

A Long Walk with René: Order out of Chaos via Avenue Lambda

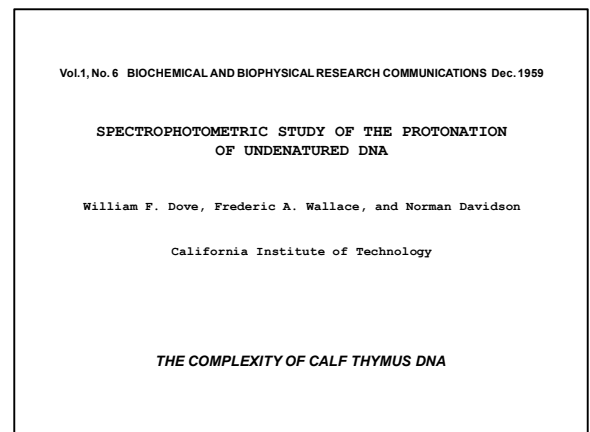
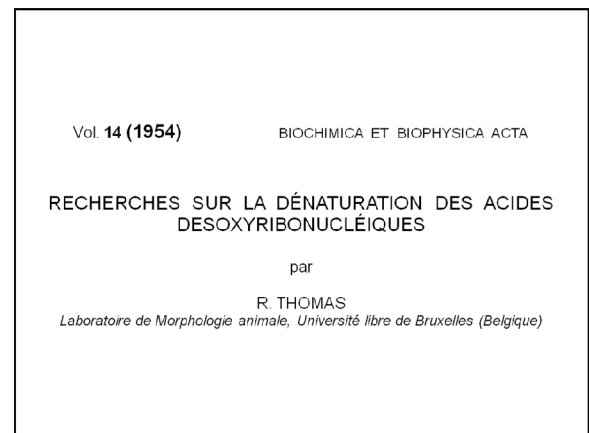
William F. Dove
May 2008

[...(chairman), colleagues, friends and family of René, I am honored and delighted to share this occasion in honoring René Thomas on the occasion of his eightieth birthday.]

I have shared with René Thomas an appreciation for the science of life for five decades. During this long walk, we have encountered concrete shared issues that each of us has addressed in his own way. More recently our communication has been more abstract; we have sent smoke signals between my efforts to understand relationships between normal self-renewal and neoplastic growth in mammals and René's efforts to understand the logical principles of biological regulation including deterministic chaos. Therefore our conversations during this long walk have generated stereoscopic visions, and has led to an appreciation of the evolved wisdom of the biological species whose study we have shared, the bacteriophage lambda. My talk today begins with a **Prologue: Connecting**. It continues with two expository sections entitled **Visions of Regulatory Principles** and **The Wisdom of Lambda**. I shall then conclude with a brief **Epilogue: The Evidence for Our Existence**.

Prologue – Connecting: I come from the State of Wisconsin in the center of the United States. This is a map of Wisconsin highlighting Madison, where the State University of Wisconsin has actively fostered research for more than a century. Green Bay, well known to fanatics of American football, is the home of the Green Bay Packers, a team owned by the people of the State, not by a capitalist. Finally, Brussels, a small town near Green Bay, is a Belgian settlement also more than a century old. Brussels still sends its young women back to Belgium to learn the art of making fine lace and other fabrics. So, though my State is far away from you in Brussels, it shares a commitment to fine textiles, to football, and to research.

My early connection with René Thomas was generated before I entered research in 1958 as a doctoral student in chemistry at Caltech. René's doctoral thesis research, carried out largely in the absence of the detailed knowledge of the structure of DNA, discovered the phenomenon of DNA denaturation. The irreversible transition of DNA from an ordered to a disordered structure was inferred from the shift in its absorbance in the ultraviolet from a hypochromic state to a one closely resembling

