

# Chapter 1

## Prologue



**Abstract** All living things are associated with a boundary defined ecological niche. Steady state conditions are rarely constant but evolutionary adaptation is too slow to adapt to daily threats so a surrogate variation mechanism is necessary. The genome defines the most basic instructions for life so that a molecular biology perspective provides the foundation for understanding resilience. Variations in the expression of RNA offers rapid variation and this book proposes this is the basis of resilience. This book attempts to illuminate mechanisms of resilience beginning with elaborating threats leading to disruption in steady state conditions. Recognition of threats and defense systems are described followed by adaptive changes in gene expression that refine responses. Finally, environmental conditions are discussed that serve to dampen the adaptive response oscillator to disruptive threats at the level of RNA expression.

This prologue is intended to acquaint the reader with my background and the genesis of optimism for an idea that the benefit of transcriptome plasticity is resilience. I grew up in several National Parks, remote regions of the United States that are set aside to preserve natural environments. I attended 12 schools by the time I graduated from high school, a fact that forced me to develop personal resilience. My career path as a scientist followed a path from ecologist to pharmacologist to molecular biologist. I was a professor that transitioned to biotechnology ensuring research subjects involving very diverse in subject matter so I appreciate the value of plasticity.

**Keywords** Pharmacology · Drug metabolism · Chemical carcinogenesis · Molecular biology · Cytochrome P450 · Exon skipping · Resilience

## Background

I have been a scientist as long as I can remember. Before starting school in Grand Canyon Arizona, I caught a horned lizard (*Phrynosoma*) and kept it in a box as a pet. These lizard's squirt a stream of blood from the corner of the eye as a novel defense strategy. I was in love. The park rangers visiting our house would tell stories of

toads and ants in the region, they convinced me to let the lizard go free. A park naturalist showed me how to lure an ant lion from his sandy cone with a blade of grass. The enthusiasm for living things and excitement adults expressed over this special place established a foundation for my life in science.

My family moved to the Petrified Forest National Monument after I completed first grade. One of the first few days after arrival, I caught a snake in the desert just outside our house. I instinctively avoided the head so I carried the snake by its tail. I had no idea what kind of snake I had, in fact I did not know there were different kinds of snake. I brought the snake home to show to the family. A new discovery was that my brother expressed severe anxiety over the snake and my proximity to him, he ran. I then discovered my mother also shared a deep fear of snakes. They encouraged me to let the snake go free but the incident was not complete until I got a snake lecture from the park naturalist. The impromptu lecture was amazing; we looked at about a half-dozen different snakes in glass enclosures including the local rattlesnake. The concept of an animal capable of delivering a poison was mind expanding.

The years in the Painted Desert planted the seeds of the resilience of life. As I grew older, my journeys into the desert become longer with greater range from home. My parents concern for my explorations centered on my lack of social development. They began exploring boarding school options for my brother and I but we stayed to attend our one-room school. Monsoon-like rains arrived late in the spring leaving pools of water in the barren clay soil. My brother and I found a muddy pool filled with hundreds of tadpoles, possibly the canyon treefrog (*Hyla arenicolor*). We watched these tadpoles mature over a two-week period and all were gone, buried in the mud, when the pool dried up. The desert provides numerous examples unique life and resilience strategies.

I had freshwater aquariums from sixth grade. The aquarium experience included routine cleaning and removal of algae from the glass at times. I added catfish to the aquarium to reduce the algae but they did not completely remove the green haze from the glass. I added snails and more living plants and found the algae problem solved. An early lesson in the living interplay, ecology. The 9th grade scientist in me and availability of a plastic sphere inspired an idea of a self-contained ecological system. Gravel in the bottom, living plants, snails, and two fish were added to the fresh water system. I cut a hole in the top so a rubber stopper with a glass pipe could allow for pressure equalization. Those fish were still alive in an algae free plastic sphere after two years when I had to take the experiment apart. I see it is now possible to purchase self-sustained aquaria on the internet, confirmation that this is routinely accomplished. These self-sustained systems represent a robust demonstration of the balance of nature.

Hiking in the high desert of Arches National Park near Moab Utah, I observed hanging desert gardens suspended in cliffs hundreds of feet above the valleys below. Juniper trees and sage brush growing in desert sand in a physically isolated space. Access available to birds and insects carried by the wind but a system in balance for dozens of hundreds of years. The message is that life will find a way. How do living things prepare or adapt to isolated environments?

