The 2008 Kyoto Prize Commemorative Lectures: Basic Sciences

"Thinking about how living things work"

Anthony James Pawson

The work for which you are honouring me with the Kyoto Prize has its origins in my fascination with living creatures and the cells of which they are composed. In particular, the idea that one could actually discover the scientific principles underlying the evolution and workings of organisms, such as ourselves, came as a revelation when I was a biology student in the 1960s, and has gripped me ever since. To my mind there can be no experience so thrilling as to uncover some unexpected, and previously undiscovered, aspect of living beings, and the universe that we inhabit. The process of scientific discovery is, I would suggest, rather like that of exploring for new continents in the age of sailing ships – there are long periods at sea, with not much happening, and then suddenly the sight of land, at first distant and mysterious, and then becoming clearer until finally one arrives at a new shore – or by my analogy at a new scientific hypothesis. But it is that moment of first seeing the land in the distance, of first realizing that one has a thread of evidence for a new way of looking at the world, that provides the greatest excitement. In school, we learn about science retrospectively, and so everything appears to make perfect logical sense. The actual course of scientific discovery, in contrast, takes many curious twists and turns, and if my experience is anything to go by, when a scientist ventures into unexplored territory and finds something new and unusual, he or she agonizes over what these findings mean and how to correctly interpret the experimental data. The philosopher Søren Kirkegaard proposed that "Life can only be understood backwards; but it must be lived forwards". So it is with science, and it is therefore surprising to a scientist to see his or her experiments described in textbooks, and being taught to students as though they are entirely straightforward, when at the time of their discovery they seemed like a very difficult puzzle box that would never be opened. It seems to me that in teaching students we should perhaps put more emphasis on what we *don't* know, than on what we already understand.

My story has to do with human cells, and how they work, and you may reasonably ask why this is important. First, cells are the basic unit of all life, such that every free-living

organism in the world is either now, or once was, a single cell. You and I, although we do not remember it, started life as one cell, that then grew and divided to form the multitude of different cell types that make up the tissues of our bodies (Figure 1). Second, most of the diseases from which we suffer ultimately result from the abnormal behaviour of cells. For example, in cancers, cells do not respect the normal controls on their proliferation and movement, and so they grow out of control and spread to distant sites in the body (Figure 2). In diabetes, cells either do not respond properly to the hormone insulin, or fail to make it in the first place. In viral diseases, the actual damage is usually done by the response of the body's own cells to the infection. As we will discuss, the inside of the cell is somewhat like a jigsaw puzzle, though one that is very complex because it is constantly changing shape. We want to know what are the important pieces, or molecules, inside the cell, and in particular how they fit together to make a complete picture – that is to say a normally functioning cell. To continue the analogy, why does disease result when an important piece of the puzzle is left out, or inserted into the wrong place?

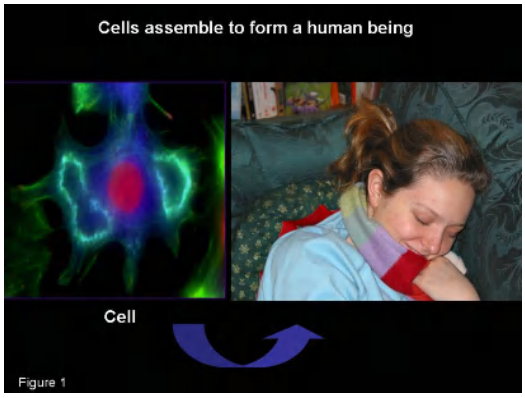


Figure1

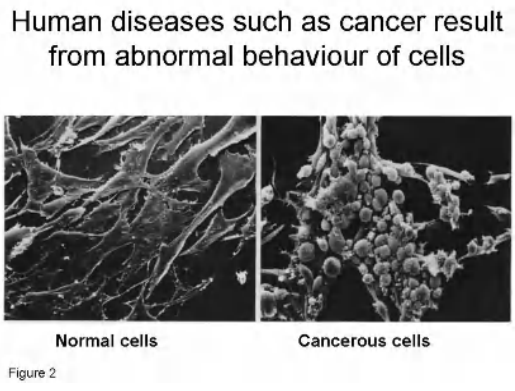


Figure2

To begin my story at the beginning, I was born in 1952 in Maidstone in the county of Kent, in England, although my family moved shortly thereafter to the town of Sevenoaks, somewhat closer to London. My mother was a botanist, and taught biology in high school;

