A Life in Science

Richard J. Roberts
New England Biolabs

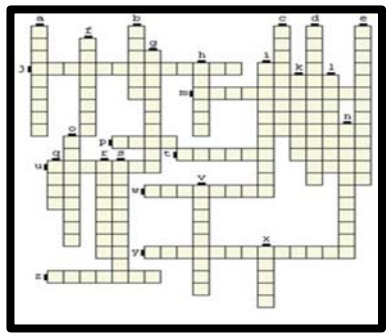
Okinawa Institute of Science and Technology

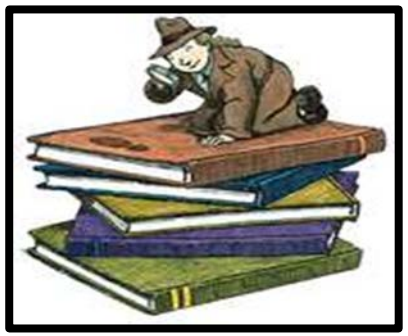
September 6th, 2016

Born in 1943 in Derby, England

Junior School – 1950-1954

St. Stephen's School, Bath





Bath, England

At age 11, I wanted to become a detective.

My parents supported my curiosity and love of puzzles.

My father helped build a chemistry lab in the kitchen where I learned to love fireworks and explosives.

City of Bath Boy's School 1954-62

I increased my love for mathematics. I learned to play the violin and joined the school orchestra.

Being close to the limestone escarpment of the Mendips (Cheddar Gorge) I became interested in caving.

I also discovered jazz for which Bath became quite famous.

Sheffield University 1962 – 1965 - 1968

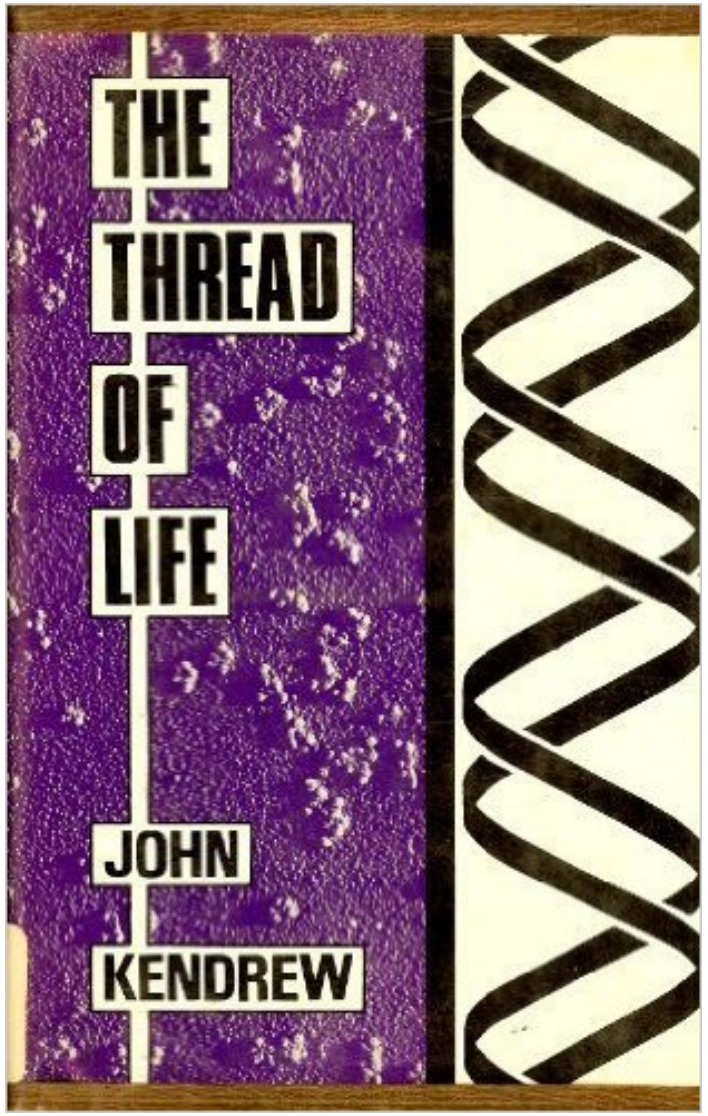
This interest in chemistry plus a fascination with games and puzzles led me to pursue a career in research.

It was a chance to be a detective and solve chemical puzzles in the world of science.

I got my B.Sc. In 1965 and stayed to do graduate work with Professor W.D. Ollis. He emphasized the use of problem-solving to make learning fun, rather than a chore.

Kazu Kurosawa

My “real” mentor
in Sheffield



Post-Doc at Harvard 1969 - 1972

Molecular Biology

Sequenced $tRNA^{Gly}$ in the laboratory of Jack Strominger

Nobel Prize in Chemistry, 1980

Walter Gilbert
Fred Sanger

"For their contributions concerning the determination of base sequences in nucleic acids"

DNA SEQUENCING

In 1969 I visited Fred's lab to learn RNA sequencing

Cold Spring Harbor Laboratory 1972-1992

In 1972, after a 10-minute interview, James Watson offered me a position at Cold Spring Harbor Laboratory. He wanted me to sequence the DNA of the virus SV40.

RESTRICTION ENZYMES

At CSHL I started investigating the enzyme Endonuclease R, which I heard about at Harvard during a lecture by Dan Nathans.

The enzyme cut DNA into specific pieces. I thought that if there were more of these enzymes, I could cut DNA into manageable-sized fragments and use them to sequence DNA. Soon my lab had a whole collection of restriction enzymes.

During the 70's and early 80's, about 75 out of the 100 known Type II enzymes were isolated in my laboratory at Cold Spring Harbor.

Werner Arber
Hamilton Smith
Dan Nathans

Nobel Prize in Physiology or Medicine 1978

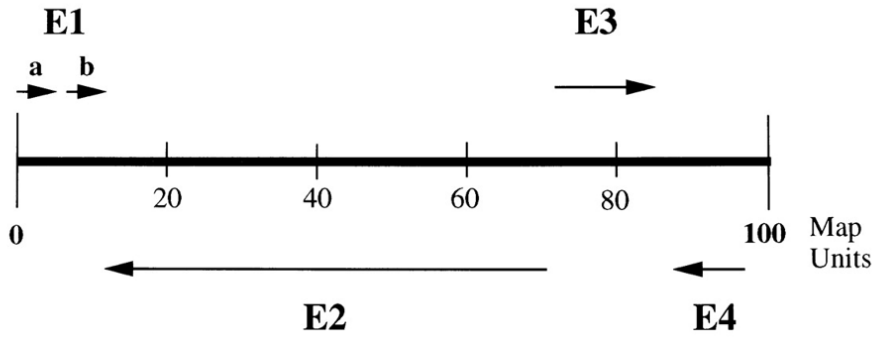
"For the discovery of restriction enzymes and their applications to problems of molecular genetics"

Cold Spring Harbor Laboratory

Some of these restriction enzymes were used to map adenovirus-2 DNA, a project in which Phil Sharp, in Joe Sambrook's lab, was also involved.

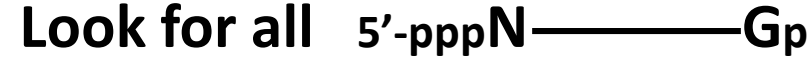
In 1974, my post-doc Richard Gelinas, started working with adenovirus-2 mRNA. We thought we could identify the DNA promoter regions by sequencing the 5' end of all the mRNAs and mapping them to the DNA. The promoter would be upstream of the 5' end of the mRNA.

