Chapter 10

Putting It All Together

With this chapter, we tie up a bunch of loose ends and ponder what lies in the future of biochemistry

Putting Everything Together

"Organic chemistry is the study of carbon compounds. Biochemistry is the study of carbon compounds that crawl"

-Mike Adam.

Looking Back

The bounds of biochemistry have expanded enormously since its inception. Wöhler's demonstration, in 1828, that urea could be synthesized outside of a living cell, showed that there was no "vital force" that distinguished the chemistry of life from that of the non-living world. Chemistry is chemistry, but the term "biochemistry" was coined in 1903 by Carl Neuberg to describe the special subset of chemical reactions that happen in living cells. This specialness derives not from any exceptions to the laws of physics and chemistry, but from the way in which the chemical reactions in cells are organized and regulated, and also from the complexity and size of biological molecules.

Faced with far greater complexity than in the inorganic world, the traditional strategy of biochemists has been "divide and conquer." In this approach, individual enzymes and other biological molecules are purified from cells so that their properties can be studied in isolation. The underlying logic of this method, sometimes described as reductionist, is that we can learn about the whole by studying its individual parts. This painstaking approach, used through most of the twentieth century, teased out chemical reactions and molecular interactions that occur within cells, one by one, gradually revealing to scientists much of what we know in biochemistry today.

Biochem is Beautiful

To the tune of "*Everything is Beautiful*"

Students study molecules with
All of the structures they possess
Proteins, fats and DNAs
There must be a million ways
To evaluate our knowledge for the test

Biochem is beautiful
Our professor says
From the sugar in our cells
To actions of HDLs

And molecules are dutiful
In every way
Substrates for the enzymes are
Converted e-ver-y day

There is no enzyme
That can lower Delta G
They just work all the time
On transition energy

Catalysis provides to cells
Metabolic jump-startin
They all capitalize
By giving rise
To reactions 'tween the carbons

Biochem is beautiful
Saying it with zest
Would be so much easier
If I could just ace the test

Biochem is beautiful
Saying it with zest
Would be so much easier
If I could just ace the test

Recorded by David Simmons
Lyrics by Kevin Ahern

As increasing numbers of biochemical reactions were worked out, biochemists began to see that they were connected together in chains of reactions that we now refer to as metabolic pathways. These metabolic pathways turned out to be remarkably similar between cells across all kingdoms of life. Though there are a few pathways that are unique to certain organisms, many more are the same, or very similar, in organisms as different as bacteria and humans.

It also became clear that metabolic pathways interacted with each other *via* common intermediates or by regulation of one pathway by molecule(s) created by other pathway(s). The similarity of the chemical reactions in all living cells was shown to extend to the common energy currency, ATP, that cells use to power their chemical reactions, as well as the mechanism by which cells make the ATP.

Metabolic pathways trace the transformation of molecules in a cell and represent the work of enzymes, which are proteins. The discovery of the structure of DNA led to understanding of how information in genes was used to direct the synthesis of these proteins. The protein-DNA interactions that determine which genes are copied into RNA at any given time were uncovered and helped explain how cells with the same DNA came to express different proteins. The genetic code, as well as the mechanisms of transcription, translation and regulation of gene expression also turned out to be remarkably similar in cells of all kinds,

leading Nobel laureate Jacob Monod to joke that what was true for *E.coli* was also true for *E.lephant*.

The "one component at a time" approach also helped biochemists understand how cells sense changes in their environment and respond to them. The ability to sense conditions outside the environs of cells extends through all groups of organisms. Even the simplest single-celled organism can follow nutrient gradients to move itself closer to food. Cells in multicellular organisms can detect chemical cues in the blood (nutrients, hormones) or impulses from nerve cells and alter their actions. These cues may trigger changes in metabolism, decisions to divide, die, or become senescent, or the performance of specialized functions (e.g., muscle contraction or enzyme secretion). Thus cells are constantly in a state of flux, adjusting their activities in response to signals from outside themselves as well as their own changing needs.

The power of the "take things apart" analytical approach is evident from the astounding pace of discoveries in biochemistry and molecular biology. The first demonstration that an enzyme was a protein was made only in 1926, and it wasn't till twenty years later that this was sufficient well established that the Nobel Prize was awarded in 1946 for this discovery. Since that time, the methods of biochemistry have uncovered all of the information that you can find in any standard biochemistry textbook, and more.

> *"We are only now beginning to acquire reliable material for welding together the sum total of all that is known into a whole "*
>
> *- Erwin Schrodinger, 1944*

Thousands of enzymes and their substrates have been identified, and hundreds of metabolic pathways traced. The structure of hundreds of proteins is known down to the position of every atom. Following the elucidation of the structure of DNA in 1953, scientists have discovered a dizzying number of facts about how information is stored, used and inherited in cells. Cloned and transgenic animals and gene therapy were a reality in less than 50 years. And the discoveries still keep coming.