her knowledge of plants and flowers is profound, and also deeply felt, and I absorbed my interest in the natural world from her (Figure 3). She was the real academic of the family. My father was a well-known sportsman, who played soccer and cricket at the highest levels, for example competing for England in the 1952 Olympic Games in Helsinki. Many people in the UK still remember seeing him play soccer, as I discovered when I visited Oxford University recently, and was asked whether I was related to the famous Pawson, the soccer player (Figure 4). He later wrote on these sports for the Observer, an English Sunday newspaper, and has published numerous books about sports, and his great love of fly-fishing, which he instilled in me at an early age (Figure 5). My father is highly competitive, in the best sense of the word, and I can perhaps illustrate this by telling you that he became the world fly-fishing champion at the age of 66. A lasting memory of my childhood is of going to soccer matches with my father, and if I was really lucky getting to sit in the press box with the other newspaper correspondents. This was in the days long before laptop computers, and I fondly recall that at the end of the game the reporters would rush madly to get to the few available telephones, to call in their stories to their newspapers in time for them to be put into print and published the next day. This was my first exposure to the competitive nature of publication, which I was later to experience in my academic life. From my father I learned how to get things done, and how to communicate. My grandparents were also important figures in my childhood. They had spent much of their lives in countries such Australia, Japan, India and the Sudan, and therefore had a remarkably rich experience, and were gifted story tellers, even if a critical listener might wonder if their tales were sometimes slightly embellished (Figure 6). They gave me a sense of identity and self-confidence, and more importantly imbued me with a desire to see the world, and discover new things about it.

Figure3

Figure4

Figure5

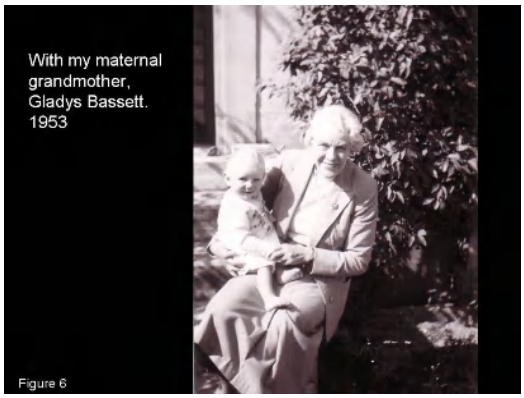


Figure6

My passion for biology was sparked by a remarkable teacher, Michael Baron, when I was a high school student at Winchester College. This is an ancient school, founded in 1394, with a tradition of teaching in a fashion that inspires free-ranging curiosity. In this mould, Michael Baron radiated enthusiasm about biochemistry and physiology, and I can still remember the exact moment in one of his classes when I realized that one could actually figure out the chemistry of life. Equally important, he related the abstruse details of the inner workings of the cell to the ways that animals and plants live in their environment. This broad view has inspired me in later life to investigate not only how individual molecules and cells function, but their collaborative impact on the whole organism, and on the process of evolution. I did my first real science project in Michael Baron's class, looking at the effects of pollution on the viability of a small aquatic organism, called Daphnia. The physics project I did at about the same time was a complete disaster, and I suspect this biased me towards the life sciences. Like my mother, Michael Baron is a devoted botanist, and to this day maintains a garden near Winchester that is open to the public and much visited by his fellow botanical enthusiasts (Figure 7). Winchester College gave me the opportunity to explore many curious aspects of English culture, among which one of my favourites involved ringing the large bells in church towers, usually six or more in number, with each bell being handled by a different person. This is an activity that requires a curious mixture of physical dexterity, mathematical acuity and teamwork. To this end we would visit historic churches of the English countryside, and ring "peals", as they are called, which could go on for several hours. It was more fun than studying.

Figure7

My first academic love was for the classical languages, and through high school I maintained a particularly interest in literature, as well as sciences. However, upon going to Clare College at Cambridge University as an undergraduate I decided to throw in my lot with biology. My first two years at Cambridge were something of an intellectual disappointment, as they were full of routine learning in large classes. What a difference it was in the third year - as part of a small class specializing in biochemistry we had lectures on the very latest research from local luminaries, such as Max Perutz, the legendary X-ray crystallographer who worked out the structure of the life-giving protein haemoglobin. I also got my first taste of real experimental science, working on a project with the future Nobel laureate Tim Hunt, on the process of protein synthesis. I found the idea that one could combine isolated fractions from a cell in a test tube, and manipulate them so as to find out the biochemistry behind what was going on as proteins were made, to be absolutely intoxicating. Hunt was also my tutor (as it is called) at Clare College, and his vitality and spontaneous love of science inspired me to go on with a career in research (Figure 8). He shared a laboratory with Richard Jackson, and the two appeared to be perfectly matched. Tim Hunt had a new visionary idea every few minutes, or so it seemed, while Richard Jackson focused on whether these ideas could actually be put to the test. For a budding scientist it was the ideal introduction to the two poles of science – creative insight on the one hand, and experimental rigour on the other. On Tim's suggestion, in 1973 I went to do my Ph.D. with Alan Smith at the Imperial Cancer Research Fund (or ICRF) in London.