Adenovirus-2 transcription in 1974

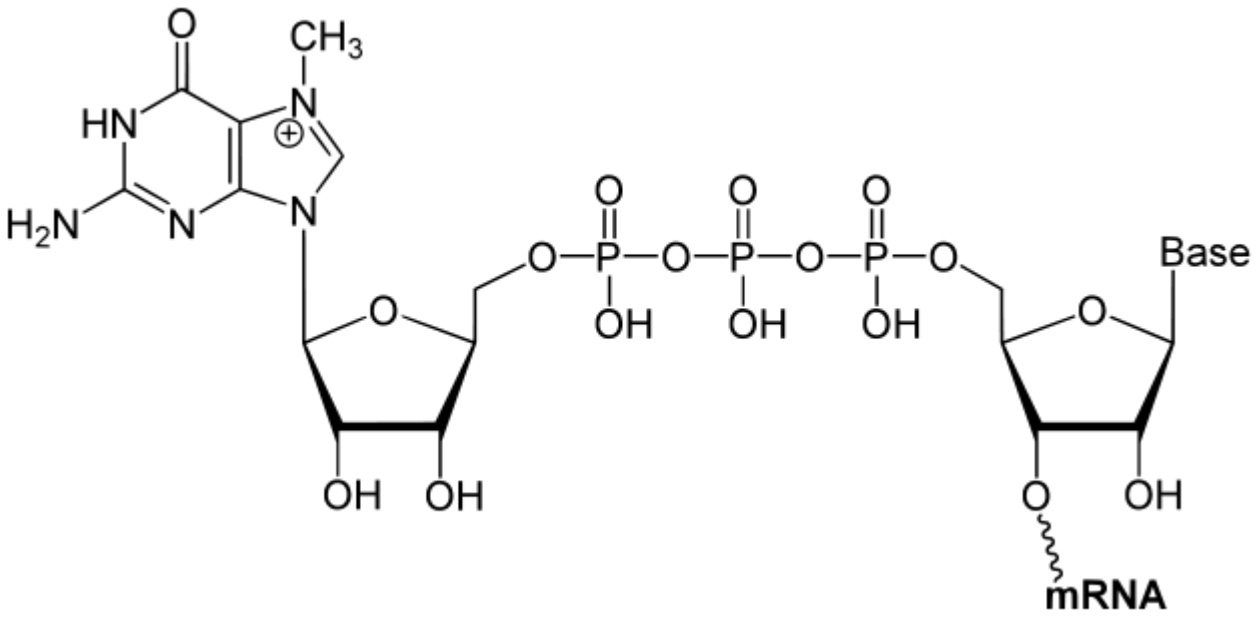


Do eukaryotic promoters look like prokaryotic promoters?

mRNA

Look for all 

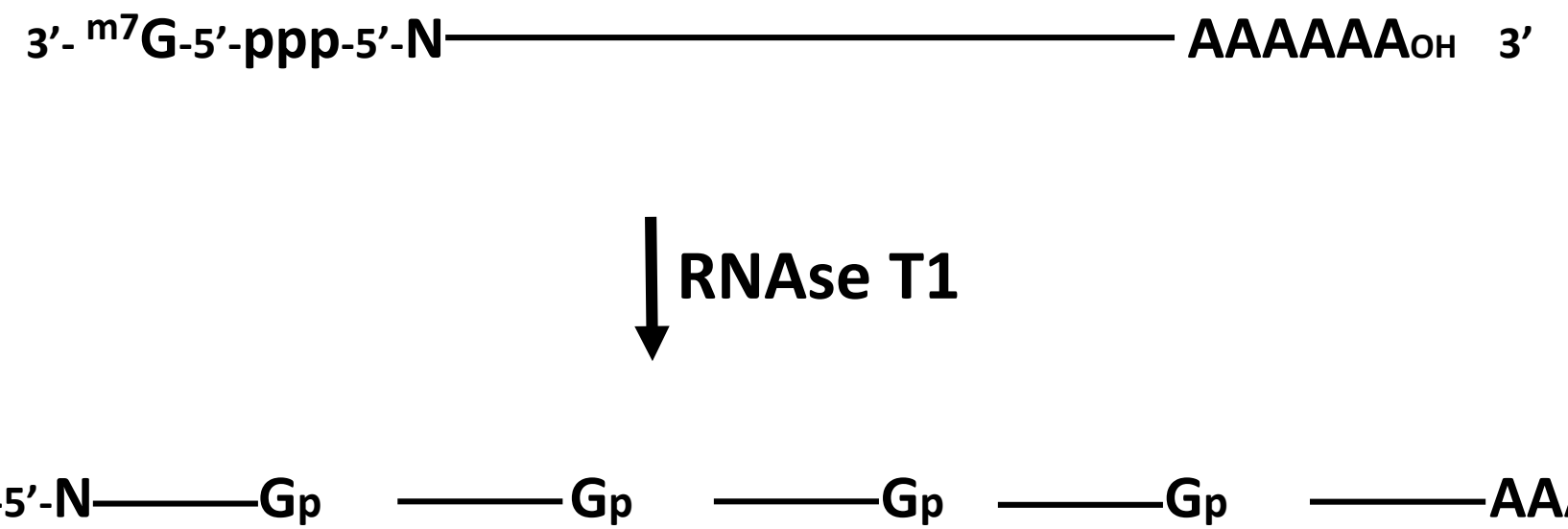
A key discovery – mRNA caps in Ad2 mRNA



S. Sommer, M. Salditt-Georgieff, S. Bachenheimer, J. E. Darnell, Y. Furuichi, M. Morgan and A.J. Shatkin *Nucleic Acids Research* **3:** 750-765 (1976) .

Moss, B., and Koczot, F. J. Virol. 17: 385-392 (1976)

