Is Molecular Genetics Becoming Less Reductionistic?

Notes from recent case studies on mapping *C. elegans* and the discovery of microRNA

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Outline

Introduction

The Central Dogma

Brenner's Worm Program (*C. elegans*)

The Discovery and Exploration of miRNA

Back to the Central Dogma: General Conclusions

Principal Thesis – slide 1

Molecular Biology is entering an age of modest ‘mechanistic holism’ in which genes and other determinants of cellular and organismal properties and behaviors ‘act’ in ways that are co-determined by integrated networks of interacting molecules and, at least in eukaryotes, cells.

Principal Thesis – slide 2

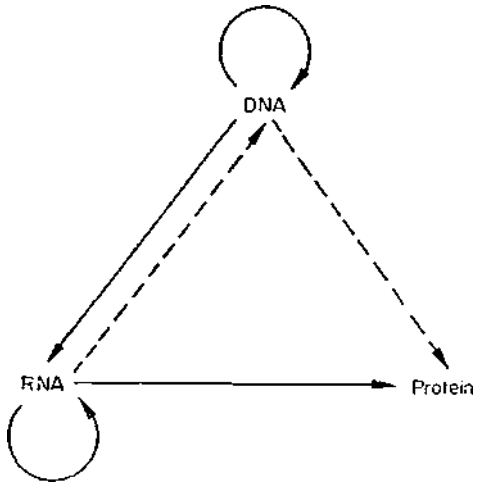
More specifically: what genes (and other determinants of cellular and organismal properties and behaviors) ‘do’ in a given cell or organism is co-determined by integrated networks of interacting molecules and cells where some of the causally effective properties of networks and cells cannot be analyzed at lower levels, at least not straightforwardly.

Sub-Thesis

A substantial part of molecular biology is natural history; it is largely descriptive rather than being based on hypothesis testing. Molecular biology, however, is strongly experimental; as the natural history of interacting biomolecules, it must rely on difficult, often highly interventive experimental protocols that often involve a great deal of hypothesis testing, deployed to establish the reliability of experimental outcomes and sound interpretations of the experiments.

The Central Dogma

Crick's Informal Diagram of the Central Dogma



Watson's Version of the Central Dogma in *The Molecular Biology of the Gene*

(transcription) translation)

dna -------> rna -----> protein

(duplication)

Brenner's C. elegans Project 1

It seems to me that, both in development and in the nervous-system, one of the serious problems is our inability to define unitary steps of any given process. Molecular biology succeeded in its analysis of genetic mechanisms partly because geneticists had generated the idea of one gene-one enzyme, and the apparently complicated expressions of genes in terms of eye color, wing length and so on could be reduced to simple units which were capable of being analyzed… In the study of development and the nervous system, there is nothing approaching these ideas at the present time… As an even more long term project, I would like to explore the possibilities of studying the development of the nervous system using insects.

[Letter to Max Perutz, 5 June, 1963]

Brenner's C. elegans Project 2

The *new major problem* in molecular biology is the genetics and biochemistry of control mechanisms in cellular development. We propose to start work in this field and gradually make it the Division's main research.

In the first place, control mechanisms can be studied most easily in micro-organisms, and this work has already begun. In addition we should like to start exploratory work on one or two model systems. We have in mind small metazoa, chosen because they would be suitable for rapid genetic and biochemical analysis.

[From a grant proposal to the Medical Research Council, October, 1963]

Brenner's C. elegans Project 3

We think we have a good candidate in the form of a small nematode worm, *Caenorhabditis briggsiae,* which has the following properties. It is a self-fertilizing hermaphrodite, and sexual propagation is therefore independent of population size. Males are also found (0.1%), which can fertilize the hermaphrodites, allowing stocks to be constructed by genetic crosses. Each worm lays up to 200 eggs which hatch in buffer in twelve hours, producing larvae 80μ, in length. These larvae grow to a length *of* 1 mm in three and a half days, and reach sexual maturity. However, there is no increase in cell number, only in cell mass. The number of nuclei becomes constant at a late stage of development, and divisions occur only in the germ line.

[From the proposal to the MRC, October, 1963]

Methodological lessons from the early phases of the worm project

- Exploratory experiments are needed to detect novel phenomena and patterns of phenomena, and to develop appropriate technologies for studying them, i.e., for molecular natural history
- The detailed characterization of novel molecular phenomena and patterns of phenomena is a key step in explaining those phenomena because the explananda constrain suitable explanatory hypotheses.
- To yield explanatory hypotheses, exploratory experimentation must be connected to hypotheses about detailed mechanisms capable of explaining the phenomena and to testing of those hypotheses.

