Book review

The old switcheroo: new tricks revealed in '*Phage Lambda Revisited*'

Adam Paul Arkin[1] and John Doyle[2]

A Genetic Switch (3rd Edition): Phage Lambda Revisited — Mark Ptashne (Cold Spring Harbor Laboratory Press, New York, 2004) ISBN 0-87969-716-4

For years, countless students have spent at least a few classes of their first senior undergraduate biology course caught up in the wonderful story of phage lambda and its role in the development of modern molecular biology and genetics. In 1986, Mark Ptashne released his now classic text on this subject '*A Genetic Switch*' to summarize the state of the field. A third edition, now subtitled '*Phage Lambda Revisited*', has been released this year.

After their too-brief introduction to lambda, students are almost always swept away into more complex systems pushing ever towards the promised land of metazoan biology. The field of molecular biology itself was, in large part, swept away from lambda and from prokaryotes generally in the early eighties, as new technologies made the temptations of understanding organisms more like ourselves too hard to resist for many biologists.

Even Ptashne himself migrated to work in yeast and other eukaryotes. In fact, in the second edition of '*A Genetic Switch*', he added two chapters comparing and contrasting transcriptional regulation in bacteria and eukaryotes. Those chapters are gone from the 3rd edition and have been expanded into a nice little book of their own, '*Genes and Signals*', in which prokaryotic regulation takes up an introductory first chapter. In their place, a chapter has been added to discuss some important new developments in lambda biology.

This return to a purer text, we wish to believe, has more to do with the sense of completeness or harmony in the lambda story than with a further severing of the eukaryotic and microbial research communities. Lambda and its life-cycle is a microcosm of all molecular biology, and the switch itself is a marvel of the reductionist approach — a nearly perfect module of developmental function. During initial infection of a bacterial cell by lambda, if the switch is flipped one way it triggers viral replication and propagation, a process called lysis; flipped another and it mediates integration of the virus into the host chromosome, a process called lysogeny, where it passively replicates with the host. Integration of the lysogen confers immunity to the host initiating proviral latency. Once integrated, the switch keeps the lysogen stable for many generations unless a stress initiates, for example, the 'SOS' system, which flips the switch back to the reproductive phase of the virus.

'*A Genetic Switch*', in all its editions, is an exquisite description of the molecular control of this switch. Ptashne is clear both in his exposition of the key experiments that elucidated the sophisticated molecular mechanisms of switch control and the general principles they imply. Despite the book's exceptional pedagogy and simple style, there is no loss in the rigor of the science. Every assertion about the control of the switch is backed-up by a description of the experiments that proved it. In doing so, the text brings the reader through many key genetic and biochemical techniques and exposes them to the standards by which knowledge is accepted about a biological system.

The clarity and care of '*A Genetic Switch*' and its organization is a paradigm for communicating the structure and behavior of complex regulatory systems such as lambda. Periodic update of a book like this is important as a reminder of the level of our understanding of a system, in this case lambda, and of how we achieved that understanding. This quality has made this text a favorite of systems biologists — scientists interested in the dynamic function of cellular pathways. '*A Genetic Switch*' provides a relatively gentle but thorough introduction to the biologist's view of pathway behavior that is accessible to those just joining the field. Lambda is a perfect example to demonstrate many of the complexities of genetic regulation and how, experimentally, they are approached.

Lambda is a system with a strong developmental phenotype — lysis or lysogeny — governed by interlocking feedback loops and a plethora of other regulatory mechanisms. These include: combinatorial control of divergent promoters (the switch itself); regulated termination and antitermination of transcriptional elongation; complex proteolytic control of regulatory molecules; antisense and convergent transcriptional control of gene expression; multiple start sites for transcription; non-traditional ribosome binding sites; staged assembly of a biological nanostructure (the final viral particle); and now, as reviewed in the new chapter five, long-range DNA looping. These features, not all described in '*A Genetic Switch*', have made lambda a favorite system for computational modeling and analysis. One of us is shamelessly guilty of one such model.

It is notable, however, that modeling and functional genomics played little or no role in any of the key developments of the field. It might be argued that some of the related technologies were not available at the time that the major discoveries in lambda were made. But the material described in the new chapter five was discovered well after the advent of biological modeling methods and microarrays. These late additions to lambda biology came from classical molecular and structural biology, rather than any high-throughput technology or model prediction.