My first real world research position was with Gradient Modelling, Inc. in Glacier National Park for the summer of 1975. Gradient Modelling was the creation of Stephen R. Kassel, a recent Cornell University Ph.D. under the direction of R. H. Whittaker. Steve wanted to exploit the marriage of computer science and ecology. He divided the environment of Glacier National Park into six continuous “gradients” including: elevation, topographical-moisture and aspect, primary succession (soil development following glacial retreat), watershed location, alpine wind-snow exposure, and time since the last forest fire (Kessell 1976). My job was as part of the Northern Forest Fire Lab for data collection to measure species diversity and fire fuel loading. The data from several teams including mine supported the computer model, which would predict forest-fire burn area, anticipate resulting plant and animal communities, and support fire management decisions in the park.

Forest fires create diverse, mosaic, and resilient forests because the fires arise from lightning strikes that occur during rainstorms and are inherently limited to small regions. Human interactions involve fire suppression measures that support maintenance of large climax forests, vulnerable to large catastrophic fires and diminished ecoregion diversity. The objective of the project was to support a “let burn” policy implemented in Glacier but limitations included stakeholders with interests at the park boundaries; the Flathead Indian Reservation, the Blackfoot Indian Reservation, Canada, and the Bob Much Wilderness Area. The job involved weekly backpacking trips to most regions of Glacier National Park and measuring plant diversity, an obligate lesson in taxonomy. The greater my capacity to identify genus and species of animals, trees, shrubs, and herbaceous plants, the more the appreciation of the impact of the environment on species diversity.

I helped write a “Student Originated Studies (SOS)” grant to the National Science Foundation (NSF), which was funded. The project evaluated the ecology around Strawberry Reservoir during the summer after graduation (Worley et al. 1977). The objective was to use aquatic invertebrates as indicator species including: caddisfly (rock rollers), stonefly nymphs, mayflies, amphipods, damselflies, midges and worms as indicators of pollution in the area. We repeatedly sampled the creeks and streams leading into (Trout, Co-op, Indian, Horse, Clyde, and Soldier Creek) and out of (Strawberry River) Strawberry Reservoir to assess the impact of recreational and agricultural uses of the area. The reservoir had been managed for 50 years by the Strawberry Water Users Association and significant problems developed. Fishing villages and campsites were established with variations of pit toilets within feet of the shoreline and these sites were to be inundated by an enlarged Strawberry Reservoir. The reservoir filled to capacity in 1975 when a series of wet years led to the first spill over since 1952 and high water inundated many trailers, outdoor toilets, and other facilities. The flood problems were associated with water quality concerns for undesirably high nutrient levels. The project supported plans to improve management of the area to ensure water quality and future safe recreational use of Strawberry Reservoir. The lessons from undergraduate research clearly pointed to humans as part of the environment and our influence on ecology. This raises a contemplative question, Are we to live in harmony with the environment or are we adversaries?

Analysis of indicator species remains an active area of environmental research. These indicator species offer a time averaged measure of an organism's adaptive response to the environment, which provides immediate practical value to environmental monitoring. Clearly, the most interesting aspect of indicator species is the boundary they define and the potential for hyper-adapted species at the boundary. Boundaries raise a question, are the adaptations a function of the survival of the fittest organisms driving evolution or can fluctuations in populations of RNA in the transcriptome provide a faster adaptive responses?

After completing my undergraduate degree, I landed a position as a biology research technician in the laboratory of Dr. Martin Rechsteiner at the University of Utah. Marty's lab developed a red blood cell preparation to deliver macromolecules including tRNA into cultured eukaryotic cells. I was fortunate to experience this laboratory atmosphere and the cellular and molecular biology environment in the Biology Department which included respected molecular biologists; Drs. Gordon Lark (plant DNA expert), David Wolstenholm (mitochondrial DNA expert), Mario Capecchi (Nobel Prize winner and gene transfer authority), and Baldimore Olivera (cone-shell toxin expert). My project involved SV40 virus and explored transformation in contrast to lytic infection in mammalian cells. The hypothesis was that mouse 3 T3 cells transformed with SV40 virus (SV3T3 cells) was due to failure of the virus to synthesize "late" proteins due to lack of specific iso-accepting tRNAs. I isolated tRNA from lytic TC7 African Green Monkey cells then loading the tRNA into red blood cells that were then fused to SV3T3 cells delivering tRNAs. We expected the SV3T3 cells to release SV40 virus into the medium but our hypothesis was incorrect (Schlegel et al. 1978). A second project involved using Sendai virus (a paramyxovirus) to fuse mouse 3 T3 cells with human HeLa cells to create mouse: human hybrid cells. I then observed the kinetics of chromosome loss as the hybrid cell line divided. I became expert at preparing metaphase spreads on microscope slides. The key observation was that mouse chromosomes, identified by their epicentric V shape, are maintained in favor of human chromosomes, identified by their metacentric X shape. The concept that the cell discards redundant genomic information is a novel rapid adaptation to changes in the cell.