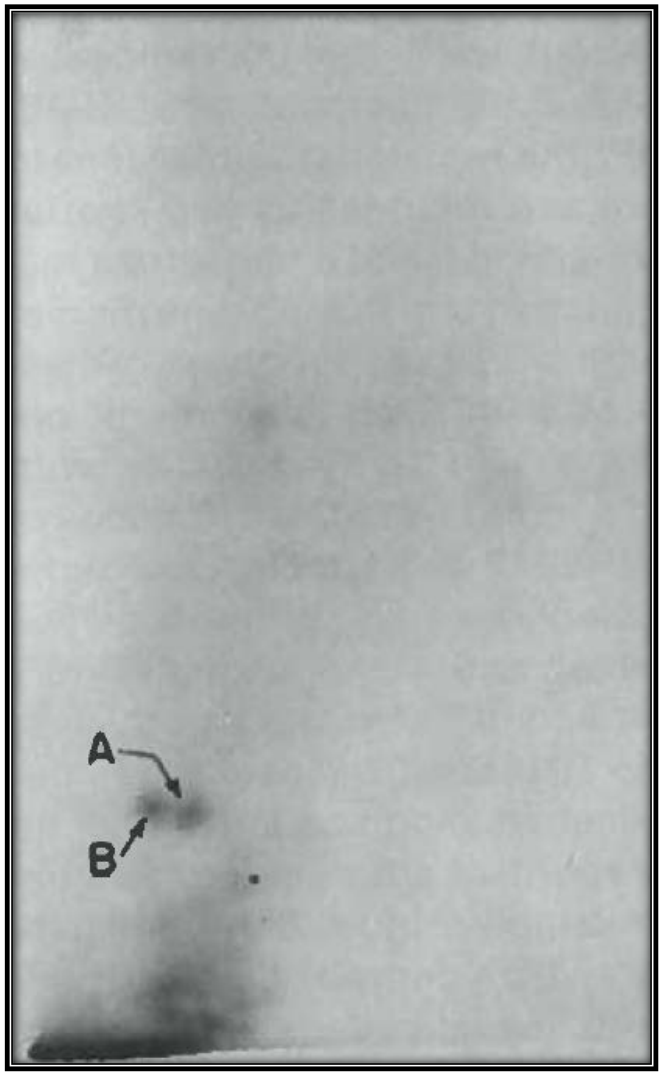
mRNA



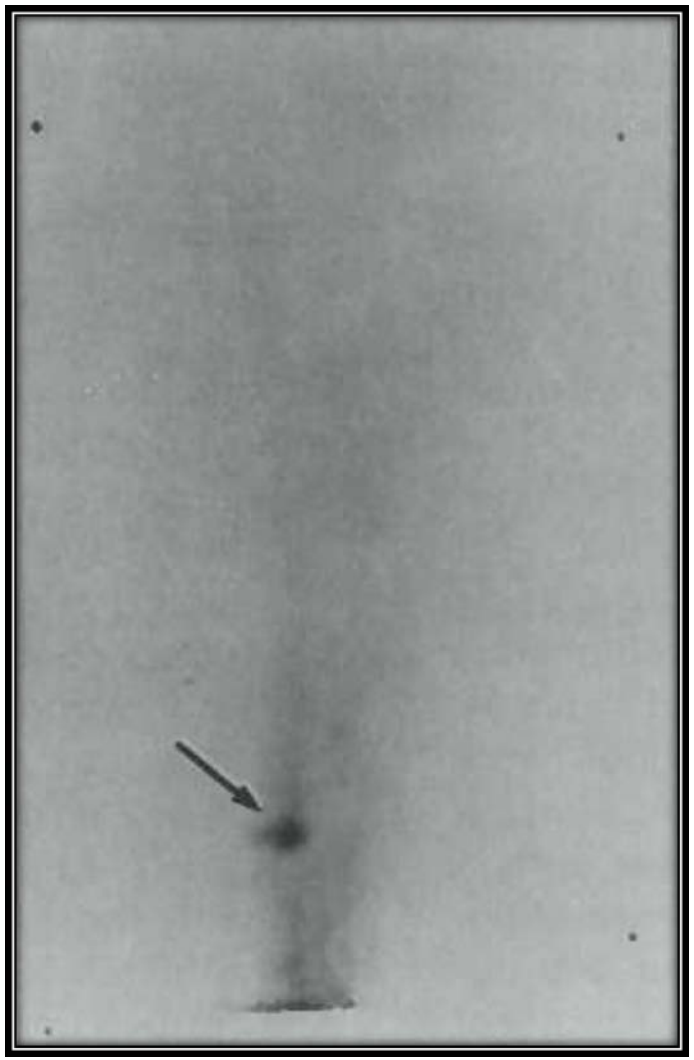
Look for all 3’– ^{m7}G-5’-ppp-5’-N———Gp

The 5’ terminal T1 oligonucleotides of late Ad2 mRNA

(2-24 hours post-infection)



(15-24 hours post-infection)



Unexpected behaviour of the capped oligonucleotide

1. Hybridization to individual DNA fragments across the Ad2 genome all gave the same cap

2. Pure mRNA for several well known proteins contained this same cap (D. Klessig)

3. When mRNA was hybridized to its main coding region and treated gently with RNAse the cap was lost

4. Ad2-SV40 hybrids showed the capped oligonucleotide was not coded adjacent to the main mRNA body (B.S. Zain)

5. Early RNAs have a different terminal oligonucleotide that is not lost by mild RNase treatment

Cell, Vol. 11, 533–544, July 1977, Copyright © 1977 by MIT

One Predominant 5′-Undecanucleotide in Adenovirus 2 Late Messenger RNAs

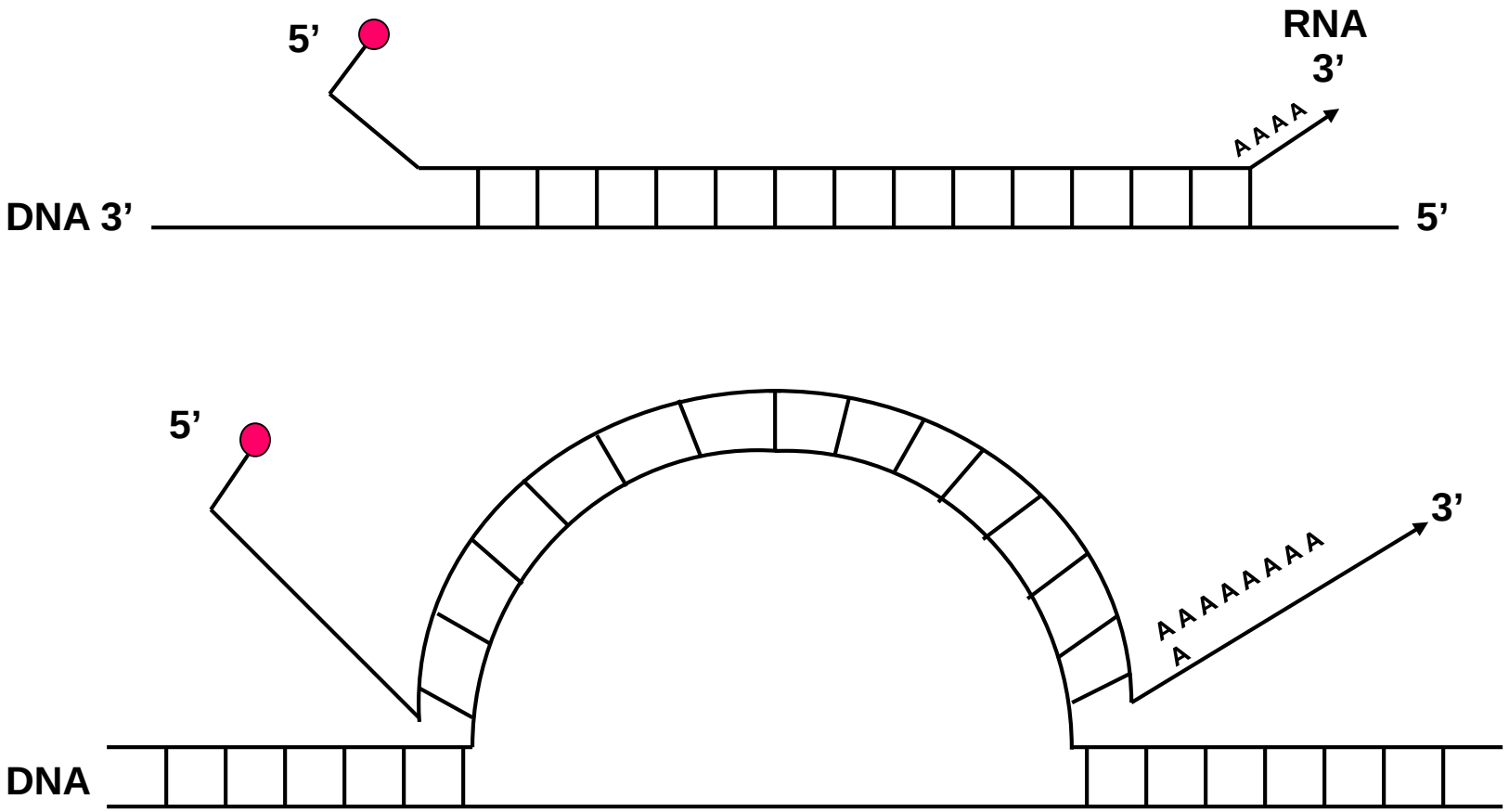
Richard E. Gelinas and Richard J. Roberts
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Summary