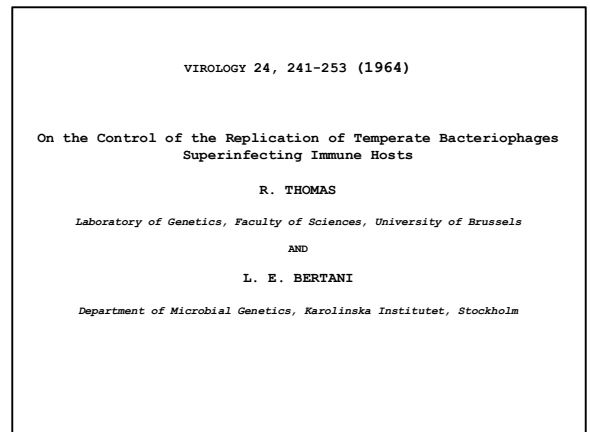
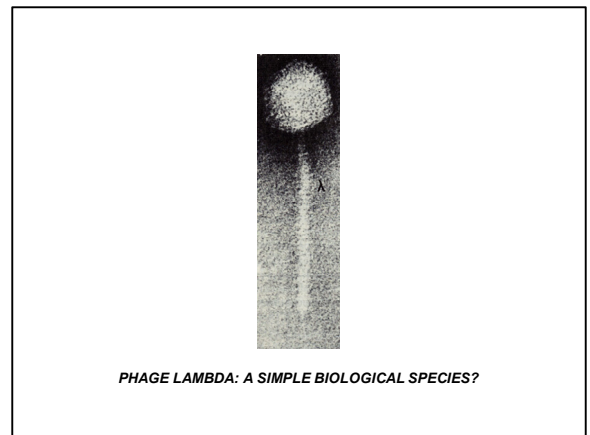
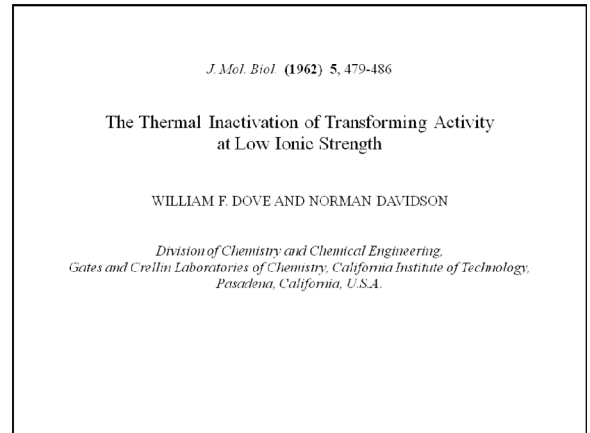


that of the constituent nucleotides. My doctoral thesis began a few years later. Many of the insights that led to my thesis on the physical chemistry of DNA were, in fact, previously seen by Thomas in his thesis. His insights also strongly influenced my Caltech predecessor Matt Meselson in the design of “the most beautiful experiment in biology” – the Meselson-Stahl Experiment. On my part, my thesis research developed an appreciation for entropy-driven hydrophobic bonds and for cooperative weak interactions. These principles of physical chemistry, shared with René even now, have combined with the language of genetics and the biology of phage lambda to generate the multilingual language of our conversations.

My research was carried out with DNA isolated from a common commercial source of the day, the thymus of the calf. Curiously, this leads me to my final introductory point. Once, while visiting René and Inga, we took a trip across the countryside to dine at one of your fine country restaurants. As we were driving along, we went past a scene exactly like this. As we passed, René was telling me with excitement, “I have discovered a solution for deterministic cows.” I blinked, looked at the scenery around us, and thought to myself, “My God, he has figured out what determines the pattern of spots on these Holstein cows.” When I shared my surprise, he explained to me that “cows” is the French way to pronounce what we, in English, call “chaos”. And so, the source of calf thymus DNA was reborn as Chaos in the Belgian countryside. That is why, ever since then, whenever I have seen René talking about chaos, he has been wearing a tie decorated with Belgian cows. More curiously, Wisconsin is known as the Dairy State!

Now, the research paths that I have pursued, and those that René has pursued, have gone from the complex (calf thymus DNA) to the simple (phage lambda) and back. I will illustrate that in the examples I will share with you. Phage lambda, here shown in electron micrograph, is a single biological particle 50% DNA and 50% coat and tail. Its genome contains only 50 genes, perhaps a simpler biological object to understand than a metazoan with 20,000 genes. As you will see, even though phage lambda might appear simple in this photograph, packed in that genome is the outcome of many a battle for survival over eons of evolution. Those of us who have studied lambda in depth have learned a number of deep lessons in biology. Though the detailed language of the lessons may be specific to lambda’s evolutionary trajectory, the laws of logic obeyed by that language are more general.

