ASPB Pioneer Member

Howard Goodman

My career as a research scientist spanned many decades and many biological systems. I started with prokaryotes (1960-75), then moved into essential genes for animal cell/tissue physiology and then yeast RNA splicing (1975-1992). Fitting with this recognition by ASPB, I spent a large part of my career working on plants (1980-2018). Given the wide range of topics covering my research interests, I have tried my best to provide highlights and a sampling of significant publications.

As an undergraduate at Williams College, I followed an honors physics curriculum, with minors in chemistry and math. I then went to graduate school in the Department of Biology at the Massachusetts Institute of Technology (MIT). After suitable time and work, I received my Ph. D. in 1964 (thesis supervisor: Alexander Rich). My dissertation consisted of several parts. First, RNA-DNA saturation hybridization showed *E. coli* tRNA is encoded by the bacterial cell DNA and there are about 40 sites per genome. The extent of tRNA hybridization between interspecies hybrids formed between *E. coli* and other bacterial DNAs reflected the relatedness between the bacterial strains. The second section of the thesis dealt with the mechanism of polyribosome action during protein synthesis.

From 1964 to 1967, I was a postdoctoral fellow of the Helen Hay Whitney Foundation and spent the entire period at the Medical Research Council (MRC) Laboratory of Molecular Biology, in Cambridge, England, in the Molecular Genetics Division (Co-directors, Francis Crick and Sydney Brenner). During this time the mechanism of bacterial amber gene suppression was under intense study in the laboratory. Given my interest and background on tRNA, I proposed a possible molecular explanation that could be tested by direct nucleotide sequence analysis of the postulated tRNA product of the suppressor gene. The only approach was to utilize the new technique Fred Sanger was developing for nucleotide sequence analysis of highly radioactive RNA. I therefore learned these techniques, and together with genetic manipulations in collaboration with other members of the MRC, I determined the sequence of the tyrosine tRNA product of the suppressor gene. These experiments resulted in the first published sequence of an *E. coli* tRNA, the first use of the radioactive method to sequence a tRNA, proof that the product of the suppressor gene is a tRNA, functional location of the anticodon, and a molecular explanation of bacterial gene suppression. The suppressor mutation results from a single base change in the anticodon of the tRNA, which allows it to read the chain- terminating codon (AUG) as tyrosine.

Then, I spent three years at the Institute of Molecular Biology at the University of Geneva, Switzerland, two years as a postdoctoral fellow of the American Cancer Society, and one year as "Assistant Professor". I turned my attention to bacteriophage Qß, for which RNA is both the genome and the messenger, and, in collaboration with Charles Weissmann, perfected techniques for determining the nucleotide sequence of *in vitro* synthesized Qß RNA. This analysis revealed the nucleotide sequence of the RNA, the secondary structure of the RNA, the regulatory signals used, chemical proof of the antiparallel complementary nature of plus and minus strands, and it revealed the presence of long-regions of RNA at both the 5' - and 3' - termini that were not translated into protein. While in Geneva, I also did experiments on the regulation of transcription of several regions of bacteriophage T4 and the mechanism by which the drug, streptolydigin, inhibits RNA polymerase.

In 1970, I accepted an Assistant Professor position at the University

continued on next page

ASPB Pioneer Member

Howard Goodman *continued*

of California, San Francisco. I was promoted to Associate Professor in 1971, and later to Professor in 1976. Although I continued some work on Qß RNA, my major interest shifted into two areas: structural analysis of tumor virus RNAs and the mechanism of action of bacterial restriction endonucleases and their use in the analysis of DNA genomes. For RNA tumor viruses, I collaborated with colleagues in Mike Bishop's lab to quantitate both "free" and 70S-associated RNA in virions of the Schmidt-Ruppin strain of avian sarcoma virus (ASV). We also investigated the initiation of DNA synthesis by the RNA-directed DNA polymerase (reverse transcriptase [RT]) associated with the virus, developed and validated three different procedures for the purification (from 70S RNA of ASV) of the primer for the RT enzyme, and showed that the primer is a tRNA. Finally, we determined conditions for reassociation between the low molecular weight RNAs and the high molecular weight subunits of ASV.

