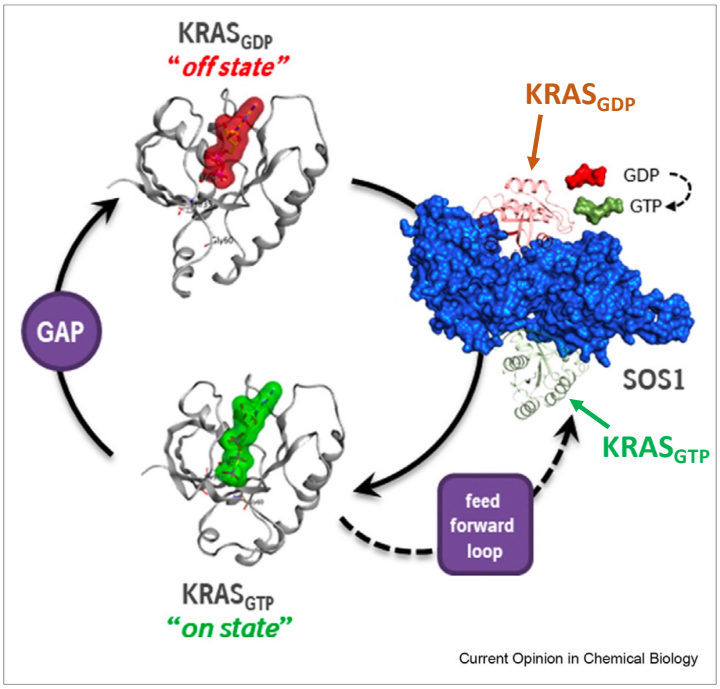

Figure 18.9. Channing Der's KRAS cycle that he thought of as *the beating heart of cancer*, with SOS as *pacemaker.* See text for explanation. From (Kessler et al., 2021) with additional labels.

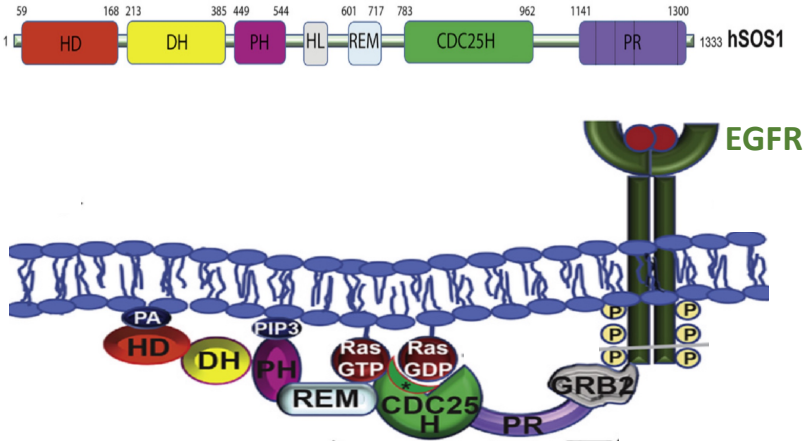

Figure 18.10. The domains of SOS and how they may interact with RAS and elements in the cell surface membrane (Baltanas et al., 2020). Starting at the carboxy (C) end of SOS (*right* end of the upper diagram), we come first to a proline-rich domain (PR), which is shown in the lower diagram as bound to the GRB2 protein, which in turn is bound to EGFR. (GRB2 has an SH3 domain that binds PR and an SH2 motif that binds a tyrosine-phosphate on EGFR.) We come next to a CDC25H domain that binds RAS_{GDP} and replaces the GDP with GTP. Then, there is a RAS-exchanger motif (REM) where a RAS_{GTP} can bind and accelerate the replacement of GDP with GTP. Finally, there are some positively charged domains that bind to negatively charged placed on the membrane, thereby stabilizing the membrane binding of SOS. From (Baltanas et al., 2020).

How RAS mutations lead to cancer.

How marvelous that human evolution of large brain and communal societies building knowledge over generations has already led us to glimpse the molecular functioning of our own bodies, their disorders and remedies. Those thoughts came to mind on contemplating the KRAS cycle, how its derangement leads to cancer, and at least in one limited circumstance to a road to therapy (Figure 18.9).