Figure8

The ICRF was an entirely different place from a university department. It was a free-standing research institute (now part of Cancer Research UK), staffed mainly by independent scientists, postdoctoral fellows and technicians, with only a small group of graduate students who were treated no differently from everyone else, as though they were already experienced scientists. So it was a daunting challenge for a young 21-year old. On the other hand, it was the most stimulating and thought-provoking environment that one could possibly imagine. The cloning of recombinant DNA was just starting, as was the tentative identification of genes and proteins that might be causally involved in the development of cancers. I shared a bench with Ed Ziff, who had just developed an early approach to DNA sequencing, and he was unfailingly generous with his time and advice. Best of all, in the fine English tradition, everything stopped for tea in the morning and coffee in the afternoon, during which all the latest ideas were hotly debated and my views were solicited as though I should know what I was talking about.

So what did I get up to at the ICRF? At this point, let me remind you that biological information is carried by genes, in the form of DNA, but that typically each gene only exerts its effects on the cell when it specifies the production of a particular protein (Figure 9). These proteins are much more complex molecules than DNA, and they organize essentially everything that goes on in the cell, in part through their ability to catalyze biochemical reactions. Almost all of the medical drugs that we have are either themselves proteins, or exert their effects by targeting proteins. Mutations that change the DNA of a particular gene can cause disease because they alter the properties of the corresponding protein. Proteins can also physically bind to one another, and thereby create a communication network that dictates how cells behave, and how they talk to their neighbours in the body (Figure 10). I became fascinated by the question of how normal human cells respond to signals from their environment to form complex tissues such as the brain. How, I wondered, does this communication process go awry in diseases such as cancer, and could understanding this process lead to better anti-cancer drugs? How do new cellular functions arise in the course of evolution? The difficulty in the 1970s

was how to find a simple way to explore these very complicated issues. Ideally, this meant finding a single protein that could alter the whole organization of the cell, with the notion that uncovering its functions might reveal something fundamental about how cells work.

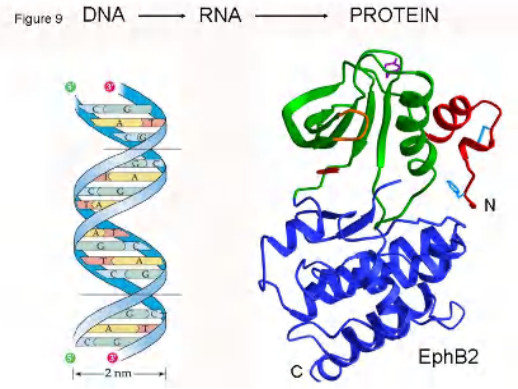


Figure9

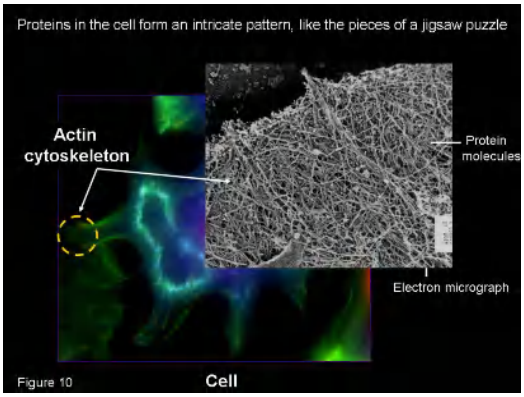


Figure10

In this regard, I was fortunate to encounter Steven Martin at the ICRF (Figure 11). Steve worked on Rous sarcoma virus, a so-called retrovirus that causes tumours in chickens, and quickly turns normal cells cultured in a dish in the laboratory into a cancerous state (Figure 12). In a stunning experiment published in 1970, he had shown that the cancer-causing activity of this virus was specified by a single gene, called Src, which Michael Bishop and Harold Varmus subsequently found to be an altered and abnormally active form of a normal cellular gene that had been captured by the virus. A protein such as that encoded by the Src cancer-causing gene seemed to be the perfect tool for my purposes, because it changes almost everything that happens in the cell. But it would be some years before I got the first glimpses of any new molecular principles that might underlie cellular organization, and its disruption in cancers.