Brenner on Genetic Programs in 1998

…[L]iving systems… are totally unlike all other natural complex systems, in that they carry an internal description of themselves written in their genes. It is this description which is passed on from generation to generation and from which the organism is 'computed'. If we compare this to the weather, for example, we find that there is no internal description of the weather that we can separate physically from the weather itself. For the weather we need the physics of matter and energy, but the existence of DNA implies something new; it is the physics of information, that is, computation. [Continued next slide.]

…[Schrödinger] was clear that the genetic material contained a programme for the development of the organism, but he thought that the genes also contained the means for its execution. They do not contain the means, but, rather, a *description* of the means for execution. This was… the distinction made by John von Neumann in his theory of self-reproducing machines... The means to translate the instruction tape is obtained from the parent machine and is used to read the description of the means and so install the means in the daughter machine. In biological systems, the egg has the means to read the genes, and the new organism makes new eggs. Thus, in addition to DNA, there is a physical continuity of the reading machinery over the total course of biological evolution, but the informational continuity is preserved in the genes [p. 107].

S. Brenner, 1998, 'Biological computation'. In G.R. Bock and Goode, J.A. (eds.), *The Limits of Reductionism in Biology*, John Wiley, 106-111.

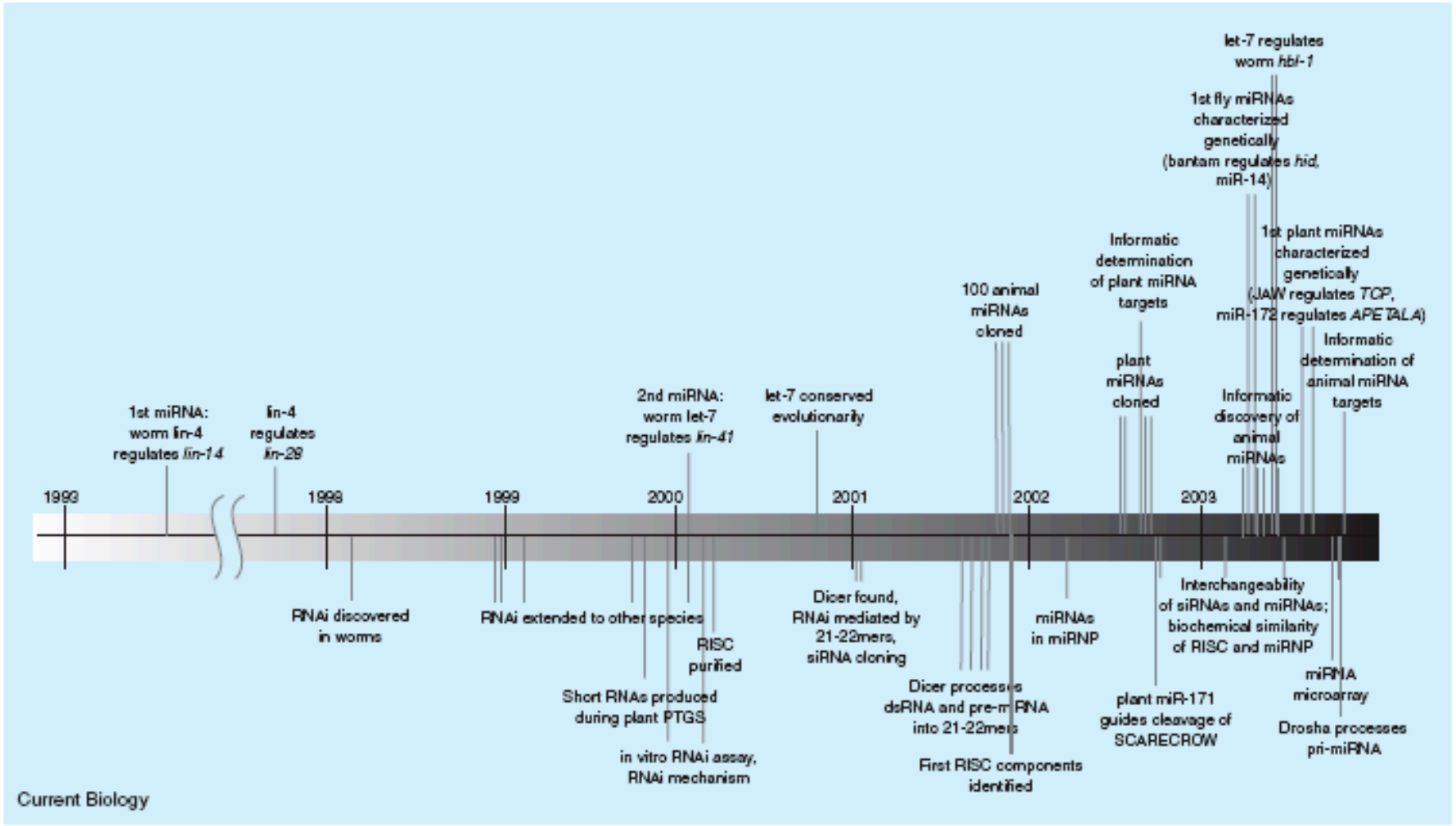


Figure 1.
A timeline of key events in miRNA and RNAi research. The events are loosely divided into miRNA/genetic studies (above) and RNAi/biochemical studies (below). Bear in mind that this overview is not intended to be an exhaustive summary of all such studies, especially those in the RNAi field.

From E.C Lai., 2003, 'microRNAs: Runts of the Genome Assert Themselves', *Current Biology*, 13: R925–R936.

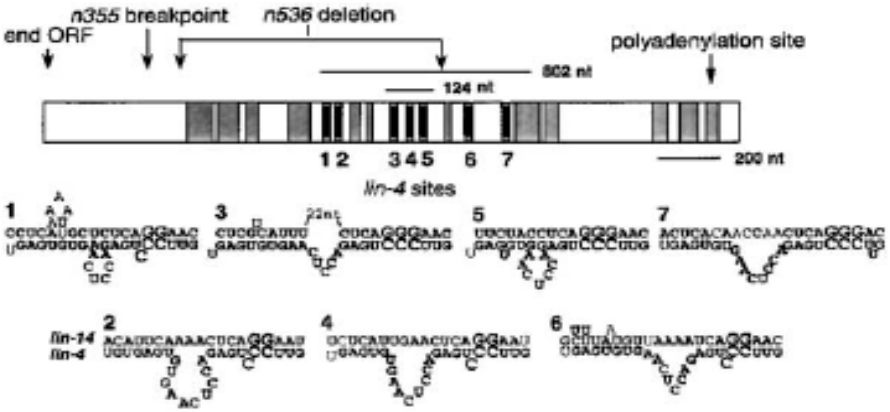


Figure 5. Conserved Sequences in the *lin-14* 3′UTR Are Complementary to the *lin-4* RNAs