I joined the laboratory of Dr. Lester Partlow in the Department of Pharmacology in the College of Medicine (1978–1979). The department was famous due to the Chairman emeritus, Dr. Louis Goodman, the author of the blue bible, "The Pharmacological Basis of Therapeutics." Utah and the Mormon culture offered a refuge for Goodman, a talented Jewish man, in the late 1930's when such ethnicity was discriminated against in the years leading up to World War II. Dr. Partlow was a cellular neuroscientist, a specialty of great personal interest. We dissected 12-day chicken embryos to remove sympathetic ganglia from adjacent to the spinal cord, a technique requiring steady hands and a stereo microscope in a sterile hood. Alternately, I removed the dorsal root ganglia from the spinal cord to recover cholinergic tissue. Once the surgery was complete, the tissues was digested to produce a single cell suspension that was then subjected to intermittent vibration so the glial cells would attach to the culture dish leaving the neurons in suspension. I developed innovative strategies to encourage neurons to adhere to the culture dish enabling

studies of pure primary neuronal cultures (Iversen et al. 1981). I became expert at measuring acetylcholinesterase, butyrylcholinesterase, and carbonic anhydrase enzyme activities. I conducted experiments with the neurons separate from glial cell populations and then mixed them to evaluate neuronal-glial cell-cell interactions (Hanson et al. 1982).

Dr. Partlow supported my application to graduate school in the Department of Pharmacology, a highly competitive process as they accepted only 3 students from nearly 600 applicants. My intention was to stay in the Partlow laboratory investigating retrograde axonal transport. The project involved characterizing the movement of 1 micron polystyrene beads that attached to axonal membranes of neurons in culture. I set up a microscope with a heated stage and videotaped the culture for hours. The beads moved along the axon towards the cell body in a saltatory manner. I also used transmission electron microscopy and scanning electron microscopy to observe the interaction of the beads with the membrane. The techniques enabled visualization of cell-cell interactions.

I joined the laboratory of Dr. Franklin, younger energetic faculty member, and investigated the cytochrome P450 (CYP) enzyme system and drug metabolism. This family of enzymes were important to drug companies because they frequently inactivated drugs by oxidative metabolism. In addition, these enzymes metabolize a wide variety of foreign substrates, xenobiotics, from the environment and in some cases; the metabolic products are chemically reactive as radical cations. The reactive products form chemical bonds with cellular molecules including DNA, which link them to mutations and chemical carcinogenesis. The CYP enzymes reside in the membrane of the endoplasmic reticulum and their metabolic action transforms highly lipid soluble substrates capable of long residence in cellular membranes into more water-soluble products that are carried out of the cell and out of the body. The CYP oxidations represent an energy efficient reaction in contrast to reduction reactions, which highlight the need for cells to manage energy utilization. The cellular content of CYP enzymes is reduced when the cell goes through the cell cycle suggesting a functional connection between CYP and growth. My dissertation investigated the expression of multiple CYP enzymes in the rat liver as it regenerates following partial hepatectomy (Iversen et al. 1985). We knew there were multiple genes encoding CYP enzymes but we did not know how many and reagents were not available to evaluate specific CYP genes. Linking chemicals in the environment to metabolic enzymes provides a fertile area directly linking gene-expression to the environment.