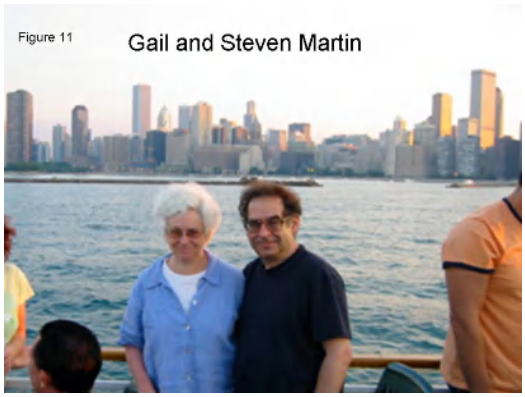


Figure11

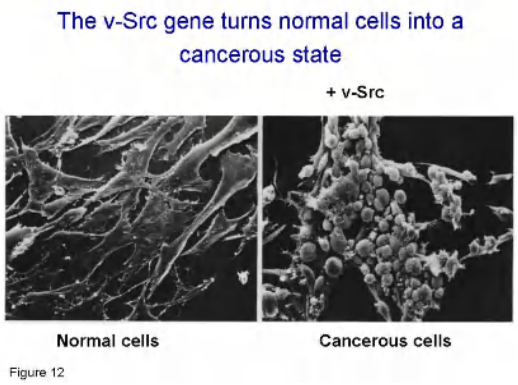


Figure12

During my Ph.D., I worked out some of the mechanisms by which retroviruses propagate themselves, which were later of more practical interest when it was realized the HIV retrovirus causes human disease. But I did not make much progress in identifying the Src protein or how it might work at that time. However, I did have the great good fortune to marry my wonderful wife Maggie, and she has inspired me in life, and has supported my scientific endeavours for more than 30 years (Figure 13). After getting my Ph.D., I went to the University of California at Berkeley, initially to work with Peter Duesberg, but I was rapidly reunited with Steve Martin, who in the meantime had moved to start a lab in Berkeley, and I began to analyze cancer-causing proteins in more detail. Calfornia, I must say, was something of a surprise to a naïve young Englishman, as I had imagined it would be rather like England, except that people would speak with American accents. I discovered that the Californian culture and countryside was much more vibrant and colourful than the rather gloomy post-war England in which I had grown up. My first earthquake also came as a great shock. Most exciting, the San Francisco Bay Area was full of laboratories working on cancer genes, and so I really felt as though I was at the centre of things.

Figure13

While I was pursuing the identification of new cancer-causing proteins, Tony Hunter at the Salk Institute found that the Src protein is a tyrosine kinase, meaning that it is an enzyme that adds a phosphate group to itself and other proteins on tyrosine, one of the 20 types of amino acids that are joined in various combinations into a linear chain to make a protein. This process of phosphate addition, or phosphorylation, is a prime means by which the properties of existing proteins are rapidly altered in response to external signals (Figure 14). Let me jump forward in time, and tell you that the hormones that control cell growth and metabolism, such as insulin, exert their effects on target cells through receptor proteins, that project from the surface of the cell like antennae to capture the passing hormone (Figure 15). These receptors traverse the outer membrane of the cell, and within the cell have a region that, like the Src protein, has a tyrosine kinase activity that transfers phosphate groups to tyrosines in the receptor itself, or on target proteins within the cytoplasm. Receptor proteins act, in effect, like the ears of the cell, to receive messages from the outside world, and to transmit the resulting information to the interior of the cell, and thereby to elicit an appropriate response. Like a light switch being rapidly turned on and off, the receptor is only active when the hormone binds, and is rapidly shut off when the signal passes (Figure 16). In cancer cells, however, a mutation in the relevant gene can give rise to a receptor protein that is locked in the active configuration, and consequently transmits a continuous signal. The cell is therefore tricked by the mutant receptor into thinking it is being told to grow, even though there is no hormone nearby (Figure 17). The cell, in effect, is hearing voices. The big issue, from my point of view, was to decipher how cells process the information emanating from these growth-inducing receptor proteins and their cancer-causing counterparts.