While in Geneva, I had started experiments on sequence analysis of the sites recognized in DNA by bacterial restriction endonucleases and methylases. My lab at UCSF went on to analyze the sites cleaved and/or methylated by several enzymes. The EcoRI host restriction-modification system was investigated in detail and provided important information about protein-DNA recognition interactions. We used these enzymes to analyze the structure of small viral genomes, such as SV40. The most exciting use of these enzymes was the development of methods to isolate large amounts of pure DNA from regions of high molecular weight eukaryotic genomes to use for *in vitro* recombination with plasmid DNA after digestion with an appropriate restriction endonuclease. Application of this "molecular cloning" technique provided an important tool for studying various biological questions.

A very significant area of my research interests began in 1977 and involved the systematic exploitation and refinement of cloning techniques developed earlier, to permit analysis of the structure and function of essential mammalian genes that direct the production of human hormones, most notably including insulin, growth hormone, and related polypeptides. Cloning these genes was immensely challenging and resulted from a coalescence of postdoctoral talents and breakthrough technologies that allowed cloning by first isolating mRNA from tissues and creating a cDNA intermediate by reverse transcription. Isolation of sufficient insulin-specific mRNA from the pancreas, which produces more RNAse than other mammalian tissues, or growth hormone mRNA from the tiny pituitary gland embedded in brain tissues, involved developing new techniques for successful mRNA extraction.

In 1977, a brief sabbatical in Ben Hall's lab at the University of Washington in Seattle allowed me, in collaboration with Maynard Olson, to clone the gene for a tyrosine tRNA from yeast. The impetus for this relatively simple cloning was to determine the molecular basis of the yeast tyrosine-inserting ochre suppressor, *SUP4-o*. After returning to UCSF, my lab demonstrated the tyrosine tRNA itself is transcribed as a precursor RNA containing an intron, and subsequently processed into mature tRNA. Déjà vu, these latter studies allowed me to revisit my long-term interest in tRNAs from my graduate and postdoctoral studies. Of course, introns are now a known and common feature of most eukaryotic mRNAs, but back in 1978 this was just beginning to become evident.

While still at UCSF, my group initiated a collaboration with the labs of Marc Van Montagu and Jeff Schell at the University of Gent in Belgium. Given my lab's expertise on molecular cloning and following on the tails of our interest in (animal) tumor biology, we embarked on cloning the plant "tumor" DNA that resulted from genetic transformation by the soil pathogen, *Agrobacterium tumefaciens.* In particular, we cloned the integration sites of the Agrobacterium-transferred DNA (T-DNA). These studies revealed there are precise sequences, the so-called T-DNA borders, that delimit the ends of the T-DNA element. These studies served as a foundation for the use of Agrobacterium T-DNA as a vector for plant genetic engineering, and my initiation into the field of plant biology.

continued on next page

ASPB Pioneer Member

Howard Goodman *continued*

In 1981, I moved my lab to Massachusetts General Hospital (MGH) and the Harvard Medical School and established the Department of Molecular Biology at MGH, where I struck a first-of-its-kind agreement with the pharmaceutical company, Hoechst AG, to fund establishment of the Department. This arrangement was unique in several ways, including the relationship we established with the Department of Genetics at Harvard Medical School. My own research began to focus more on aspects of plant biology, although we continued to do animal research until about 1991. Several studies were continued on the structure and evolution of the human growth hormone and insulin gene families, as well as more diverse work on the mumps virus fusion protein and attempts to develop a model gene therapy system.

Our plant biology effort was also rather eclectic, but at its core were gene structure and function studies. These included pathogenesisrelated proteins, nodulespecific genes in alfalfa symbiotic root nodules, nuclear genes encoding chloroplast and cytosolic glyceraldehyde3phosphate dehydrogenase, nitrate reductase, 1aminocyclopropane1 carboxylic acid synthase, and glutamine synthetase. The cloning of some of these genes required, in collaboration with others, the development of some new techniques such as isolation of the *Arabidopsis thaliana ABI3* gene by positional cloning and the *Arabidopsis* GA1 locus by genomic subtraction. But perhaps our most significant contributions during this period involved the early development of methods for analysis of the *Arabidopsis* genome. This included an integrated genetic/RFLP map, an early version of a database (AAtDB), mapping of YAC clones, and construction of an ~2Mb contig in the region around 80 cM of *Arabidopsis* thaliana chromosome 2. These early studies helped facilitate the sequencing of chromosome 2 and eventually the entire genome by others.