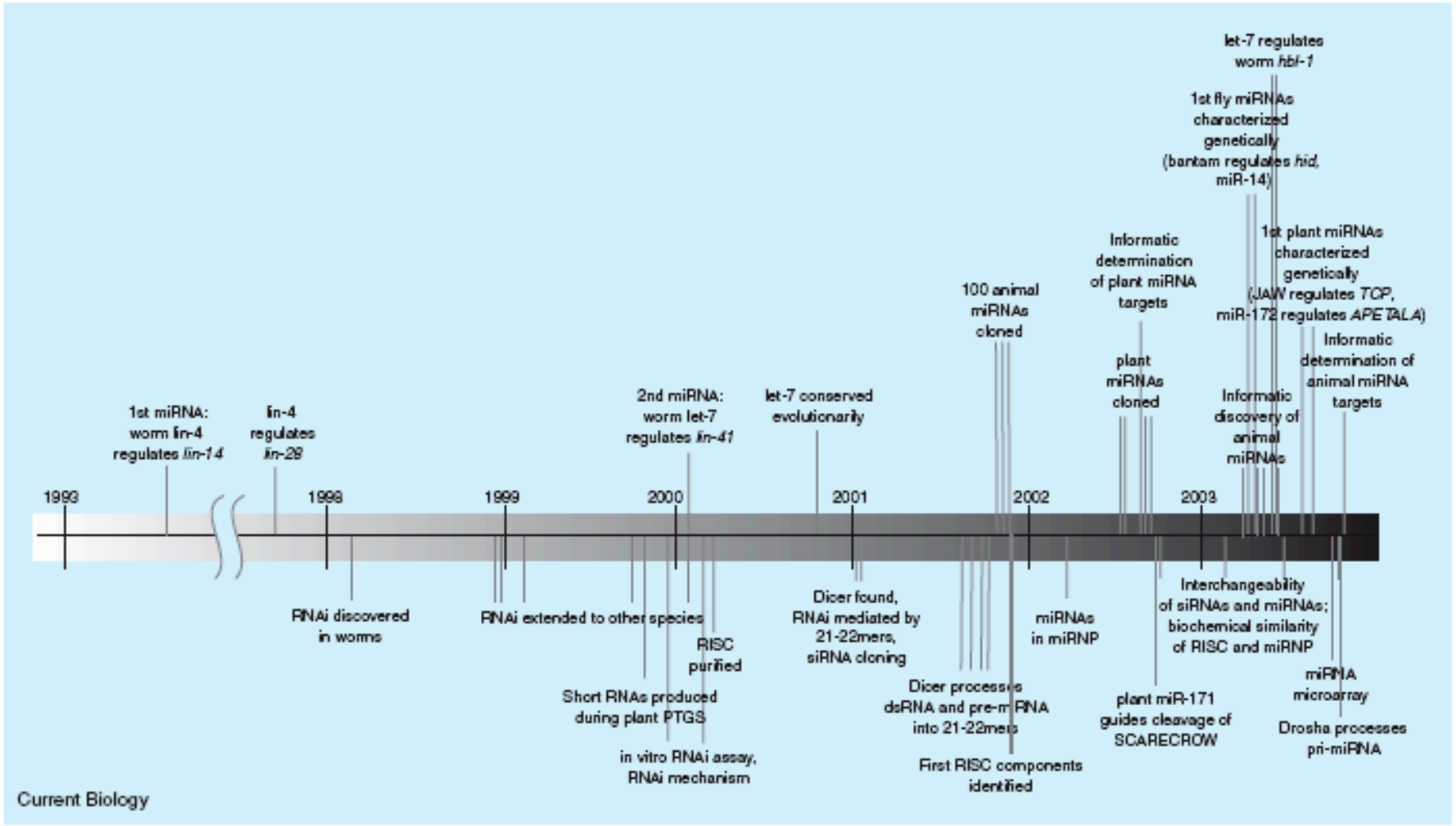
(Top) A representation of regions in the C. elegans 3′UTR that are conserved in C. briggsae. Shown in gray are regions of 10 nt or more that are conserved exactly. Shown in black overlaid on gray are conserved regions that are complementary to the *lin-4* RNAs. These potential *lin-4*-binding sites are numbered to correspond to those shown in detail below. The deletion breakpoints and polyadenylation site for the C. elegans sequence are labeled above the line. The region of the *lin-14* 3′UTR assayed in pC10L14/802 and pC10L14/124 is also shown. (Bottom) Predicted *lin-4–lin-14* RNA duplexes in the 3′UTR of a *lin-14* mRNA. The stacked G::C base pairs in the core element and the potential *lin-4* bulged C at RNA duplexes 1, 2, 4, and 6 are shown in large type. *lin-4* and *lin-14* nucleotide positions that are conserved between C. elegans and C. briggsae are indicated in bold type. Only the 5′-most nucleotides of the *lin-4* sequence are shown.

From: Wightman B.C., Ha, I. and Ruvkun, G.B., 1993, 'Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans', *Cell*, 75: 855-562.

Ruvkun on the Failure to Obtain Decisive Tests of the Mechanism for *lin-4* Regulation of *lin-14*

"Ruvkun continues to argue that elegance in molecular genetics is aesthetically pleasing, but scientifically overrated."

[G.B. Ruvkun, C.C. Wightman, and I. Ha, 'The 20 years it took to recognize the importance of tiny RNAs', *Cell*, S116 (2004): S93-S96, at p. S94]



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Steps in the biogenesis and processing of miRNAs from the initial transcript (pri-miRNA) of an miRNA gene. Processing in the nucleus produces the ~70 nt pre-miRNA hairpins and signals prepares the resulting pre-miRNA to the cytoplasm. There it enters into a complex with Dicer, which cuts out the miRNA and enters into the RISC (RNA-Induced Silencing Complex), which uses the miRNA's sequence similarity to its target to find and bind to the target and process it according to its location on the target molecule and local biochemical circumstances.

From E.C. Lai, 'microRNAs: Runts of the genome assert themselves', *Current Biology*, 13 (2003): R925–R936.