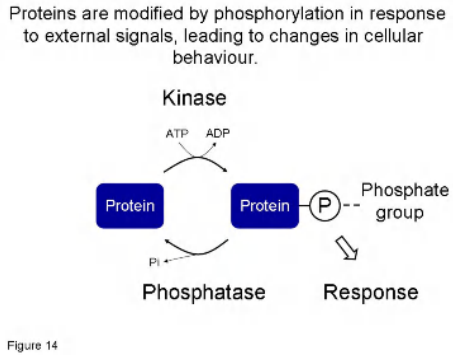


Figure14

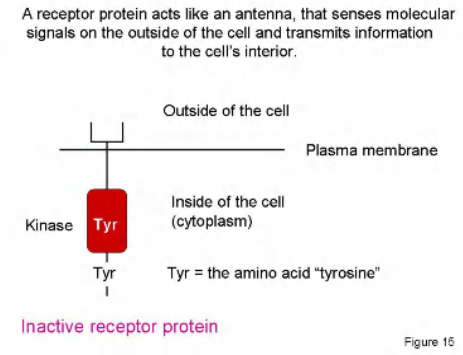


Figure15

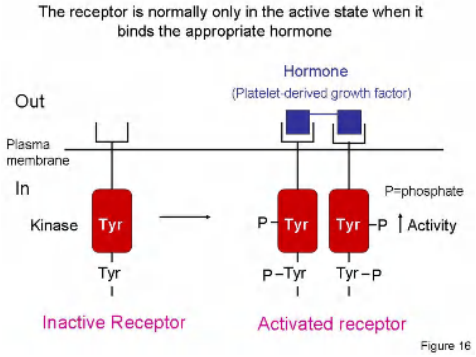


Figure16

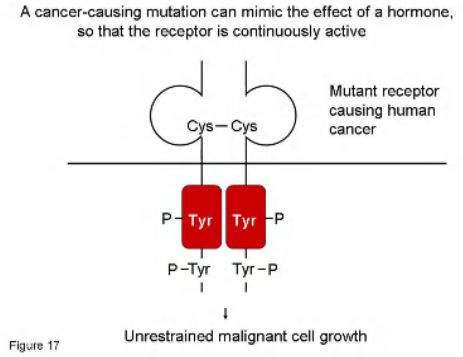


Figure17

This was what I set out to explore when I moved in 1980 to the University of British Columbia in Vancouver, Canada, as an Assistant Professor in the Department of Microbiology. The campus of the University of British Columbia is in an idyllic setting; it seems to be perched on the edge of the world, looking out over the Pacific Ocean, and is rimmed with snow-capped mountains. Unfortunately I was too busy in the lab to spend much time looking at the view (Figure 18). Before departing from Berkeley, I had started to work on another cancer-causing retrovirus that, serendipitously for this Prize, originated in Kyoto. This was the Fujinami sarcoma virus, isolated by Professor Akira Fuijinami, who founded the Pathology Department of Kyoto University in 1900. We had discovered that the cancer-causing protein of the Fujinami sarcoma virus (termed Fps) is an active tyrosine kinase, like the Src protein, and a similar observation had been made by Hidesaburo Hanafusa, then at the Rockefeller University in New York, and more recently the Director, and now Director Emeritus, of the Osaka Bioscience Institute. Indeed, I would like to pay particular tribute to Dr. Hanafusa and his many illustrious trainees for their inspiring work on retroviral cancer genes, which has profoundly influenced much of my own research. At the University of British Columbia I had a stroke of good fortune to collaborate with Michael Smith, who was just inventing a technology called site-directed mutagenesis, for which he won the Nobel Prize in

Chemistry in 1993. This technique allows a scientist to make any desired change to a gene, and thus to the sequence of amino acids in a protein, and to test the effects of these alterations on the protein's function. Conceptually, this is rather like using a word processor to change the text of a written document, and thus the possible meaning of a sentence. Today, any high school student could do such a site-directed mutagenesis experiment, but at the time we were in a unique position to apply this powerful approach to the problem of cell signaling. So, by good luck I was in the right place at the right time to use this emerging technology to ask how Professor Fujinami's Fps protein might plug into the cell's communication pathways.