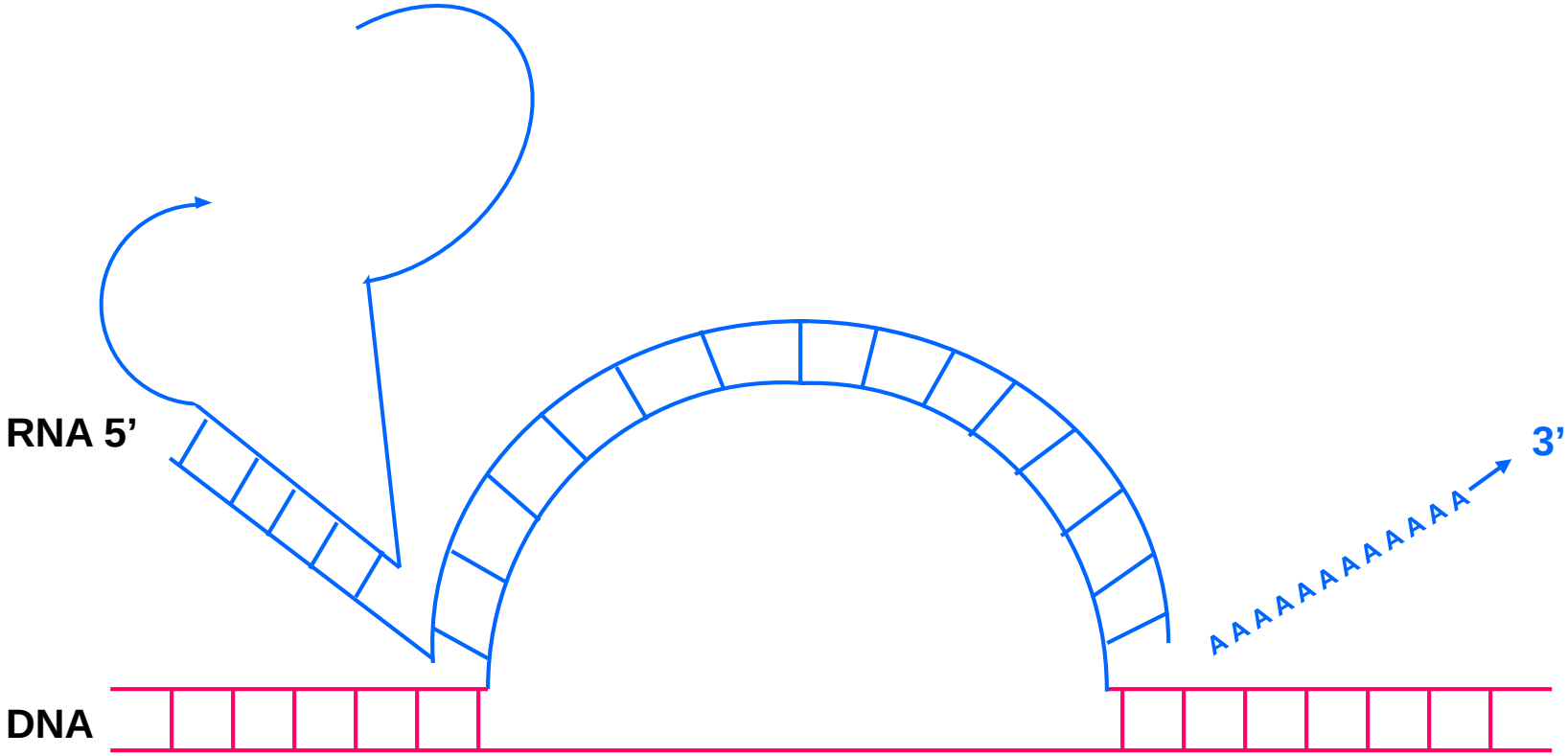
Oligonucleotides containing the 5′ termini of adenovirus 2 mRNA are selectively retained on columns of dihydroxyboryl cellulose. When total late adenovirus 2 mRNA was treated with RNAase T1, a single 5′ terminal oligonucleotide was isolated, although in several states of methylation. This oligonucleotide has the general structure $m^7G^{5'}ppp^{5'}\overset{*}{A}mCmU(C_4,U_3)G$. Since at least twelve individual species of mRNA must be present late after infection, this finding was unexpected and its significance is discussed.

the presence of a 7-methylguanosine residue linked via a 5′,5′-triphosphate bridge to the 5′ terminal residue of the mRNA chain. The unusual linkage of the 7-methylguanosine residue provided a way of isolating 5′ terminal oligonucleotides containing these structures. These oligonucleotides are unique in that they contain a 3′-phosphate at one terminus (for example, after RNAase T1 cleavage, they contain 3′-Gp) and a 2′,3′-*cis* diol from the 7-methylguanosine at the other terminus. Oligonucleotides containing a 2′,3′-*cis* diol have previously been isolated on affinity columns of dihydroxyboryl cellulose (DBAE-cellulose) (Weith, Wiebers and Gilham, 1970), and this method has been of value for the isolation of 3′ terminal oligonucleotides from ribosomal RNAs (Rosenberg, 1974) and capped structures generated by RNAase T2 cleavage of avian sarcoma virus mRNA (Furuichi et al., 1975b). We have extended their approach and used

An explanation and an experiment



THE 1977 R-LOOP EXPERIMENT



Cold Spring Harbor Laboratory

Through the course of these molecular biology experiments, we discovered that the genes in adenovirus must be split into at least two pieces. I then devised an electron microscope experiment that proved visually that this was true. Fortunately, Louise Chow and Tom Broker worked in a lab close to ours

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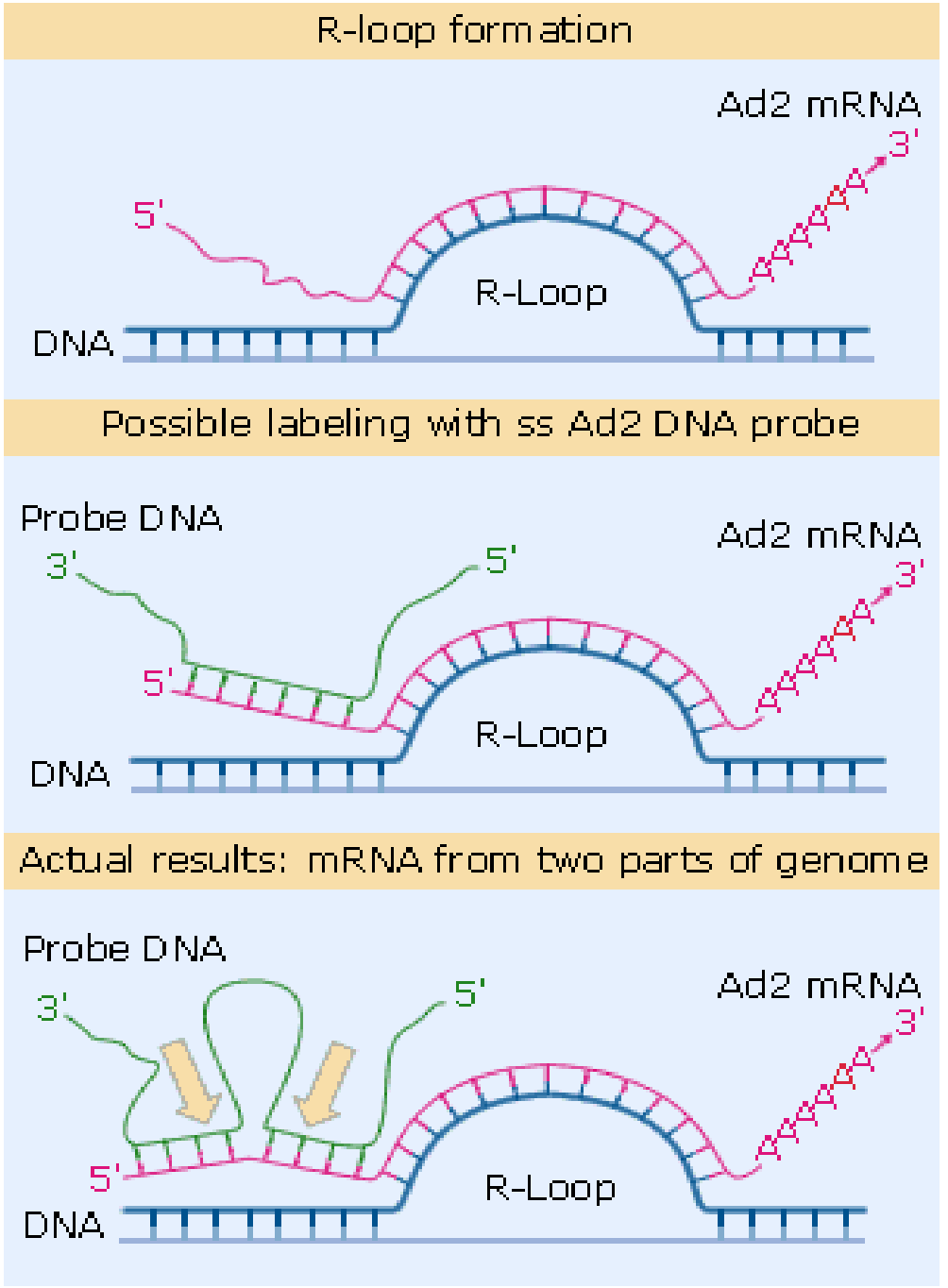