Looking Forward

But toward the end of the twentieth century, new methods began to change the face of biochemistry. The launching of the Human Genome Project and the development of faster and cheaper sequencing technologies provided biochemists with entire genome sequences, not only of humans, but of numerous other organisms. Huge databases were set up to deal with the volume of sequence information generated by the various genome projects. Computer programs cataloged and analyzed these sequences, making sense of the enormous quantities of data.

Thank God There's a Video

To the tune of "*Thank God I'm a Country Boy*"

Students sing text in RED

There's a bundle of things a student oughta know
And Ahern's talk isn't really very slow
Learnin' ain't easy / the lectures kinda blow
Thank God there's a video

Well we've gone through the cycles and their enzymes too
Studying the regulation everything is new
I gotta admit that I haven't got a clue
What am I gonna do?

So I got me a note card and bought me a Stryer
Got the enzymes down and the names he requires
I hope that I can muster up a little more desire
Thank God there's a video

Just got up to speed about the NAD
Protons moving through Complex Vee
Electrons dance in the cytochrome C
Gotta hear the MP_3

Fatty acid oxidation makes the acetyl-CoA
Inside the inner matrix of the mitochondri-ay
It's very complicated, I guess I gotta say
Thank God there's a video

So I got me a note card and bought me a Stryer
Got the enzymes down and the names he requires
I hope that I can muster up a little more desire
Thank God there's a video

Replication's kind of easy in a simple kind of way
Copyin' the bases in the plasmid DNAs
Gs goes with Cs and Ts go with As
Thanks to polymerase

And the DNA's a template for the RNA
Helices unwinding at T-A-T-A
Termination happens, then the enzyme goes away
Don't forget the poly-A

So I got me a note card and bought me a Stryer
Got the enzymes down and the names he requires
I think that I can muster up a little more desire
Thank God there's a video

Recorded by David Simmons
Lyrics by Kevin Ahern

Protein coding regions of genomes could be identified and translated “in silico” to deduce the amino acid sequence of the encoded polypeptides. Comparisons could be made between the gene sequences of different organisms. In parallel with the growth of sequence information, more and more protein structures were determined, by using X-ray crystallography and NMR spectroscopy. These structures, too, were deposited in databases to be accessible to all scientists.

The accumulation of vast amounts of sequence and structure information went hand in hand with new and ambitious goals for biochemistry. Modern biotechnology techniques have provided tools for studying biochemistry in entirely new ways. The old ways of dividing and conquering to study individual reactions are now being supplemented by approaches that permit researchers to study cellular biochemistry in its entirety.

These fields of research, which collectively are often referred to as the ‘-omics’ include genomics (study of all the DNA of a cell), proteomics (study of all the proteins of a cell), transcriptomics (study of all the transciption products of a cell), and metabolomics (study of all the metabolic reactions of a cell), among others. As an example, let us consider proteomics. The field of proteomics is concerned with all of the proteins of a cell. Since proteins are the ‘workhorses’ of cells, knowing which ones are being made at any given time provides us with an overview of everything that is happening in the cells under specific conditions.

“It is an old saying, abundantly justified, that where sciences meet there growth occurs. It is true moreover to say that in scientific borderlands not only are facts gathered that [are] often new in kind, but it is in these regions that wholly new concepts arise.”

– Sir Frederick Gowland Hopkins

How is such an analysis performed? First, one extracts all of the proteins from a given cell type (liver, for example). Next, the proteins are separated in a two-step gel method, where the first step resolves proteins based on their charge and the second separates them by mass. The product of this analysis is a single gel (called a 2-D gel) on which all of the proteins have been separated. In the left-right orientation, they differ in their original charge and in the up/down orientation, they differ in their size.

By using such a technique, as many as 6000 cellular proteins can be separated and visualized as spots on a single gel. Robotic techniques allow excision of individual spots and analysis on mass spectrometers to identify every protein present in the original extract.

Why is this useful? There are several ways in which this information can be illuminating. For example, by comparing the proteins in a normal liver cell with those in a cancerous liver cell, one can quickly determine if there are any proteins that are expressed or missing only in the cancer cells. These differences between normal and cancerous cells may provide clues to the mechanisms by which the cancer arose or suggest ways to treat the cancer. Or, the same sort of analysis could be done on cells to find out about the effects of a hormone or drug treatment. Comparison of the proteins found in untreated and treated cells would give a global view of the protein changes resulting from the treatment.

Similar analyses can be performed on the mRNA of cells, employing devices called microarrays. In this case, all the RNAs that are being made at the time that the cell extract is made can be identified by the signals generated when the RNAs hybridize with oligonucleotides complementary to their sequence, that are immobilized in ordered arrays on the surface of a plate. The position and strength of these signals indicates which RNAs are made and in what amounts.

The techniques of proteomics and transcriptomics, together with other "global view" approaches of molecules like lipids, carbohydrates, etc., are allowing biochemists to have, for the first time, a "big picture" view of the activities of cells. While these techniques have already provided valuable new insights, they are still incomplete, as a description of what goes on in cells. This is because they provide us with a snapshot that captures what is happening in cells at the moment that they were disrupted to make the extract. But cells are not static entities. At every moment, they are adapting their activities in response to changing combinations of internal and external conditions. Changes in response to any one signal are modified and influenced by the every other condition, within and outside the cell, and understand these complex systems as an integrated whole is the new holy grail of biochemistry.