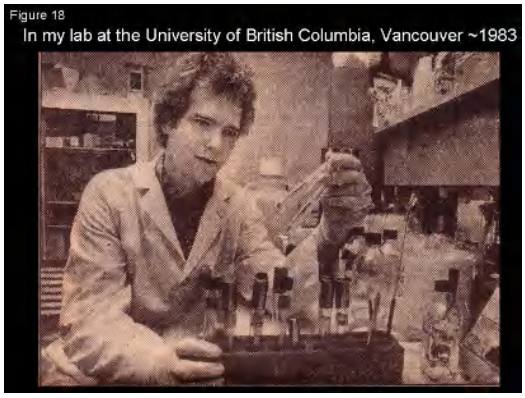


Figure18

Ivan Sadowski, James Stone and Geraldine Weinmaster in my lab found that the Fps protein, which like Src is entirely confined to the inside of the cell, has three different regions, or domains, all of which are important for its biological cancer-causing activities (Figure 19). A domain is a fragment of a larger protein that retains its biochemical properties even when it is made in isolation. Our work has indicated that a typical protein has a modular construction, resembling a child's building toy, made out of several blocks, where each block represents a domain. The tyrosine kinase region shared between Src, Fps and receptor proteins is one such domain. In Fps we found that this is preceded by a distinct domain, which interacts both with the adjacent kinase domain and with other cellular proteins, and thereby serves as a critical bridge to guide the Fps protein to its intended targets, which it phosphorylates, and thereby converts normal cells to a cancerous state. By looking at the sequence (or order) of amino acids in this new region of Fps, I realized that it was very similar to a corresponding region of my old friend the Src protein, as well as a related protein involved in human cancers called Abl, and so I called it the Src Homology 2 domain (or SH2 domain for short) (Figure 20). Had I known how important it was to be, I would have tried to think of a more memorable name. As an aside, after trying for 22 years, we finally got the three-dimensional structure of the linked SH2 and kinase domains of the human Fps protein, which we just published two months ago; this shows in atomic detail how this very first SH2 domain

actually looks, and fortunately confirms the hypotheses we made in the 1980s about how it works (about which I had been slightly nervous for the ensuing two decades) (Figure 21).

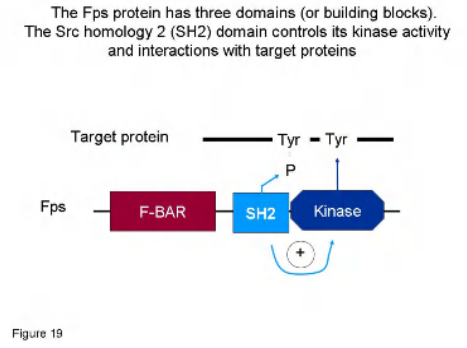
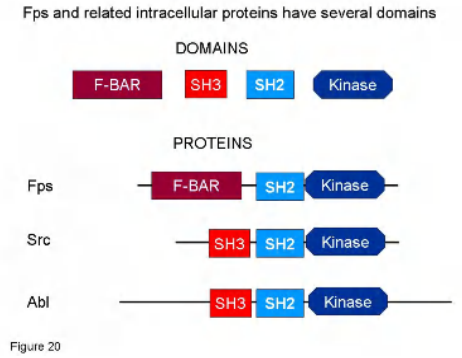


Figure19



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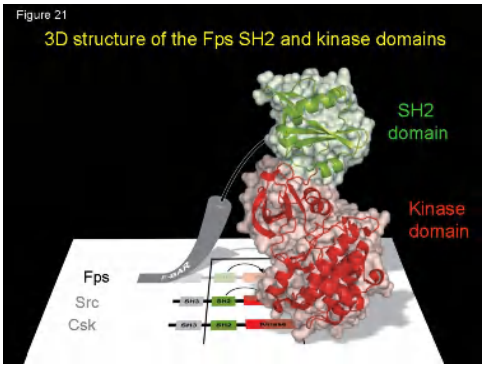

Figure21

In 1985 I moved from Vancouver to Toronto, as one of the founding members of the Samuel Lunenfeld Research Institute of Mt. Sinai Hospital, affiliated with the University of Toronto, where I found a remarkable group of colleagues, including Lou Siminovitch, Alan Bernstein, Janet Rossant and Alex Joyner (Figure 22). Their biological interests were related to mine, but they were more focused on areas such as genetics, embryonic development and stem cells. The most innovative research often seems to come from collaborations between scientists with distinct ways of looking at things, and so it was at the Lunenfeld. This was especially true as we started to realize that the signaling proteins I was working on also control processes such as embryonic development and formation of the blood system, in which my colleagues were interested.