Once I completed my dissertation, I joined the laboratory of Dr. Edward Bresnick at the Eppley Institute for Cancer Research in Omaha Nebraska. Dr. Bresnick was an aggressive scientist exploring the molecular biology of the CYP enzymes with interest in details of chemical carcinogenesis. I participated in a project to explore the molecular biology of mouse cytochrome P450c, now known as CYP1a1 (Hines et al. 1985). My project quickly turned using the mouse gene as a probe to find and clone the human version of CYP1A1. The group effort was successful in isolating the first gene encoding a human drug-metabolizing enzyme. I spent 9 months sub-cloning and sequencing this gene (Iversen et al. 1987), a task that would take hours

today. The molecular biology of CYP1A1 was exciting, rapidly expanding scientific questions that exceeded my capacity to conduct studies. Ed was an outstanding mentor but his responsibilities as director of the Eppley Institute diminished the time we could meet. Dr. Ron Hines was a new investigator at the institute having recently completed his postdoctoral fellowship with Ed and I relied on Ron as my mentor for the remainder of my postdoctoral fellowship. Reagents and expression vectors were rapidly becoming commercially available and served to expand perspectives in molecular biology and pharmacology. New techniques including the polymerase chain reaction (PCR) and automated DNA synthesizers and sequencers dramatically enhanced the pace of our research.

## RNA Based Therapeutics

Dr. Manuchair (Mike) Ebadi invited me to join the faculty in the Department of Pharmacology at the University of Nebraska Medical Center (UNMC) in 1987. I combined the essence of molecular biology with pharmacology in the exploration of antisense inhibitors of gene expression. The concept that a short single-stranded DNA molecule could be synthesized to bind to its complementary single-stranded RNA transcript to inhibit translation had the potential to lead to a vast new area of pharmacology. My friend, Dr. Mario Stevenson, was growing the Human Immunodeficiency Virus-1 (HIV-1) in cell culture and we decided to collaborate on a project to use antisense oligonucleotides to inhibit HIV-1 viral growth (Stevenson and Iversen 1989). I then worked with a group at the NIH to reveal the sulfur modified DNA oligomer inhibited the HIV-1 reverse transcriptase activity independent of the oligomer sequence (Egan et al. 1991). An emerging belief that antisense oligomers could be RNA-based drugs became the focus of my future research.

I wanted to see if a synthetic oligonucleotide would have drug-like properties so I developed a method for synthesis of a radioactive phosphorothioate synthetic DNA molecule and then injected it into rats to estimate various pharmacokinetic properties (Iversen 1991; Iversen et al. 1994). I was convinced a synthetic oligonucleotide would distribute into multiple organs but would it inhibit targeted gene expression? I investigated multiple gene targets for inhibition in animal models including a mouse tumor model of chronic myelogenous leukemia (Skorski et al. 1994) and rat models of CYP2B1 (Desjardins et al. 1995) and CYP3A2 (Desjardins and Iversen 1995). With feasibility and *in vivo* efficacy completed, I explored the potential therapeutic limitations; finding a cardiovascular toxicity (Cornish et al. 1993) and reactive metabolite adduct formation from small molecule therapeutics (Copples et al. 1995). These limitations define a fulcrum in the risk to benefit balance for a patient with a lethal leukemia. I worked with a group at UNMC to investigate an antisense inhibitor of p53, an overexpressed gene in acute myelogenous leukemia (AML; (Bayever et al. 1993a)). I directed the effort to prepare an investigational new drug (IND) application to the US Food and Drug Administration (FDA) and gained permission to proceed to conduct the first evaluation of a systemically administered synthetic oligonucleotides in humans (Bayever et al. 1993b). The first-

in-human milestone with antisense led to an international press release, which aired, on CNN International in 1992. The appearance of my name in the interview process led to a request from King Hussain of Jordan to treat his lymphoma as well as hundreds of requests to treat patients with this emerging technology. The international recognition of my accomplishment supported my promotion to full professor, resulted in numerous invitations to present these first-ever clinical observations, and a position on the national board of directors of the Leukemia Society of America. I participated in the creation of a new biotechnology company, Lynx, as a spin off from Applied Biosystems and prepared IND documents for antisense targeting *bcr-abl* in collaboration with Bruno Callebretta at Thomas Jefferson University, *c-myc* with Andrew Zalewski at Thomas Jefferson University, and *c-myb* in collaboration with Alan Gewertz at the University of Pennsylvania. My research effort for each of these projects involved firsthand experience with the concept of translating synthetic DNA into a drug product by exploiting the questions of dose, dose interval, route of administration, and dose limitations by conducting the first pharmacokinetic and preclinical toxicology studies.