My first encounter with beautiful experiments from René came in the study of what is called the Thomas-Bertani Effect. I first heard of this effect in a symposium organized by René in Brussels in 1963 when I was a postdoctoral fellow in Cambridge.



Thomas described this within the context of the dominant Jacob-Monod model for regulation, including the regulation of phage lambda by its repressor, the cI protein. What Thomas and Elizabeth Bertani showed is that, if one infects bacteria carrying a repressed phage by a related lambdoid phage insensitive to the lambda repressor, the infecting phage would replicate itself, but it did not help the resident repressed phage to replicate. It seemed that the cI repressor blocked replication more deeply than simply by preventing the synthesis of lambda's replication machinery. The phenomenon was called "replication inhibition" or ri for short. My laboratory studied this phenomenon in depth, isolating mutants that identified the origin of replication and others that escaped replication inhibition - replication inhibition constitutive or ri^c . The ri^c mutation creates a secondary transcription site near the replication origin. These studies demonstrated that lambda has developed two layers of protection from autonomous replication when it takes the alternative pathway of establishing a stable lysogenic relationship with its host. One layer is that given by the CI repressor to prevent the synthesis of the replication proteins, the other layer is the effect of that repressor on preventing transcription in the region of the origin of replication. This is an example of the principle first enunciated by the embryologist Spemann - "double assurance." The importance of double assurance was illustrated by experiments done with Masako Ohashi, in which we showed that when bacteria were infected by wildtype lambda under conditions where lysogenization was frequent, that the newly formed lysogens would grow and form microcolonies in a few hours. By contrast, when bacteria were infected by the lambda ri^c mutant the infected complexes failed to grow. They did not go through lytic development but took a nonproductive pathway involving filament formation. This little experiment involving the watching the biology of lambda unfold over time, under the microscope, echoes the style of science that René has enjoyed early in his training under Jean Brachet in Brussels. I shall revisit this style of science at the end of my talk today.

In 1966 Thomas and I published simultaneously our studies on the control of development in phage lambda. René's work focused on the important role of the positive regulator gene N in controlling a cascade of viral functions. Mine focused on the importance of replication and gene Q in controlling late gene expression. I showed that both phage replication and the positive regulator gene Q controlled late development, giving rise to a non-linear temporal trajectory to phage growth.

The third vision that René and I have shared during our long walk involves interacting with the next generation of scientists in our fields. The phage course at Cold Spring Harbor, was a series of interactive workshops begun by Max Delbrück just after World War II and continued for 25 years until 1970 when René

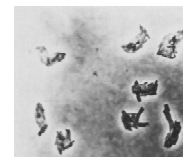
JAPAN J. GENETICS VOL. 44, SUPPL. 1: 11-22 (1969)

REPLICATOR ACTIVATION IN LAMBDA

WILLIAM F. DOVE, ERMILE HARGROVE, MASAKO OHASHI,
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"REDUNDANT" NEGATIVE REGULATION



Lambda wildtype
lysogenization



Lambda ci^+ rIF
lethal lysogenization

Genetics 131:515-518 (1992)

Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics
Edited by James F. Crow and William F. Dove