Figure22

I was now able to pursue the deeper significance of the SH2 domain. An important clue emerged from the realization that the SH2 domain is also present in an otherwise diverse group of proteins that transmit biochemical information from activated receptors at the cell surface to the cell's interior. A key moment for me came in 1989, when Michael Moran in my lab took the isolated SH2 domains from a number of different signaling proteins, and found that they all bound selectively to growth-inducing receptors, but only when the receptors were in the active state, in which case the receptors are themselves phosphorylated on tyrosine. On seeing these results I felt positively light-headed, as they immediately suggested a general mechanism by which receptor proteins at surface of human cells transmit their signals. Based on such experiments, we proposed the following scheme – Receptors activated by their appropriate hormones, or by cancer-causing mutations, are clustered together and consequently phosphorylate one another on tyrosine. This acts as a signal, so that the receptors physically bind the SH2 domains of proteins within the cell, which then signal to their targets to elicit a change in cellular behaviour (Figure 23). One can think of the activated receptor as a magnet for proteins with SH2 domains. The addition of phosphate groups to proteins was thereby revealed as a kind of selective molecular glue, that makes particular proteins stick to one another.

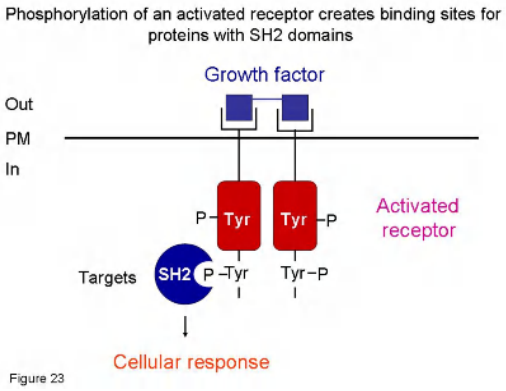

Figure23

From these simple beginnings has emerged a principle of cellular organization, of the sort that I wondered about as a student. Human proteins are typically composed of a few domains or building blocks, of which there are several hundred different types in total. These domains are linked in different combinations in distinct proteins, and it is this combinatorial effect that endows proteins with their varied and complex biological functions. In the context of communication, protein domains can be viewed as words in a sentence, that give different meanings depending on their identity and the order in which they are assembled. Like the SH2 domain, many different types of domains primarily act by binding to particular sites on other proteins within the cell. One can view them as the bumps and holes on the pieces of a jigsaw puzzle, that allow the protein pieces to fit together in a unique way. This domain-based network of interacting proteins provides an organizing principle that controls the properties of normal cells and allows them to rapidly respond to external signals. This network is usurped by mutant receptors to elicit a cancerous state. Indeed, some human cancers are driven by mutant proteins in which domains are artificially joined together in combinations never seen in normal proteins, causing a mis-wiring of signals (Figures 24 and 25). This network of signaling proteins is also hijacked ways by pathogenic viruses and bacteria, which trick the cell into behaving to the advantage of the micro-organism.

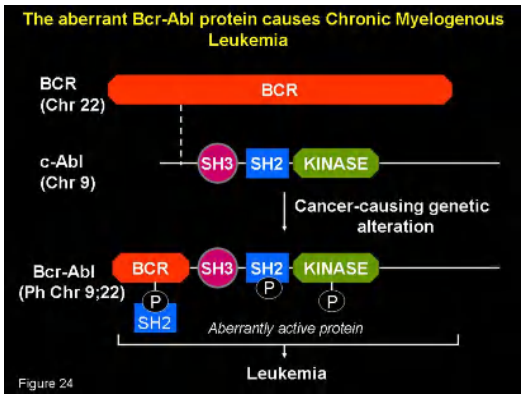

Figure24

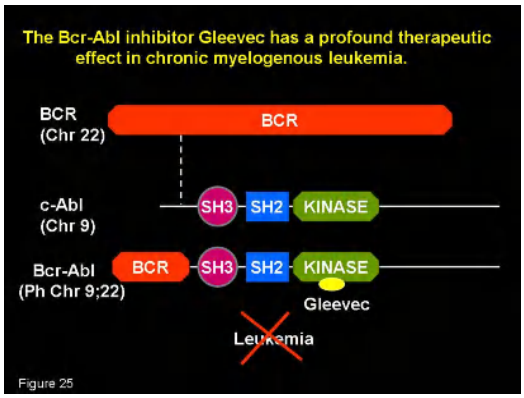

Figure25

Perhaps the purest example of this phenomenon involves proteins that we have called adaptors. Typically, these are composed exclusively of domains that bind other proteins. By simultaneously engaging an activated receptor on the one hand, and cytoplasmic target proteins on the other, they form an intimate grouping in which the right proteins are brought together at the right time to direct the flow of molecular information within the cell (Figure 26). An advantage of this set-up, I believe, is to have facilitated the evolution of complex animals, such as ourselves. Rather as human languages have become more sophisticated through the creation of new words, so the emergence of protein domains with new functions, and the joining of domains in new combinations, has provided cells with new lines of communication. Francois Jacob said that "evolution is a tinkerer", meaning that it is rare in biology that something completely novel appears,

but rather that cells evolve by finding new uses for old molecules. This principle of tinkering is evident in the emergence of novel proteins by the reorganization of domains into new combinations.