I identified numerous sequence-independent limitations associated with phosphorothioate (PSO) chemistry including: activation of complement, inhibition of coagulation, inhibition of cell surface receptors, stimulation of immune responses, and the generation of radical oxygen in cells producing 8-oxo-guanine adducts in RNA, DNA and the oligonucleotide. I looked at the radical oxygen production in a V79 cell line selecting for cells capable of growing in 6-thioguanine (HGPRT<sup>-/-</sup>) as well as oubain (Na/K ATPase<sup>-/-</sup>) and identified enhanced mutagenesis frequency. Later studies with phosphorodiamidate morpholino oligomers (PMO) compounds did not produce this radical oxygen response. While I felt publication of this mutagenesis activity was important, I was not able to convince our regulatory affairs people to explore this further for fear of finding something we did not want to know. An extension of this limitation was in the role of GLP studies in which contract research organizations do not recommend non-traditional mutagenesis assays in part due to lack of validated V79 cell lines. Mutagenesis assays are generally done with no intent to understand the process and the FDA does not demand much of these assays since they have some difficulty determining how to interpret the data. Basically, don't ask and don't tell until something arises in treated humans. While these off-target effects might be acceptable in the treatment of life threatening disease, the PSO as DNA chemistry would not be appropriate for the broad evaluation of genome function.

My first NIH RO1 research grant involved using synthetic oligonucleotides to inhibit the expression of cytochrome P450 enzymes (CYP). The observed phenotype was prolonged midazolam-induced sleep in rats as CYP3A2 is the only enzyme responsible for metabolism (clearance) of midazolam. The grant compared the *in vivo* efficacy, pharmacokinetics, and toxicity of a variety of oligomer chemistries including phosphorothioate oligoribonucleic acids, ribozymes (Desjardins et al. 1996), and PMO (Arora et al. 2000). The PMO are steric blockers and the hypothesis was that they would be less potent due to their lack of a catalytic mechanism of action such as RNase H, RNase P, or RISC. The PMO were significantly more potent inhibitors and more sequence selective. The research finding led to my

leaving the University to join AVI BioPharma, a biotechnology company in Corvallis Oregon where the PMO chemistry had been invented. In retrospect, the diverse actions of antisense oligomers illuminated the emerging sophistication of RNA in a living cell.

The first research project at AVI BioPharma used PMO to replace the PSO approach used by Lynx to targeting *c-myc* (Hudziak et al. 2000) for the prevention of restenosis of coronary arteries following balloon angioplasty (Kipshidze et al. 2002). This product, AVI-4126, was licensed to Medtronic and then to COOK Cardiology where phase II studies were completed and while the results were positive, AVI-4126 (Kipshidze et al. 2007) was not as effective as rapamycin coated stents in competing clinical trials.

## A New Paradigm

The idea that tumors arise when the rate of new cells exceeds the rate cells die led to interest in genes that regulate apoptosis. A simplistic view that an imbalance between genes promoting apoptosis like BAX and genes that prevent apoptosis like BCL-2. Reminiscent of inhibiting p53 to induce leukemia cell death, I targeted BCL-2 to inhibit anti-apoptosis and enhance BAX dominance to kill tumor cells. I boarded a jet in Eugene Oregon to San Francisco to meet my connecting flight 92 to Newark. It was September 11, 2001; my flight never left the runway in Eugene and the plane I planned to board in San Francisco had crashed in southern Pennsylvania. I was stunned, walking in a stupor out of the airport like a zombie. As I returned to my home, I could only think of the terrorists next steps. I resolved to investigate rapid development of anti-infectious disease agents with our antisense technology. I established a collaboration with Dr. Alvin Smith at Oregon State University to explore inhibition of Calicivirus in cell culture (Stein et al. 2001). I also engaged Dr. Bruce Geller to investigate the feasibility of targeting bacteria (Geller et al. 2003). Our anti-viral studies expanded to include SARS (Neuman et al. 2004), Dengue (Kinney et al. 2005), and West Nile Virus (Deas et al. 2005). The feasibility that an antisense molecule could be quickly designed with antiviral activity began to reshape the directions of AVI BioPharma.