Cold Spring Harbor – 1972-1992

1977 discovery of RNA splicing

Cell, Vol. 12, 1–8, September 1977, Copyright © 1977 by MIT

An Amazing Sequence Arrangement at the 5′ Ends of Adenovirus 2 Messenger RNA

Louise T. Chow, Richard E. Gelinas, Thomas R. Broker and Richard J. Roberts
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Summary

The 5′ terminal sequences of several adenovirus 2 (Ad2) mRNAs, isolated late in infection, are complementary to sequences within the Ad2 genome which are remote from the DNA from which the main coding sequence of each mRNA is transcribed. This has been observed by forming RNA displacement loops (R loops) between Ad2 DNA and unfractionated polysomal RNA from infected

teristic of that of the host genome (Lewin, 1975a, 1975b). For example, long polyadenylated transcripts appear in the nucleus, but only a small percentage of this nuclear RNA appears as polyadenylated mRNA on cytoplasmic polysomes (Philipson et al., 1971). These mRNAs are "capped" at their 5′ ends (Moss and Koczot, 1976; Sommer et al., 1976). Gelinas and Roberts (1977) found that most Ad2 mRNAs isolated at late times during infection contain the same "capped" 11 nucleotide sequence at their 5′ ends. This sequence was sensitive to ribonuclease cleavage in mRNA:DNA hybrids (Gelinas and Roberts, 1977; Klessig, 1977) and led to the suggestion that this 5′ terminal sequence might not be coded immediately adjacent to the main body of the mRNA.

Cold Spring Harbor Laboratory – 1978-1992

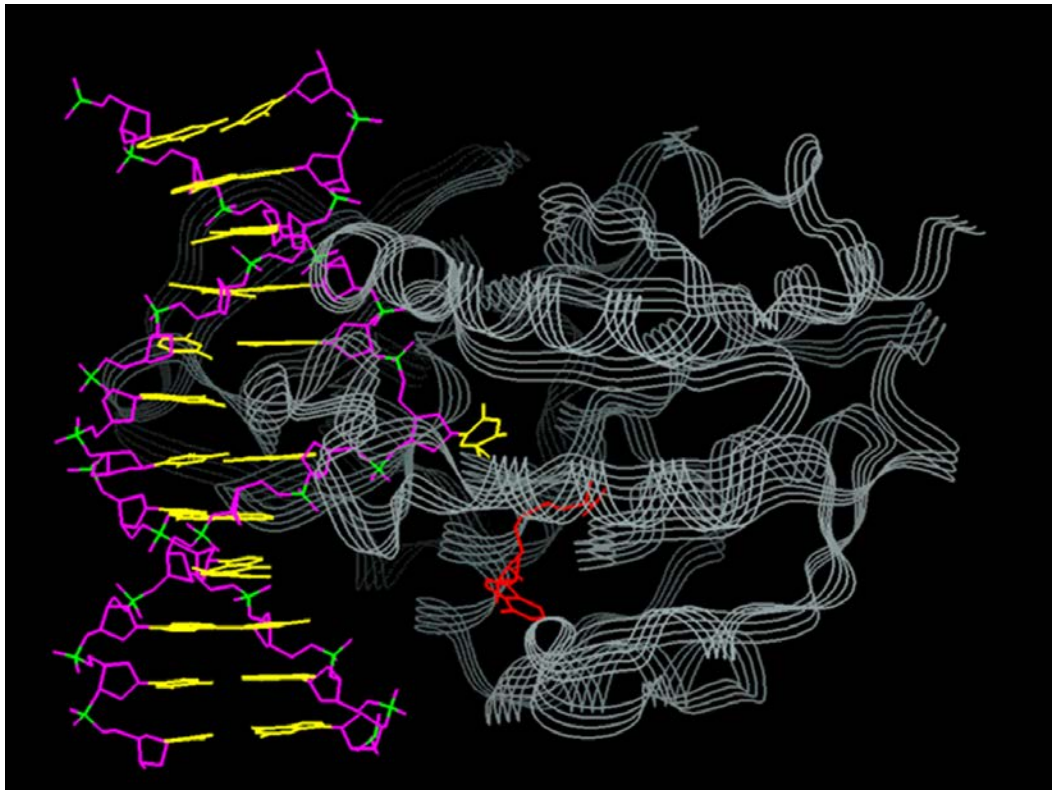
1. Sequenced some splice junctions
2. Sequenced Adenovirus-2 DNA
3. Pioneered sequence assembly programs
4. Began developing BIOinformatics

Nobel Prize in Physiology or Medicine 1993

“The Nobel Assembly at the Karolinska Institute in Stockholm, Sweden, has awarded the Nobel Prize in Physiology or Medicine for 1993 jointly to Richard J. Roberts and Phillip A. Sharp for their discovery of split genes.”

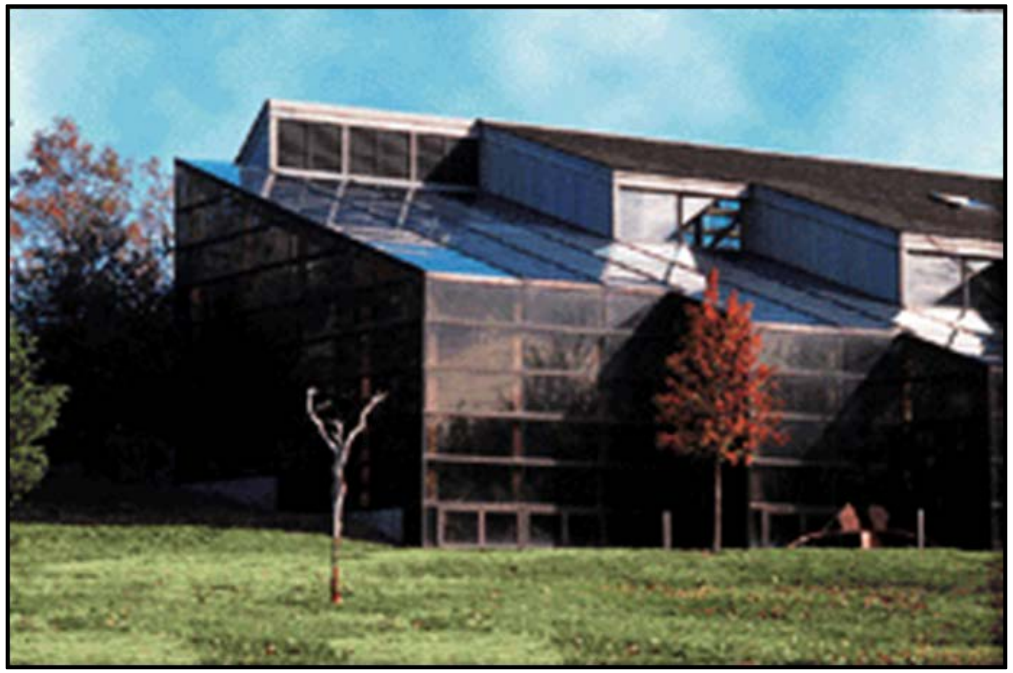
Cold Spring Harbor/NEB – 1991-1993

With Xiaodong Cheng - discovery of base flipping in M.HhaI



1974 - New England Biolabs

1984 – North Beverly, MA



In 1992 I moved to New England Biolabs as a Research Director. I am now the Chief Scientific Officer.