Of the five sections in the new chapter, three represent significant

modifications to the understanding of switch regulation; the other two further elucidate the structural basis of key mechanisms already hinted at in the first edition. The first and perhaps most dramatic modification is the discovery of a whole new level of control of the switch itself. Repressor molecules bound at the left operator O_L can form octamers with repressor bound at the right operator O_R through a DNA looping event, thereby changing, in a fairly significant way, the autoregulation of the promoter P_{RM} (which directs 'maintained' transcription of the repressor gene during lysogeny). Second, the role of differential binding of repressor to the three sites in O_R during the induction phase of the switch is more quantitatively explored. Third, the role of the transcriptional regulator CII in controlling the lysis–lysogeny decision is further clarified.

For the systems biology community one question has to be: how did we miss the added stability conferred by DNA looping? In defense of functional genomics, it might be argued that lambda was enough out of fashion that there wasn't much effort to apply these expensive technologies to the study of the phage. But it is unclear that such experiments would have uncovered these extra biochemical mechanisms. While they almost certainly could give a more comprehensive picture of host/virus gene expression (for example), if population heterogeneity and viral RNA concentration issues could be dealt with, these tools do not really ask the right questions of the system. For example, a global (average) view would likely wash out measures of relative stability of the prophage quite a bit.

For the modelers, a generous answer is they were getting to it. Another generous answer is that the right experiments hadn't been done. The less generous answer is that they can only recapitulate what is already known. We, of course, favor the generous interpretations. Mathematical models ask very detailed question about lambda function, such as: can we really explain with our current understanding the fraction of lysogens as a function of average phage input, or can we explain the extraordinary stability of the lysogen? Modelers were beginning to see problems explaining both simultaneously with the current (pre-looping) understanding of the switch. The elegant experiments by John Little described in the fourth section of Chapter 5 threw a real wrench in the works of the standing models — for reasons too complicated to go into here, they implied that the parameters of models that allowed the lysis–lysogeny data to be explained could not explain the stability of the lysogen. Models provide a formal way to combine assertions about a system, the consistency and implications of which may be checked mathematically and computationally. Inconsistencies discovered through such analysis can both focus attention on areas that need further elucidation and test whether newly discovered mechanisms resolve the inconsistencies (and under which conditions they do so). Looping may provide one of the necessary mechanisms to resolve the conflict.

The point of this digression into systems biology is that reasonable people can disagree on what it means to understand a system. '*A Genetic Switch*' in its first edition was an example of a very satisfactory level of understanding of biological function to the classical biologist; however, we think even the classical biologists were surprised by the new discovery of the looping regulation. In retrospect, the 'satisfactory' understanding communicated in the 2nd edition was not satisfactory. The detection of such discrepancies will only become harder in more complex systems. Something of the formal approaches for building and testing models against data is necessary. This will require that the right data be collected and the right level of modeling applied. This is still an art, but an increasingly important one as the number of mechanistic assertions and the wealth of associated data about a pathway increase. But it seems it is still the precisely designed genetic or biochemical experiment that has the most impact on understanding cellular function and in discriminating between alternative models of behavior rather than data developed from the current high-throughput functional genomic technologies. '*A Genetic Switch*' makes a good case for this point of view.

'*A Genetic Switch*' is wonderful for what it is — a beautiful, concise (only 154 pages) exposition of the major molecular processes involved in lambda's lysis–lysogeny and induction decisions. While it is well and properly scoped, there is room for a more expansive modern text, and certainly more research, on the subject of lambda and other bacteriophage. Lambda and its host, the bacterium *Escherichia coli* are perhaps, individually, the most 'understood' organisms we have. Still there is a great deal we do not know about their interaction and molecular function. We have not sufficiently explored if the mechanisms we have determined adequately explain the gene expression and population dynamics of the phage and its host. We have certainly not fully explored the viral ecology, evolution and competition of lambdoid and other bacteriophages with the same depth and rigor as we have the switch despite compelling early findings and the experimental tractability of the system.

Lambda still stands as perhaps the best system to test theories of how genotype is mapped to phenotype and phenotype to fitness. It is a shame that more researchers haven't taken on this system with modern experimental and computational approaches to test the limits of biological knowledge. The 3rd edition of '*A Genetic Switch*' prompts us to remember the power of this under-utilized model system in discovering general principles of cellular function and behavior. Switch back!

[1]Howard Hughes Medical Institute, Departments of Bioengineering, University of California and Physical Biosciences Division, E.O. Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA. [2]Control and Dynamic Systems, California Institute of Technology, Pasadena, California 91125, USA.