Following retirement from MGH (2004), I moved to California and joined the lab of Pat Zambryski at UC Berkeley (2005-2018), where I embarked on an entirely new (to me) area of research, the study of intercellular communication via cell wall spanning plasmodesmata (PD) channels. I specifically studied embryo defective mutant lines of *Arabidopsis* that exhibited either increased or decreased cell-to-cell movement via PD. These lines were generated by chemical mutagenesis that produced single nucleotide alterations, making identification of their affected genes challenging, necessitating the creation of genetic "mapping" populations, determination of linked single-nucleotide polymorphisms, and finally genome sequencing. All the mutations proved to be in essential genes in a diversity of critical cellular processes and are currently under study by former postdocs of the Zambryski lab.

Obviously, none of this would have been possible without the wonderful postdocs, students, and collaborations I have had the honor to work with over my career.

Among my significant publications (251) are the following:

Goodman HM, Rich A. Formation of a DNAsoluble RNA hybrid and its relation to the origin, evolution, and degeneracy of soluble RNA. Proc Natl Acad Sci USA 1962; 48:21012109.

Goodman HM, Rich A. Mechanism of polyribosome action during protein synthesis. Nature 1963; 199:318322.

Goodman HM, Abelson J, Landy A, Brenner S, Smith JD. Amber suppression: a nucleotide change in the anticodon of a tyrosine transfer RNA. Nature l968; 2l7:l0l9l024.

Goodman HM, Billeter MA, Hindley J, Weissmann C. The nucleotide sequence at the 5'terminus of the Qß RNA minus strand. Proc Natl Acad Sci USA 1970; 67:921928.

Faras AJ, Dahlberg JE, Sawyer RC, Harada F, Taylor JM, Levinson WE, Bishop JM, Goodman HM. Transcription of DNA from the 70S RNA of Rous sarcoma virus: II. Structure of a 4S RNA primer. J Virol l974; l3:ll34ll42.

Morrow JF, Cohen SN, Chang ACY, Boyer HW, Goodman HM, Helling RB. Replication and transcription of eukaryotic DNA in *Escherichia coli.* Proc Natl Acad Sci USA l974; 7l:l743l747.

Dugaiczyk A, Boyer HW, Goodman HM. Ligation of EcoRI endonuclease generated DNA fragments into linear and circular structures. J Mol Biol l975; 96:l7ll84.

Ullrich A, Shine J, Chirgwin J, Pictet R, Tischer E, Rutter WJ, Goodman HM. Rat insulin genes: construction of plasmids containing the coding sequences. Science l977; l96:l3l3l3l9.

Seeburg PH, Shine J, Martial JA, Baxter JD, Goodman HM. Nucleotide sequence

continued on next page

ASPB Pioneer Member

Howard Goodman *continued*

and amplification in bacteria of structural gene for rat growth hormone. Nature l977; 270:486494.

Goodman HM, Olson MV, Hall BD. Nucleotide sequence of a mutant eukaryotic gene: the yeast tyrosineinserting ochre suppressor *SUP4o*. Proc Natl Acad Sci USA l977; 74:54535457.

O'Farrell PZ, Cordell B, Valenzuela P, Rutter WJ, Goodman HM. Structure and processing of yeast precursor tRNAs containing intervening sequences. Nature l978; 274:438445.

Seeburg PH, Shine J, Martial JA, Ivarie RD, Morris JA, Ullrich A, Baxter JD, Goodman HM. Synthesis of growth hormone by bacteria. Nature l978; 276:795798.

Bell GI, Pictet R, Rutter WJ, Cordell B, Tischer E, Goodman HM. Sequence of the human insulin gene. Nature l980; 284:2632.

Zambryski P, Holsters M, Kruger K, Depicker A, Schell J, Van Montagu M, Goodman HM. Tumor DNA structure in plant cells transformed by *A. tumefaciens.* Science l980; 209:l385l39l.