NEW ENGLAND BioLabs® Inc.

2005 – Ipswich, MA

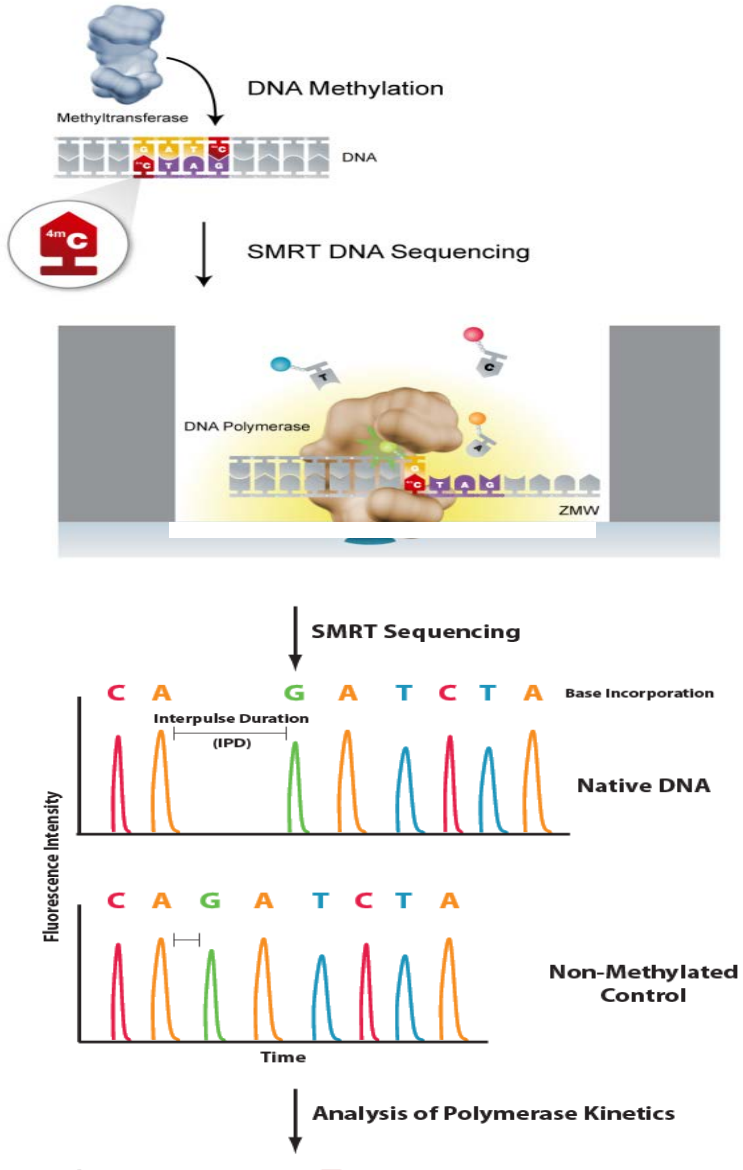
New England Biolabs – 1992-present

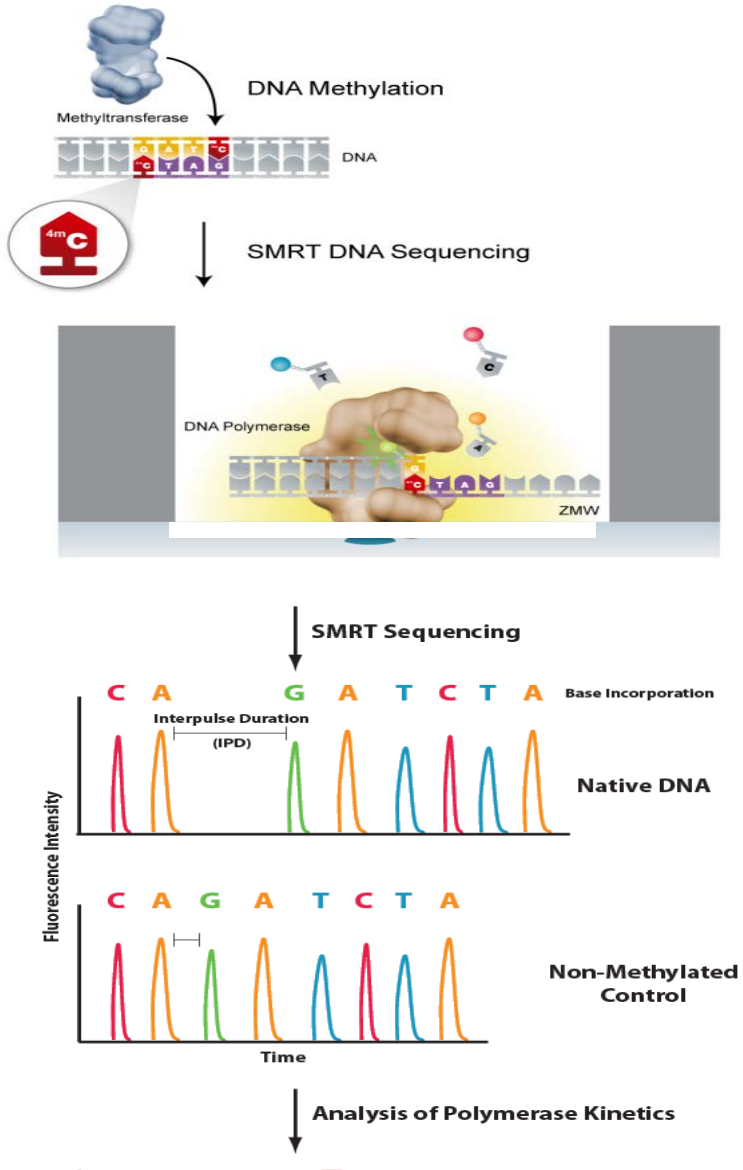
Cloning, BIOinformatics and genomics

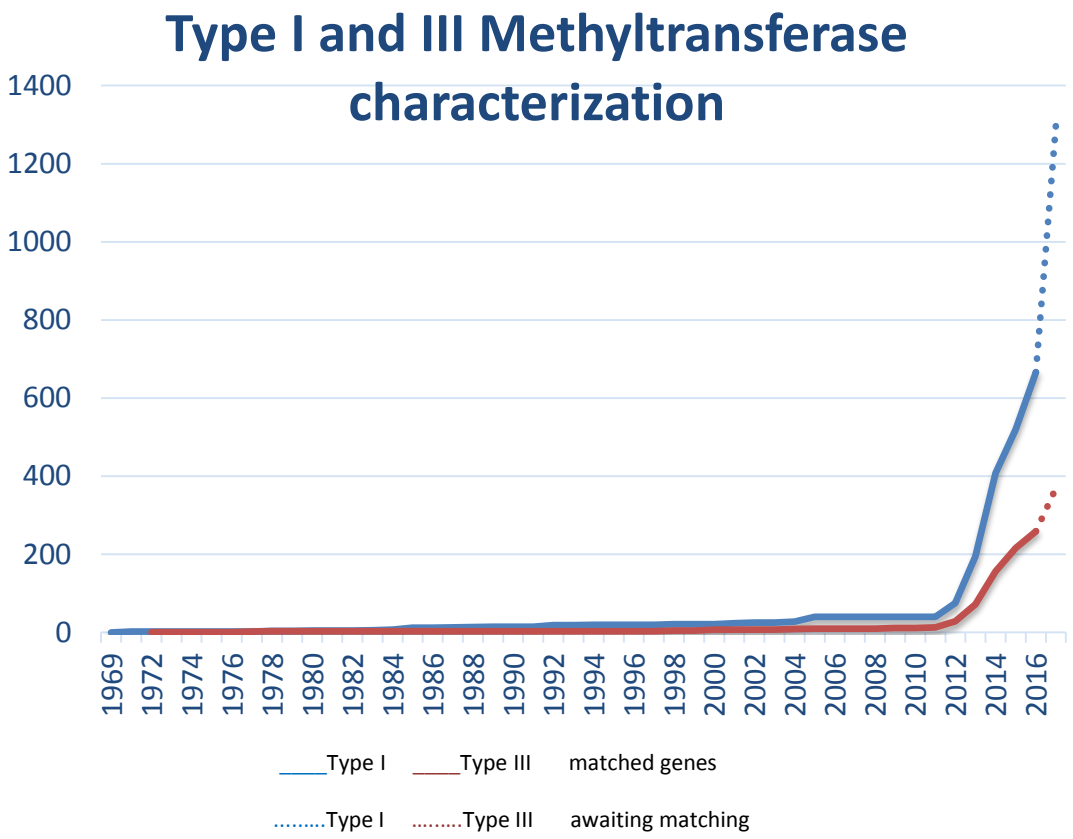
1. Cloning restriction enzyme genes for commercial production

2. Exploring the limits of bioinformatics

3. Genome sequencing to find restriction enzyme genes


DNA Methylation
Methyltransferase
DNA
4mC
SMRT DNA Sequencing
DNA Polymerase
ZMW
SMRT Sequencing
C A G A T C T A
Base Incorporation
Interpulse Duration
(IPD)
Native DNA
Fluorescence Intensity
C A G A T C T A
Non-Methylated
Control
Time
Analysis of Polymerase Kinetics
IPD Ratio
(Native/Control)
Forward Strand
Reverse Strand
G A T mC
mC T A G


DNA Methylation
Methyltransferase
DNA