**Molecular Genetics Under an Embryologist's Microscope:
JEAN BRACHET, 1909-1988**

René Thomas

J. Mol. Biol. (1966) 22, 79-95

Control of Development in Temperate Bacteriophages

I. Induction of Prophage Genes following Hetero-immune Super-infection

R. Thomas

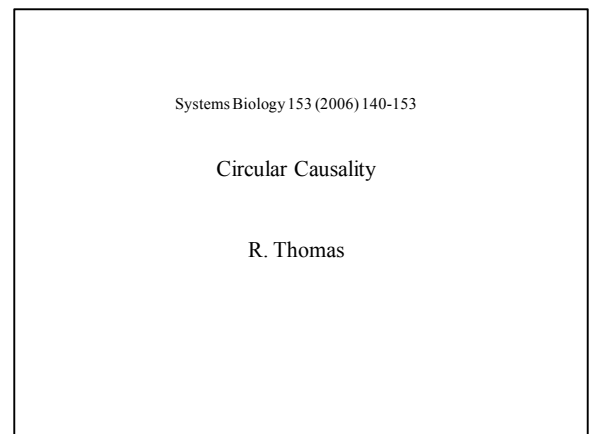
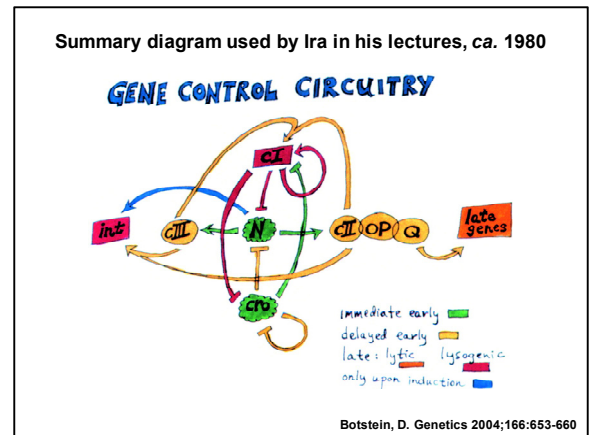
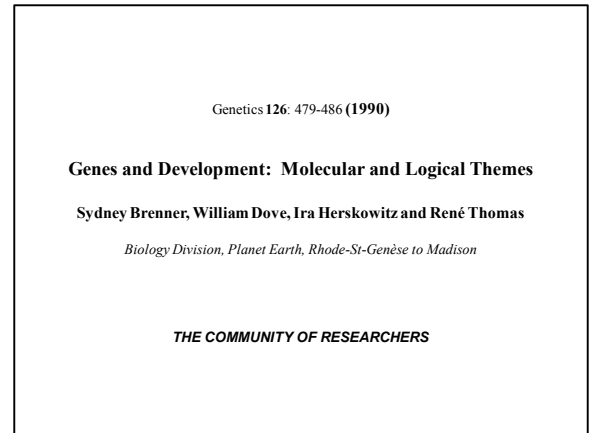
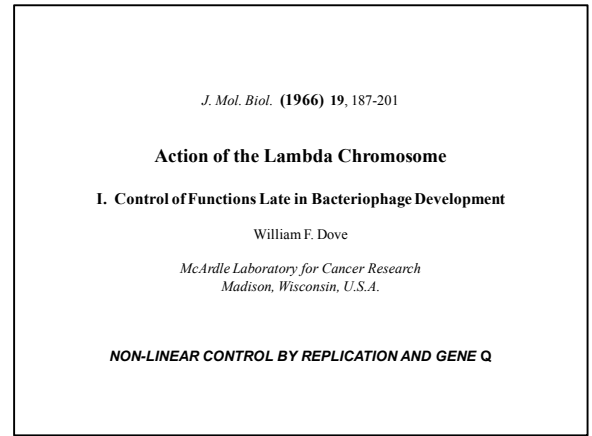
Laboratoire de Génétique, Faculté des Sciences
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and I taught the final course assisted by Arianne Toussaint and John Lehman from our labs. In that context we were able to train a cohort of people who later ended up as leaders in the field of molecular genetics in the United States and elsewhere. A very satisfying experience in the community of science was generated at this time a publication edited by Alfred Hershey, The Bacteriophage Lambda, where both René and I published synoptic visions of the issues that we had been independently studying. This synoptic effort for the community was echoed 20 years later when René and I joined others in the field of regulatory and developmental genetics to honor François Jacob on the occasion of his 70th *anniversaire*.

From the visions that we have shared along the way comes a recognition of the wisdom of lambda, “This simple virus”. This circuit, summarized by Ira Herskowitz several decades ago, is Byzantine. Is it chaotic? How did it evolve? I see this as a record of the series of challenges for survival that the 20th century version of phage lambda has overcome over its evolutionary history. As Sewall Wright reminded us, “There is no formula for serendipity.” But this diagram contains universal lessons.