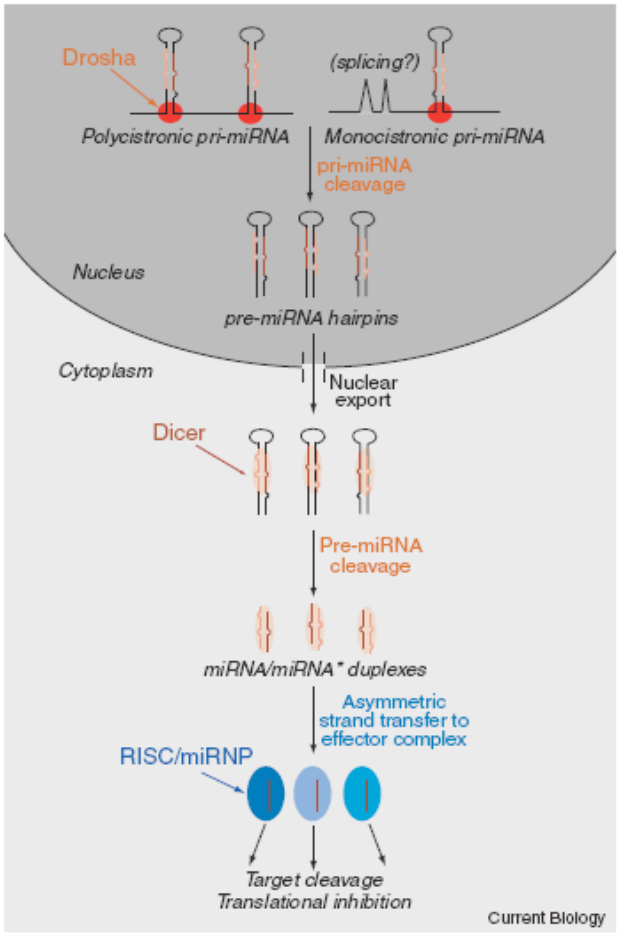

Figure 3.
Biogenesis of miRNAs involves stepwise processing by the RNase III enzymes Drosha and Dicer (red ovals). Most miRNAs are likely initially transcribed as longer primary-miRNA (pri-miRNA) transcripts containing one or more miRNAs; some of these may even be spliced (denoted by 'splicing?'). Nuclear Drosha cleaves pri-miRNAs to release the pre-miRNA as a 70 nt hairpin. These are exported to the cytoplasm, where they are then processed by cytoplasmic Dicer to give a ~22 nt duplex RNA with 2 nt 3′ overhangs. Only one of the two strands is predominantly transferred to the RISC/miRNP effector complex, which mediates target regulation. Although effector complexes contain a common protein core, there may be functional varieties of effectors, which are symbolized by various shades of blue.

Two modes of miRNA Interaction with mRNAs

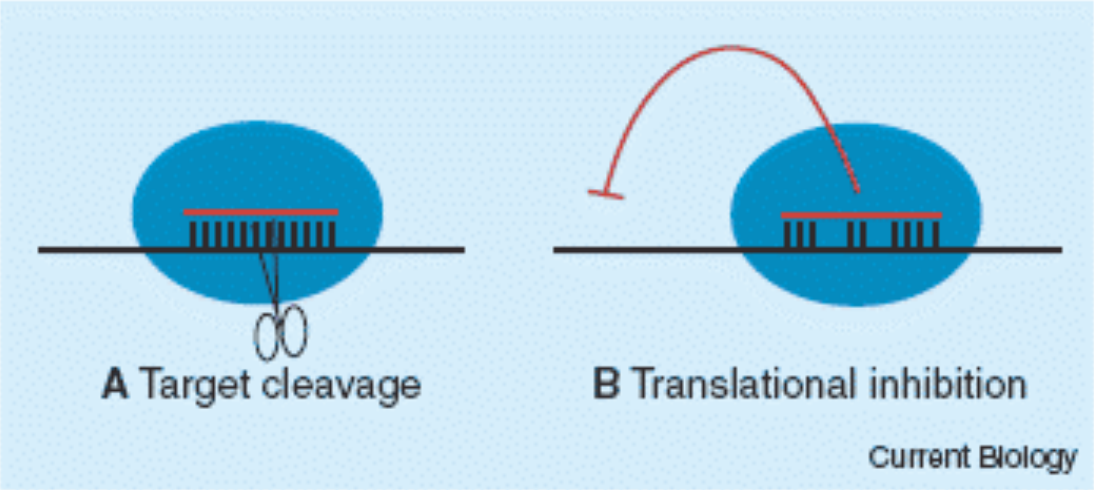

Figure 2.
Two major functional outputs of miRNA-mediated regulation. The miRNA (red line) is associated with a regulatory protein complex (blue oval) that recognizes complementary mRNA targets (black line). Perfectly complementary targets are subject to cleavage (A, scissors) and subsequent degradation, while less-complementary targets are subject to translational inhibition (B, red curve). Note that the regulatory output may depend on other factors (see text for details) and that other regulatory consequences are not yet ruled out.