Monday, February 9, 2004 I left my home in Corvallis at 5:00 am to check in for a flight from Portland (PDX) to Dulles (IAD) leaving at 8:00 am. I arrived at 4:00 pm (EST) collected my luggage and took a taxi to Fredrick Maryland in time for a 7:00 pm dinner meeting with Sina Bavari. We met Tuesday morning to discuss a letter of collaboration, begin preparing a CRADA (cooperative research and development agreement), and I gave a seminar describing AVI BioPharma technology to Sina's research group. I met with Dr. Javad Aman at 2:00 pm to discuss Ebola VP40 as a potential antisense target and a supplement to ongoing vaccine efforts. I met with Rekha Panchal at 4:00 to explore Anthrax bacterium as a project and then back to Sina at 5:00 pm to discuss targeting orthopox viruses. I had a short meeting with Dr. Kelly Warfield on Wednesday morning to discuss strategies for Ebola and



Marburg viruses and possible animal models for early studies. I left USAMRIID before lunch *en route* to Dulles for my flight back to PDX. Around 5:30 pm on that Wednesday, February 11, I was settling in on my flight Kelly Warfield was in the BSL-4 lab experiencing an accidental needle stick with a syringe that had been used to inject Ebola infected mice. On Thursday I met with Dr. David Hinrichs to discuss an immunology project involving c-FLIP and then on Friday, February 13th I had invited Ben Newman to give a seminar on our collaborative project involving SARS. Tom Stewart came to Corvallis for the seminar and to meet Ben. The morning was busy with plans for studies to evaluate the potential for SARS to become resistant to our antisense treatments.

Tom Stewart, Alan Timmins and I were in my office having returned from lunch when Sina Bavari called, “Would you guys do me a favor. Someone had an accident with Ebola virus in the BSL-4, a needle stick.” “Can you make enough PMO to treat?” We agreed to consider the feasibility. We recruited Dr. Denis Burger, CEO, and Dr. Dwight Weller, VP of Chemistry and Manufacturing, and we all agreed, “We should at least try.” This moment launched us into the hemorrhagic fever filovirus business. I felt two targets would be feasible and give us the best chance for success and proceeded to look at the GenBank sequence for Ebola genome. It was about 2:30 pm when I called Sina to confirm these would be a reasonable strategy. Next step was Dr. Dwight Weller and initiation of the effort for synthesis of two PMOs, one targeting the 3'-end of the viral genome and one targeting VP35. Synthesis was complete on Monday morning. I left Corvallis on Saturday to arrive in London Sunday morning to attend an international antisense meeting. On Wednesday at 8:00 pm I joined a conference call involving USAMRIID (Judy Pace, Sina Bavari, Dr. Mark Kortepeter, and Dr. James Martin), the FDA (Jeff O’Neil, Doug Throckmorton) and AVI BioPharma (Janet Christenson, Dwight Weller, Christina Fox, Doreen Weller, and Alan Timmins) to request compassionate use authorization to treat Kelly. The FDA requested endotoxin analysis for the substance, a search to ensure there are no human sequences targeted, an informed consent form, and an investigators brochure which we sent by FAX to Jeff O’Neil. We all agreed on a clinical event timeline predicated on a PCR positive sample from Kelly. Alan Timmins took a redeye flight to Dulles and hand delivered the two PMOs to USAMRIID on Wednesday morning. Elapsed time since the call from Sina, six days.

My group successfully targeted nearly every family of virus known to infect humans. We created AVI-4020 for the treatment of West Nile Virus and AVI-4065 for the treatment of hepatitis C virus (HCV) and both were evaluated in phase I clinical trials. Three more advanced examples include AVI-7537 for the treatment of Ebola Zaire, AVI-7288 for the treatment of Lake Victoria Marburgvirus (Warren et al. 2010), and AVI-7100 for the treatment of influenza A. The more advanced examples have completed single and multiple dose escalation human phase I trials (Heald et al. 2014, 2015). The use of viral genome sequence in the design of antiviral therapeutics led to the prospect of rapid response antiviral therapeutics for the development of countermeasures for emerging infectious disease. While these projects are currently dormant as there is no current business model to drive this work

forward. My research interest is in finding a workable model with either improved oligomer synthesis and or a global antiviral based on targeting host genes. Alternately, research to identify therapeutics to high value infections such as HIV, HBV, and HCV and to a lesser extent EBV, CMV, and HPV which would likely represent therapeutics to be used in combination with current and emerging standards of care.