First, it demonstrates experimentally the properties of positive and negative circuits, seeding the theoretical research that René, Marcelle Kaufman, and colleagues have since carried out in Brussels to establish the necessity and sufficiency of each of these two classes of circuit for particular biological behaviors. A particular manifestation of this formal analysis is that of “circular causation.” This notion changes the standard mode of thinking in the field involving linear biochemical pathways of material transfer from A to B to C to Z. Instead, there are situations of information transfer in which A causes B causes C causes A. An example of such a circle is being encountered in mammalian biology: is the relationship between gene silencing by polycomb chromatin complexes and that by DNA cytosine methylation. It seems that one can promote cytosine methylation by entering this circuit through polycomb silencing and, *visa versa*, one can promote polycomb silencing by methylating cytosines. This example of circular causation creates redundant entry points into the stable silencing of genes, giving robustness to epigenetic transitions such as X-chromosome inactivation.

A second message from the wisdom of lambda that we share is that of double assurance, as mentioned in my discussion of the Thomas-Bertani Effect. In our current research on intestinal neoplasia, we have found that the transition from normal self-renewal in the intestinal epithelium towards neoplastic growth requires more than one event. It requires hits to both alleles at the *Apc* locus. Furthermore, the transition is enhanced by clonal cooperation between independent neighboring clones, each independently transformed, giving rise to a polyclonal early



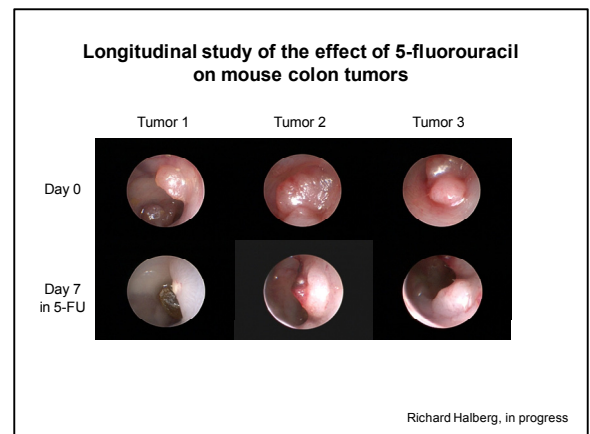
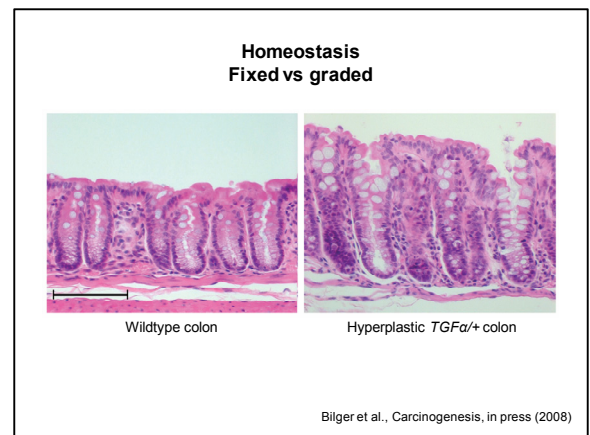
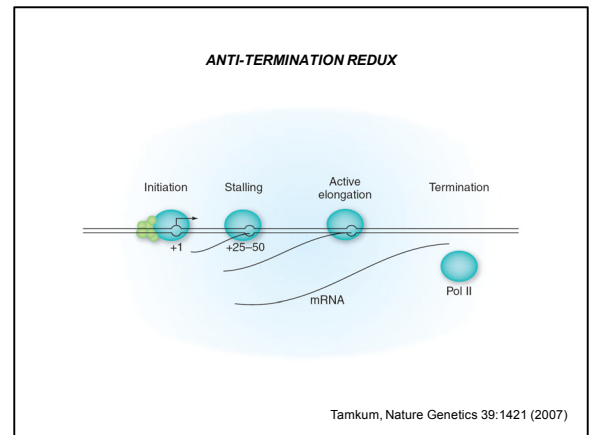
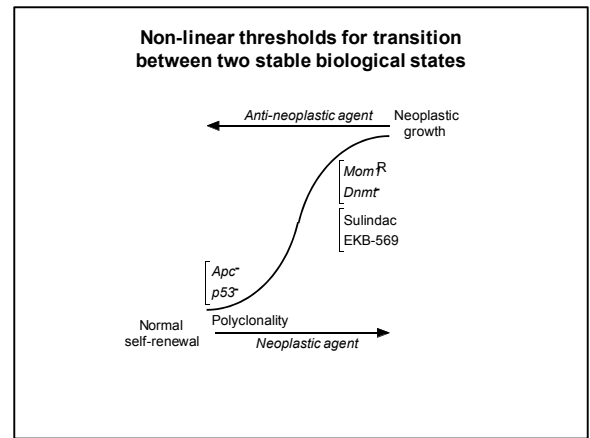
adenoma. On the other side of the biological equation, starting with the neoplastic state, we find cooperation between different factors, for example, between two different genetic modifiers, *Mom1*, and DNA methylase, or between the non-steroidal anti-inflammatory agent, sulindac, and an epidermal growth factor inhibitor EKB-569. Nonlinearity is a key lesson for understanding the biological transitions between one mode of growth and another.