A path to cancer happens when cells have excessive amounts of KRAS in its "*on state*", $KRAS_{GTP}$, where KRAS has GTP bound -- because $KRAS_{GTP}$ signals cell division and must be controlled to avoid the excessive cell growth of cancer. Excessive $KRAS_{GTP}$ could accumulate if the rate of $KRAS_{GTP}$ production is too high or if the rate of its loss by conversion to the "*off state*", $KRAS_{GDP}$, is too low. It turns out that it is the latter case that is most often the trouble. In particular, it is because the mutant KRAS has lost its ability to bind well to "GTPase accelerating protein" or GAP that accelerates the conversion of $KRAS_{GTP}$ to $KRAS_{GDP}$. The mutant KRAS therefore accumulates in its "*on state*", $KRAS_{GTP}$, and stimulates excessive cell division.

A drug that targets a particular KRAS mutation was designed using detailed knowledge of the chemistry and molecular structure of the mutant protein. The new drug combined covalent and non-covalent binding designed specifically to fit the mutant protein's structure and may be a step forward in the design of targeted drugs. Its story follows.

Mutant KRAS as anticancer drug target.

I have for the most part focused on history because current events often soon become obsolete, but make an exception now because of the recent molecular design of a drug that targets specifically a particular mutation of the KRAS protein and binds tightly, apparently covalently, only with the protein that has that mutation and only when KRAS is in the GDP-bound state. The drug was deemed so promising that just two months ago, in March 2021, the U.S. Food and Drug Administration (FDA) granted it a *Breakthrough Therapy* designation as the first promising anticancer drug targeting a KRAS mutation. The drug is Amgen's AMG510 "Sotorasib" that specifically inhibits the KRAS G12C mutant. The preliminary approval was for treatment of patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) with the KRAS G12C mutation who had received at least one prior systemic therapy. By blocking the $KRAS^{G12C}$ mutant in its GDP-bound state, the drug prevented the replacement of GDP with GTP (Figure 18.9, the part in blue labelled SOS1). The drug thus traps $KRAS^{G12C}$ in an inactive state and prevents it from sending signals that would stimulate cell division.

The remarkable specificity of the drug comes from a combination of two factors (Canon et al., 2019). First, it fits and binds in a hydrophobic groove in the protein with a geometry

specific to the mutant protein in its GDP state (Figure 18.11). Second, the drug can bind covalently to a sulfur atom of a cysteine that is only present in the mutant protein – because the mutation puts a cysteine in place of a glycine at position 12 in the amino acid sequence (Figure 18.12).

A phase I trial of sotorasib was conducted in 129 patients who had advanced cancers with a KRAS G12C mutation (non-small-cell lung (59), colorectal (42), or other (28)) (Hong et al., 2020). Of the 129 patients in the study, 88% had evidence of response or had stable disease. The cancer was held in check (progression-free survival) for a median of 6 months. However, 12% of the patients had serious side-effects, perhaps due in part to the alkylation and hydrophobic binding abilities of the drug to attack sites on normal cell components – a side-effect that might be reduced by modifying the drug's structure. Better results might be expected after further studies to determine optimal dosage and to add drugs that could prevent resistance to the drug. Still, the drug would be effective only in the relatively low, albeit significant, fraction of patients who have cancers with that particular KRAS mutation.