Zambryski P, Depicker A, Kruger K, Goodman HM. Tumor induction by *Agrobacterium tumefaciens:* analysis of the boundaries of TDNA. J Mol Appl Genet 1982; 1:361370

DasSarma S, Tischer E, Goodman HM. Plant glutamine synthetase complements a glnA mutation in *Escherichia coli.* Science 1986; 232:12421244.

Selden RF, Skoskiewicz MJ, Burke Howie K, Russell PS, Goodman HM. Regulation of human insulin gene expression in transgenic mice. Nature 1986; 321:525528.

Cheng C, Dewdney J, Kleinhofs A, Goodman HM. Cloning and nitrate induction of nitrate reductase mRNA. Proc Natl Acad Sci USA 1986; 83:68256828.

Shih MC, Heinrich P, Goodman HM. Intron existence predated the divergence of eukaryotes and prokaryotes. Science 1988; 242:1164 1166.

Nam HG, Giraudat J, den Boer B, Hauge B, Loos W, Moonan F, Goodman HM. Restriction fragment length polymorphism linkage map of *Arabidopsis thaliana.* The Plant Cell 1989; 1: 699705.

Van Der Straeten D, Wiemeersch LV, Goodman HM, Van Montagu M. Purification and partial characterization of 1aminocyclopropane1 carboxylic acid synthase from tomato pericarp. Eur J Biochem 1989, 182: 639647.

Hwang I, Kohchi T, Hauge B, Goodman HM, et. al. Identification and map position of YAC clones comprising onethird of the *Arabidopsis* genome. The Plant Journal 1991; 1: 367374.

Hauge B, Giraudat J, Hanley S, Hwang I, Kohchi T, Goodman HM. Physical mapping of the *Arabidopsis* genome and its applications. In: Plant Molecular Biology. Plenum Press, ed., R.G. Herrman; 1991.

Sun TP, Goodman HM, Ausubel FM. Cloning the *Arabidopsis* GA1 locus by genomic subtraction. The Plant Cell 1992; 4: 119128.

Zhang H, Scheirer D, Fowle W, Goodman HM. Expression of Antisense or sense RNA of an ankyrin repeatcontaining gene blocks chloroplast differentiation in *Arabidopsis* . The Plant Cell 1992; 4: 1575-1588.

Cherry JM, Cartinhour SW, Goodman HM. AAtDB, An *Arabidopsis thaliana* Database. Plant Mol Biol Reporter 1992; 10: 308.

Hauge B, Hanley S, Cartinhour, Cherry J, Goodman HM, Koorneef M, Stam P, Chang C, Kepmin S, Medrano L, Meyerowitz E. An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. The Plant Journal 1993; 3: 745-754.

Goodman HM, Ecker J, Dean C. The genome of Arabidopsis thaliana. Proc. Natl.Acad.Sci.USA 1995; 92: 10831-10835.

Zhang H, Wang J, Nickel U, Allen R, Goodman HM. Cloning expression of an *Arabidopsis* gene encoding a putative peroxisomal ascorbate peroxidase. Plant Molecular Biology 1997; 34: 967-971.

Lin Y, Sun L, Nguyen LV, Goodman HM. The *PEX* 16 Homolog *SSE*1 and storage organelle formation in *Arabidopsis* seeds. SCIENCE 1999; 284: 328-330.

He J, Baldini RL, Deziel E, Saucier M, Zhang Q, Liberati NT, Lee D, Urbach J, Goodman HM, Rahme LG. The broad host range pathogen *Pseudomonas aeruginosa* strain PA14 carries two pathogenicity islands harboring plant and animal virulence genes. PNAS. 2004;101:2530-2535.

Bevan M, Bancroft I, Bent E, Love K, Goodman H, et. al. Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana.* Nature 1998; 391: 485-488.

Lin X, Kaul, S, Rounsley S, Shea TP, Goodman HM, et. al. Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana* Nature 1999; 402: 761-768.

Shen B, Goodman HM. Uridine addition following miRNA-directed cleavage. Science 2004; 306: 997

Brunkard JO, Xu M, Scarpin, MR, Chatterjee S, Shemyakina EA, Goodman HM, Zambryski P. TOR dynamically regulates plant cell-cell transport. Proc Natl Acad Sci USA. 2020; 117: 5049-5058.