"Almost all aspects of life are engineered at the molecular level, and without understanding molecules we can only have a very sketchy understanding of life itself."

— Francis Crick

The aim, then, is to develop models that depict these dynamic interactions within cells, and to understand how such interactions give rise to the properties and behavior that we observe. This is the goal of the emerging field of systems biology that constructs mathematical models and simulations, based on the large data sets generated by transcriptomic, proteomic and other broad-range techniques. Systems biology is truly an interdisciplinary venture, drawing as it does on mathematics and computer science as much as traditional "bench biochemistry". While the original laboratory techniques of biochemistry are by no means obsolete, they will

no longer be the sole tools used to understand what goes on inside of cells.

These newer approaches are already leading to applications that are of tremendous value. Understanding the system level differences between normal and diseased cells can lead to major changes in the way diseases are detected, treated or altogether prevented.

One recent triumph of systems biology has been in an intriguing discovery about how antibiotic drugs work. System level studies of many classes of antibiotics revealed that, regardless of how we think they work to kill bacteria, all of the drugs appear to have a common effect – that of increasing the level of oxidative damage, leading to cell death. This observation suggested that the potency of antibiotics could be enhanced by blocking bacterial responses that protect against oxidation damage. This idea was tested by screening large numbers of compounds for the ability to inhibit a pathway that bacteria use to repair their oxidation-damaged DNA. This screen yielded several compounds, the best of which was able to increase the effectiveness of the drug gentamicin by about a thousand-fold. Such compounds will be of increasing value in a world where antibiotic resistance is on the rise.

Another application of systems biology is in the development of more effective vaccines. Till recently, most vaccines have been developed with little understanding of how exactly they stimulate the immune response. As systems biology approaches give us a better understanding of the changes that vaccines bring about to mediate immunity, it will be possible to identify the patterns that characterize stronger immune responses or adverse reactions to vaccines and even to predict how well particular vaccines

BB Wonderland

To the tune of "*Winter Wonderland*"

Milam Hall - It's 12:30
And Ahern's gettin' wordy

He walks to and fro'
While not talkin' slow
Givin' it to B-B-4-5-0

I was happy when the term got started
Lecture notes and videos galore
MP_3s got added to my iPod
But recitations sometimes were a bore

And exams bit me roughly
When the curve turned out ugly

I don't think it's so
My scores are too low
Slidin' by in B-B-4-5-0

Final-LY there's an examination
On December 9th at 6:00 pm
I'll have my card packed with information
So I don't have to memorize it then

And I'll feel like a smarty
With my jam-packed note-cardy
Just one more to go
And then ho-ho-ho
I'll be done with B-B-4-5-0

Recorded by David Simmons
Lyrics by Kevin Ahern

The tert alcohol was a fool
Turned to ketone in making a fuel
The work is complete
The bond gave up heat
Losing all of its family joules

may work in specific populations or individuals. Similarly, system level studies can help identify which drugs might be most effective, with the fewest side-effects, for a given patient, leading to a new era of personalized medicine.

Related to systems biology, and heavily dependent on it, is synthetic biology, which aims to use the knowledge gained from the former to engineer novel biological systems and pathways. Because the technology now exists to synthesize extremely long pieces of DNA, entire genomes can be made synthetically and used to program cells that they are inserted into. It also allows for the possibility of custom-designing an organism to create particular chemical compounds through artificially assembled pathways.

These methods have already been used to produce the drug artemisinin, which is used to treat malaria. The pathway for making a precursor of artemisinin was created by combining a metabolic pathway from yeast with part of another derived from the plant *Artemisia annua*, the natural source of artemisinin. Similar efforts are underway for anticancer drugs, novel drugs, flavoring compounds, etc. One major goal is to create organisms programmed to make biofuels that could potentially replace petroleum.

The driver's ed student gave holler
For a driving course worth every dollar
When she passed it today
Her friends had to say
It made her some kind of road scholar

The successes of systems and synthetic biology, even in their infancy, promise great advances both in our understanding of living systems and in the applications that arise out of that knowledge. The next fifty years in biological research may well eclipse even the amazing accomplishments of the last. The practice of medicine will be transformed. Regenerative medicine will improve, as a better knowledge of stem cells allows us to use them more effectively to replace cardiac muscle lost in a heart attack, neurons damaged in Parkinson's or Alzheimer's, or even to regrow limbs lost in accidents or war. Treatments for our illnesses can be tailored to be optimal for each individual. Biofuels may bail us out when oil supplies run out and engineered organisms may help clean up a polluted planet. And research on longevity may give us the best gift of all- lives extended long enough to witness these advances and to participate in the creation of a new and better world.

The science researcher would whine
At his data darn near every time
When it comes to a graph
There's no cause to laugh
He wonders where he should draw the line