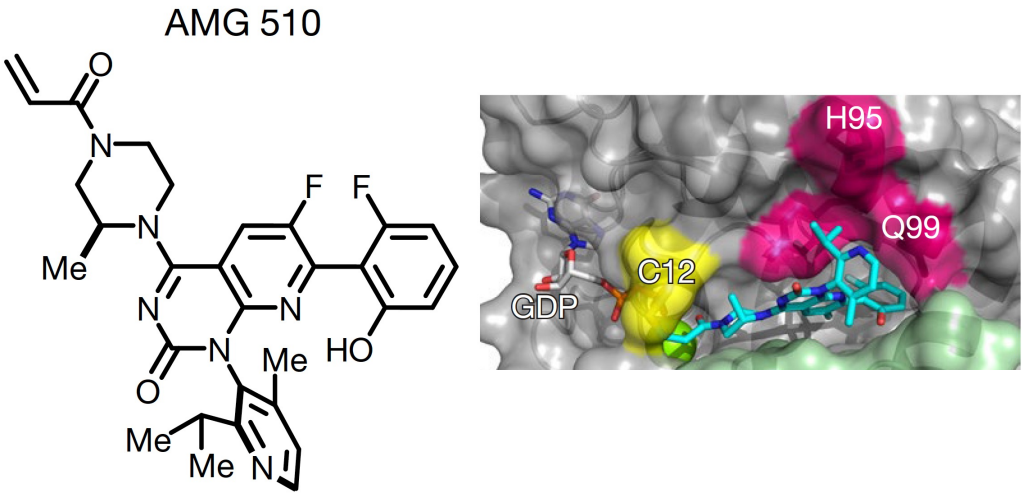

Figure 18.11. The structure of sotorasib (AMG510) and how it fits in a groove in the G12C mutant KRAS protein (Canon et al., 2019). The cysteine that replaces the glycine at position 12 in the mutant protein is shown in yellow. The carbon atom double-bonded shown at the upper end of the structure (*left*) is close to the sulfur atom of cysteine-12 (*right*). The hydrophobic part of the drug fits nicely in a hydrophobic groove in the protein.

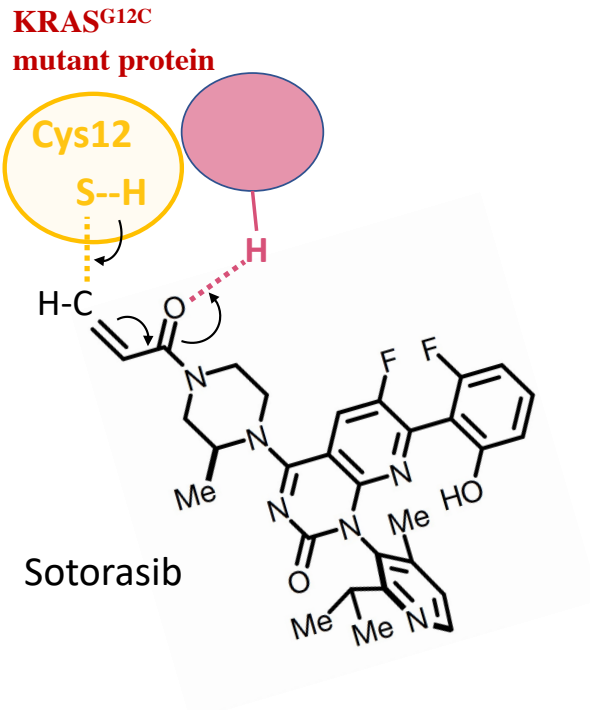

Figure 18.12. How sotorasib could bind covalently to the sulfur of the cysteine that replaces the glycine in the G12C mutation of KRAS. A hydrogen bond from the KRAS protein (red color) helps sotorasib to suck in an electron from the sulfur atom (yellow color) to from a covalent bond between drug and protein.

Summary

The three *RAS* genes are the most frequently mutated genes that drive human cancer – they are the most frequent oncogenes activated by mutation. Their importance drove strong efforts to develop inhibitors of the overactive RAS functions. However, these efforts, extending over more than three decades, were disappointing, giving rise to the opinion that mutant RAS proteins were "undruggable." Armed with new technology and deeper understanding of the complexities of RAS functions, attempts to develop therapy targeted against RAS oncogenes were renewed (Papke and Der, 2017).

Earlier studies – before 2015 -- had revealed that the strength of signals from RAS proteins depended on control of RAS activity. RAS proteins send signals to the cell nucleus to initiate cell division, but this happens only when RAS is in its GTP-bound state. Importantly, the amount of RAS-GTP was tightly controlled, so that cells did not divide too often. That was accomplished by control of RAS cycling between the active GTP-bound state and the inactive GDP-bound state.

This Chapter looked back at how mutations of fruit fly genes led to the discovery of human versions of genes functioning in an analogous pathway. The fruit fly protein altered by the *sevenless* mutation was found to be a receptor tyrosine kinase that corresponded to

human EGFR (Simon et al., 1991). The *Son of sevenless* (SOS) mutation was especially revealing, because it disclosed previously unknown genes that turned out to be central to the cause and treatment of many human cancers. Particularly important was the discovery of the *RAS* genes. The relevance of SOS to cancer was shown by finding that it transmits signals from EGFR that activate RAS. RAS in turn activates RAF (the topic of Chapter 19). It is remarkable how that arcane route from fruit fly eye mutations to the RAS oncogenes, together findings about cancer-causing viruses, led to the discovery of human oncogenes and their importance in cancer cause and treatment. Who would have imagined that the SOS gene of fruit flies would become thought of as the *pacemaker* of a *beating heart of cancer*?

The most important of the RAS proteins was found to be KRAS whose mutations are prominent in several common cancers. KRAS therefore became a major focus of research, and efforts were made to find drugs that would be effective against KRAS-mutated cancers.

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