4mC
SMRT DNA Sequencing
DNA Polymerase
ZMW
SMRT Sequencing
C A G A T C T A
Base Incorporation
Interpulse Duration
(IPD)
Native DNA
Fluorescence Intensity
C A G A T C T A
Non-Methylated
Control
Time
Analysis of Polymerase Kinetics
IPD Ratio
(Native/Control)
Forward Strand
Reverse Strand
G A T mC
mC T A G


Type I and III Methyltransferase characterization
1400
1200
1000
800
600
400
200
0
1969
1972
1974
1976
1978
1980
1982
1984
1986
1988
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1994
1996
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2002
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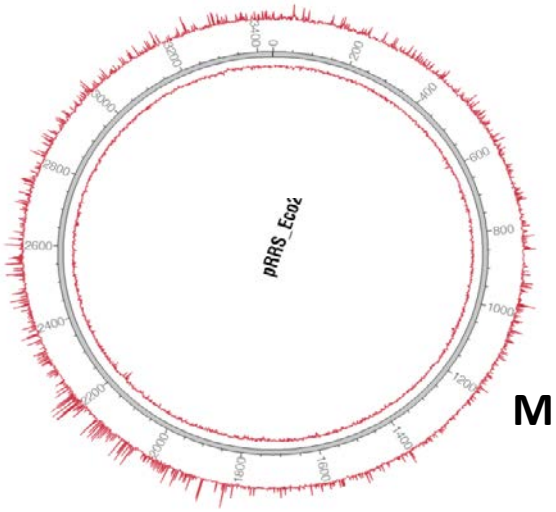
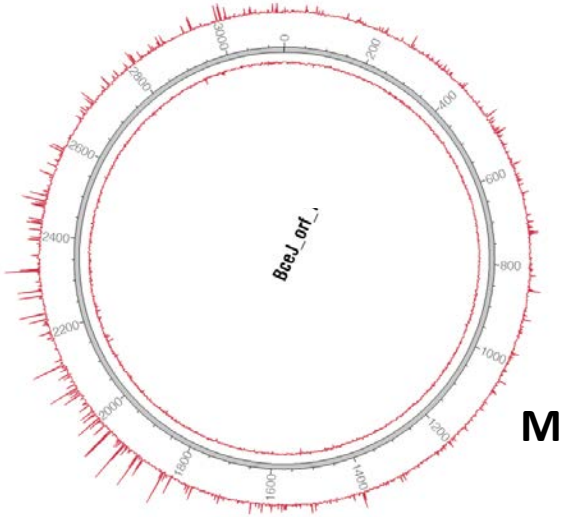
2006
2008
2010
2012
2014
2016
Type I
Type III
matched genes
Type I
Type III
awaiting matching