A remarkable rebirth of one of the regulatory principles taught by lambda is the recent discovery of the major importance of transcriptional stalling on developmental genes in metazoans, followed by anti-termination. The positive regulators of lambda that René and I have initially observed, N and Q, are now known to act as anti-terminators of short transcripts, not as transcription initiation factors. Again, this illustrates the principle of non-linearity by combining two independent regulatory mechanisms into the decision whether or not to express a gene.

Homeostasis, an important topic for the theoretical research in Brussels, has appeared for my laboratory in studying the normal self-renewal of the intestinal epithelium. We find that the homeostasis is graded: one can identify different steady states created by the balance between the gain and loss of cells in the normal self-renewal process, and its perturbation by the imposition of added growth factors. On the left it the homeostasis observed under normal conditions. On the right, the imposition of transforming growth factor alpha leads to a new steady state of normal growth. It does not enhance the neoplastic transformation unless the process of cell loss, apoptosis, is reduced. I call this graded homeostasis: the homeostatic mechanisms must be able to respond to more than one steady state.

The final wisdom learned from lambda is the advantage created by the fact that it has been studied in depth by a large number of people. We cannot hope to carry out such study on more than a few of the millions of species in the biological universe. If one wants to understand an organism deeply, must be studied deeply, from the vantage point of many independent investigators.

Our research on intestinal cancer aims to involve this principle in two different ways. First, we have developed ways to study individual tumors longitudinally, just as three decades ago we studied microcolonies of bacteria being lysogenized by wildtype and mutant lambda. These studies are enabled by the capacity for endoscopy in both the mouse and particularly the laboratory rat, to observe and biopsy tumors as a function of time, and to acquire individual molecular and biological phenotypes from these tumors. One can then study tumors that have different fates and ask, what is the molecular phenotype of tumors that



will respond to 5FU, versus the molecular phenotype of tumors that are resistant to 5FU?

That is the end of my story of Visions seen and Lessons learned during my long walk with René Thomas that continues to this day. In my Epilogue, I comment on the response I would give as a contemporary biologist to the conjecture offered four centuries ago by another René, Descartes, when he pronounced “Cogito ergo sum.” I have talked about is the evolution of my own research from lambda to Physarum, to the mouse and rat, and to the human. So, my research, just like lambda’s regulatory circuits, has evolved then along a long evolutionary trail. And what one learns for life on earth: **It evolves, therefore it exists.**



We can say the same for we earthlings who pursue research. René has been able to continually climb this mountain decade after decade, giving us a longer, more global view of biological regulation. Though much of this path has been solitary, René has shown abundantly the pleasure of taking this journey in the company of others, especially Inga. **I can say in closing that I am very grateful for the privilege of having accompanied René Thomas over a long period of time, and I look forward to continuing this long walk.**