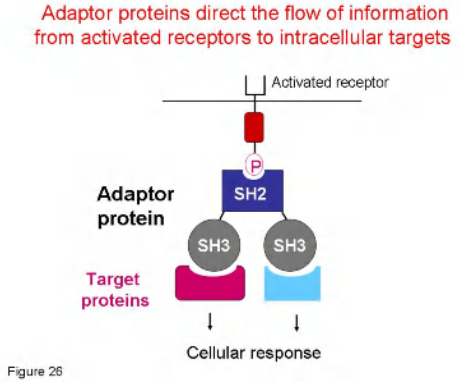

Figure26

Remarkably, the basic science that has been pursued over several decades into the nature of cell communication, and the mis-wiring of signaling pathways in disease, is starting to yield new targeted therapies that are changing the way that we treat cancers for the better, and will be applicable to many human ailments. Although these are early days, I believe that this progress underscores the importance of giving free rein to human inventiveness. It would have been hard to predict that work on a curious chicken virus would have ultimately led to new ways of thinking about how human cells are organized, and to new drugs to treat one of mankind's most persistent enemies. Governments increasingly want to see immediate returns on the research that they support, but it is worth viewing basic science as a long-term investment that will yield completely unexpected dividends for humanity in the future. Indeed, I think that new and unpredictable ideas flowing from fundamental research in the sciences and humanities will be essential for us to transcend the problems we face in combating disease, climate change and social upheaval. I personally envisage a world in which the diseases that afflict mankind are fully brought to bay, so that every child born into the world has the promise of a full and natural life span. At this challenging time in history, this may seem overly optimistic, but I believe that humankind has the genius, the altruism and the passion to make this a reality. As I said in my acceptance speech yesterday, we are a young and inventive species.

In part, my optimism stems from the view that, despite our seeming sophistication about medical matters, we are still profoundly ignorant. It has been argued that sequencing of the human genome has given us the book of life, but we still have only a cursory understanding of how this book is read. As I have indicated, our work on protein domains has given some insights into the meanings of the individual words of the book, but in this

and many other areas, such as our understanding of the brain and human behaviour, we are only at the beginning. I think this is good news, as the future is sure to be full of surprises. The clinician-scientist Lewis Thomas pointed to the effort it takes to bring a new scientific idea into existence, and to overturn longstanding myths, when talking about tuberculosis – he remarked that "without the long painstaking work on the tubercle bacillus, we would still be thinking that tuberculosis was due to night air and we would still be trying to cure it by sunlight". With this in mind, the recent decades of molecular biology have revealed the deep and close interconnection of all living things at the most fundamental level of their genes and proteins. In the long run, this new understanding, and the work yet to come, must inevitably alter our relationship to the natural world, and to one another, in addition to opening up entirely new areas in medicine and biotechnology. The fun and excitement in biology is just starting. I believe that nothing is more important to us human beings than to know where we came from, and how we relate to the vast diversity of other species with which we share the planet.

It has been a privilege and a great good fortune to pursue a vocation that is both fascinating and, I believe, important. On this matter, the playwright George Bernard Shaw wrote "This is the true joy of life, the being used for a purpose recognized by yourself as a mighty one". None of the progress that I have made has been achieved solely as an individual. As I have mentioned, I was trained by brilliant and inspiring mentors, and I have had the benefit of a collegial group of scientists around the world, who have made the field of cell signaling exceptionally enjoyable and productive. I could have done nothing without the dedication, insights and hard work of outstanding students and postdoctoral fellows, many of whom are now highly successful independent scientists in their own right. Most important, I thank my wife Maggie for her love and support, and our three children Nick, Catherine and Jeremy, of whom we are very proud (Figure 27). My children have the job of keeping me humble, and they will have to work overtime after the magnificent and overwhelming events of the Kyoto Prize week.

Figure27