Single-strand plasmid methylation



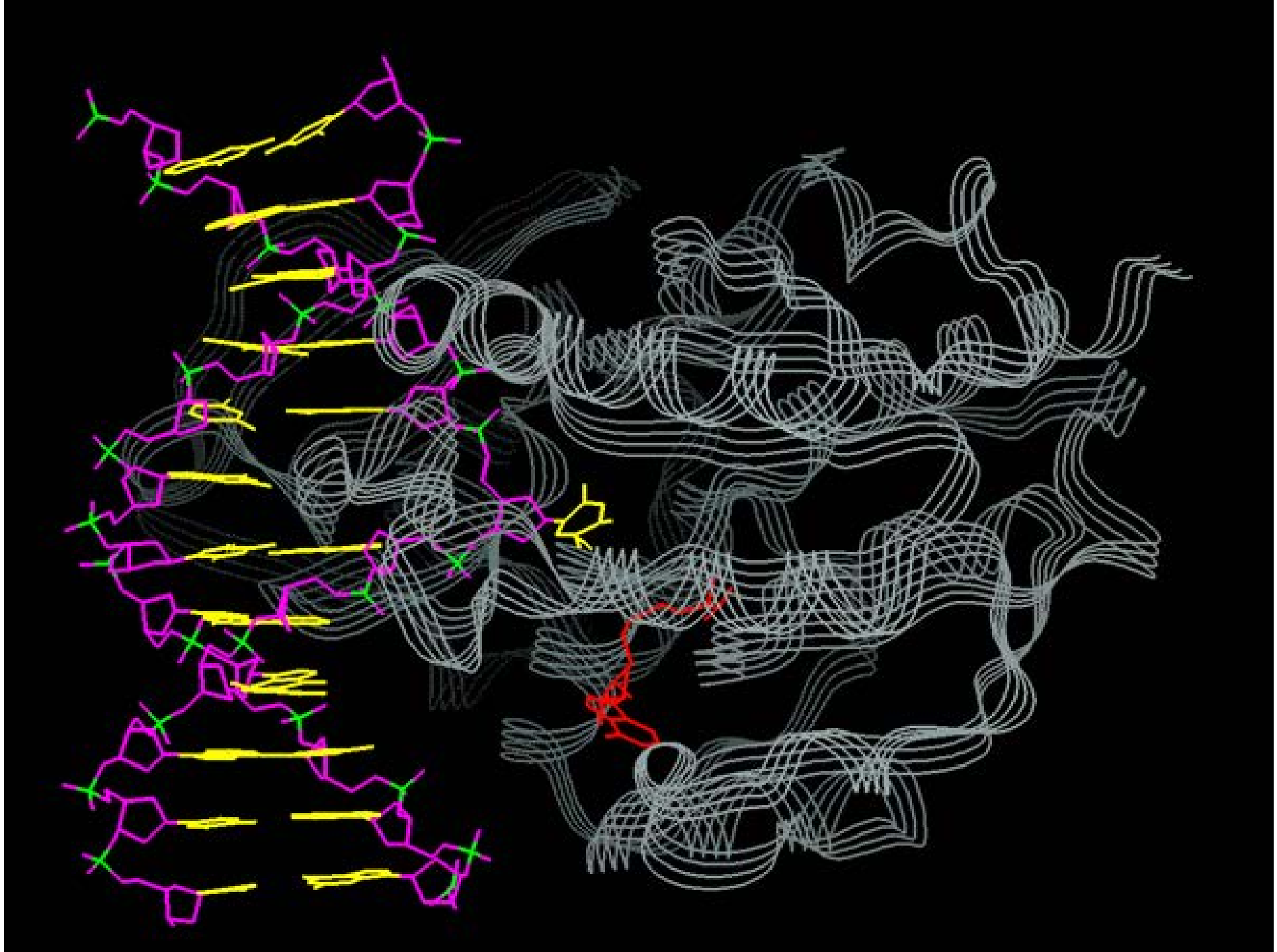
M.EcoGIX

Escherichia coli TY-2482

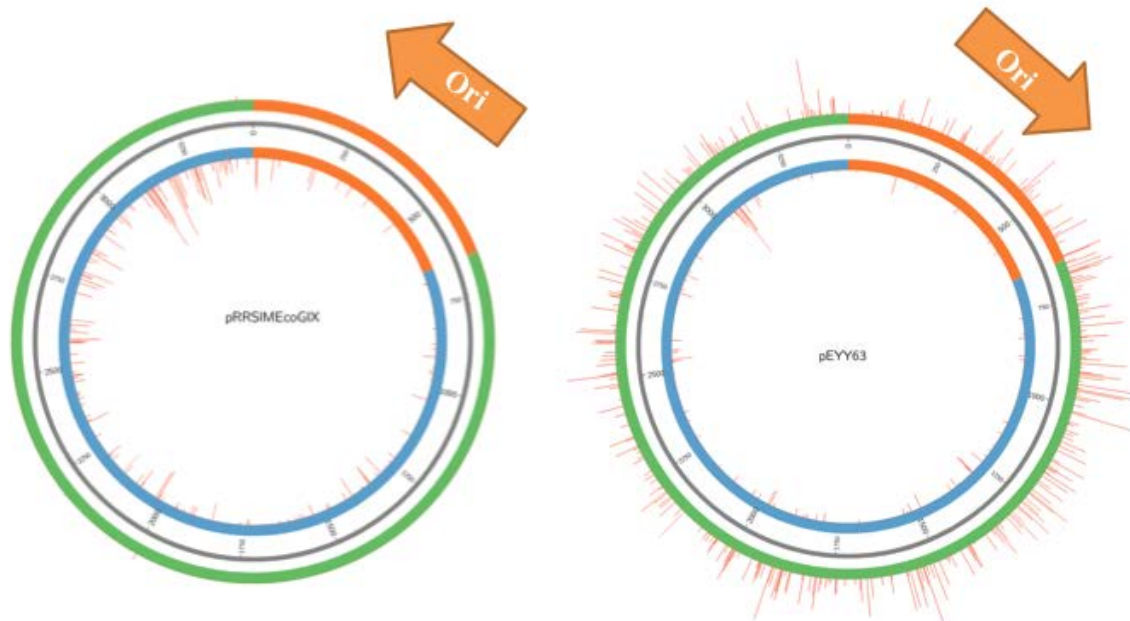


M.BceJIII

Burkholderia cenocepacia J2315



M.EcoGIX: Inverting Origin Switches Modified Strand



What has been learned so far?

1. Many solitary MTases are found in bacteria and archaea.
   Could they be involved in epigenetic phenomena?

2. Much more variability in recognition sequences than expected.

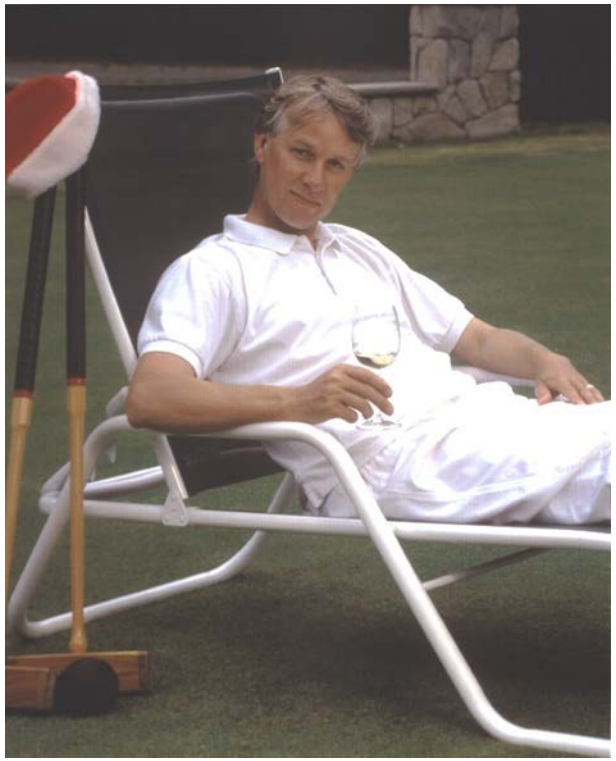
3. Type II MTases specificity matches that of the REase

4. Many new non-specific MTases discovered on prophages

Advantages of PacBio sequencing

1. Methylase recognition sequences are easily determined
2. Functional annotation of methylase genes becomes straightforward
3. Closing bacterial genomes is greatly simplified
4. Accuracy of sequencing is enhanced

Puzzles and Games



I have always been fascinated by puzzles and games.

One of my favorites is croquet, which combines the skill of snooker with the strategy of chess.

My problem-solving nature is accompanied by a dry sense of humor as evidenced by my appearance as Dr. December in the 1997 Calendar “The Stud Muffins of Science”, and my regular trips to the IgNobel Awards (the “opposite” of the Nobel Prizes) at Harvard University.

Honours and Humanitarian Work

I was awarded an Honorary Degree (Doctor of Science) by the University of Bath in 1994.

A refurbished science department at Beechen Cliff School (previously the City of Bath Boys' School) was also named after me.

In 2005, a large expansion to the Chemistry Department at the University of Sheffield was named after me.

I was knighted in Queen Elizabeth's 2008 Birthday Honours List.

I have helped to organize the Nobel Laureates for several good causes including gaining the release of some Bulgarian nurses from jail in Libya.

Join the Nobel Laureates Pro-GMO campaign

110 Nobel Laureates have written an open letter to Greenpeace and every UN Ambassador urging an acknowledgment that GMO technology is basically safe and should be supported for the sake of the Developing World, who desperately need better yielding crops with added nutritional value.

http://supportprecisionagriculture.org/

The Importance of Luck!

1. Billiards
2. Kazu Kurosawa
3. Harvard not Wisconsin
4. Looking for eukaryotic promoters in Adenovirus-2
5. Taking an early plane
6. Studying bacterial RM systems

Acknowledgments

Richard Gelinas
Louise Chow
Tom Broker

Sayeeda Zain (Ad2-SV40 hybrids)
Dan Klessig (fiber mRNA)

Many lab members, colleagues collaborators and friends over 50 years

Karen Otto (Slides)