TABLE 1. *Some crystallins and their protein counterparts.*

αA-Crystallin	(vertebrates) small heat shock protein homologue
αB-Crystallin	small heat shock protein
ϵ-Crystallin	(ducks, crocodiles) lactate dehydrogenase B_4
τ-Crystallin	(turtles, *Mola mola* fish) α-enolase
δ1-Crystallin	(birds, reptiles) argininosuccinate lyase homologue
δ2-Crystallin	argininosuccinate lyase
ζ-Crystallin	(guinea pig, camel, llama) quinine oxidoreductase
π-Crystallin	(gecko) glyceraldehyde-3-phosphate dehydrogenase
η-Crystallin	(elephant shrew) retinaldehyde dehydrogenase
Ω/L-Crystallin	(cephalopods, scallop) aldehyde dehydrogenase homologue
S-Crystallins	(cephalopods) glutathione S-transferase homologues
J3-Crystallin	(cubomedusan jellyfish) saposin homologue

From J. Piatigorsky, 'Gene Sharing, Lens Crystallins and Speculations on an Eye/Ear Evolutionary Relationship', *Integrative and Comparative Biology*, 43 (2003): 492-499.

My Pathways to this Thesis

- Interactions among developmental biology, evolutionary biology and genetics
- Exploratory experimentation in biology
- Molecular biology as natural history
- Combining exploratory and classificatory methodologies with hypothesis testing
- Genetics has been hoist on its own petard: Proper analysis of gene action and its consequences proceeds through interactions in integrated networks
- Epigenetics and miRNAs exemplify these complexities and the holism that results

Brenner on Limits of the Central Dogma

Suppose there are no completely disjoint sets of genes but that most genes participate in all, or many, developmental processes. These, we assume are structured in two layers; one set of *kernel* processes which are inaccurate and another set of *refinement* processes which can reduce and compensate for the unreliability of the first… [B]iological processes must be intrinsically noisy. Thus it is not possible… for a cell reproducibly to synthesise a set of exact numbers of molecules of one protein… Such systems can find an optimum but do not need to take particular account of any intricate internal structure in achieving this end; they have distinct advantages for evolution. Thus, one begins with a messy, inaccurate 'sort of fly' which is then progressively refined into a fly. Of course, in this process many changes will have 'unpredictable' consequences, but, unlike computer programming, natural selection is cheap and has plenty of time to work (pp. 6-7). [continued next slide]

Brenner on Limits of the Central Dogma 2

> From these considerations and others based on our own experimental work, I am drawn inexorably to the conclusion that it is unlikely that we will easily find general principles of wide application to the developmental and other problems of higher organisms. It seems that answers to questions about the relation between genomes and complex organisms will come from the detailed structure and expression of individual genes and from an insight of how their products participate in the biochemical and cellular processes underlying development (p. 7).

S. Brenner 'Genes and development'. In C.W. Lloyd and D.A. Rees, (eds.), *Cellular Controls in Differentiation*, Harcourt Brace Johanovich, 1981, 3-7.

Brenner on Limits of the Central Dogma 3

…Not only is the cell the only physical locus for gene action but it is the correct level of abstraction to construct a framework for understanding functions… [We should seek to construct] a map in many dimensions [which] is at once a map of the cells in the organism onto which are projected the map of instantiations, as well as a map of the molecules in the cell… [and] also a temporal map connecting cells with their predecessors and successors in development. By studying how such cells are connected with their homologues in different organisms we can see how these maps are layered in evolutionary space and what has been added to or removed from any particular subsystem as we move up and down on the evolutionary scale. [Such a map has the potential to yield] a predictive system, and, in the future, a system that we could use for the synthesis of new cell types and new organisms.

Sydney Brenner, 'Nature's Gift to Science (Nobel Lecture)', *ChemBioChem*, 4 (2003): 683-687, at p. 686.