## Induced Exon Skipping

We discovered AVI-4126 induced a downstream shift in the exon 2 splice acceptor of *c-myc*. The downstream site was downstream of the AUG translation start site and led to in-frame initiation of translation even further downstream resulting in a dominant negative variant of *c-myc*. I wanted to explore the utility of oligomer-induced manipulation of exon use. The first project involved collaboration with Steve Wilton in Perth Australia which led to the discovery and development of Eteplirsen, designed to induce skipping of exon 51 in the dystrophin transcript in boys with Duchenne Muscular Dystrophy (DMD). This project led Sarepta Therapeutics to the first FDA approved drug for the treatment of DMD, ExonDys 51. This research area is leading the way to finding solutions to an extensive array of rare genetic diseases by the pharmaceutical industry. I feel we have only scratched the surface of oligomer induced exon skipping studies. Our recent studies have exploited “alternate exon inclusion” to induce ligand independent receptors including the T cell co-receptor CTLA4 and ligand independent nuclear hormone receptors including the vitamin D receptor and the glucocorticoid receptor.

## Viruses with RNA Genomes

I spent years developing therapeutics for emerging RNA viruses (Ge et al. 2006; Iversen et al. 2012; Kinney et al. 2005; Lai et al. 2008; Neuman et al. 2005; Paessler et al. 2008; Stone et al. 2008). These viral genomes are unstable producing thousands of defective virions and a few successful infectious particles. This property results in inevitable resistance to antiviral therapeutics but demonstrates an important capacity to adapt. I joined Dr. Craig Marcus in the Department of Environmental & Molecular Toxicology at Oregon State University in 2012. We recruited Dr. Andrew Annalora as a research assistant professor to add structural biology expertise to our effort. The core lab effort centers on the cytochrome P450 enzymes and their interactions with nuclear hormone receptors. We explored transcript variants in the cytochrome P450 and nuclear hormone receptor families of genes finding unappreciated diversity in functional transcript variants (Annalora et al. 2017). An emerging hypothesis is that like RNA viruses, these genes express multiple transcript variants to enhance human adaptation to a changing environment. A key

distinction is that RNA provides the adaptive response to the environment and not alterations in DNA.

I now wish to integrate ideas, methods, technology, and emerging hypotheses. The early notions of a relationship between expression of CYP enzymes and the cell cycle have become more interesting. The CYP enzymes are generally suppressed during infection, a response that is rapid and observed after viral, bacterial, fungal, or parasitic infection. The hypothesis is that an endogenous CYP substrate is an inhibitor of DNA and RNA polymerases and suppression of CYP activity means elevated concentrations of endogenous inhibitor- a protective response. Aphidicolin, a fungal diterpenoid compound, is a potent inhibitor of DNA polymerase and a potential valuable anti-cancer agent but it is rapidly metabolized by CYP enzymes. The hypothesis is that various polymerases are linked to biological molecules in the environment and the CYP family modulate that activity. The diterpenes are chemically diverse with broad biological activity. Are these activities modulated by CYP expression? Can environmental exposure to chemical congeners disrupt the modulation? Alternately, the chemical carcinogenesis process I studied 20 years ago restricted interest to chemical adducts forming on DNA with disregard for the chemical congener RNA.

## Dark Matter of the Genome

Historically, RNA sequencing (RNASeq) was focused on the transcriptome, or the population of RNA with a poly (A) tail. However, recent improvements in our understanding of the genome have revealed that over 80 percent of the genome is transcribed into RNA, focusing new attention several emergent classes of non-coding RNA. Hence, much of the key information from GWAS has been overlooked inadvertently, as many variants are found in noncoding regions or unannotated regions of the genome (St. Laurent et al. 2014). Non-coding RNA (Table 1.1) can be divided into long non-coding RNA, lncRNA, and small non-coding RNA. The small ncRNA include: (1) miRNAs that are 18–21 nt in length and are involved in regulation of gene expression, (2) siRNA that are 21 nt segments that regulate gene expression and transposon activity, (3) rasiRNA are 24–27 nt that help form centromeres and orient heterochromatin, (4) piRNA or piwi-interacting RNA are 26–30 nt molecules that regulate transposon activity and chromatin, (5) snoRNA are 60–300 nt in length that create 2'-O-methyl and pseudo uridylation modifications of other RNAs, and (6) snRNAs that are 100–300 nt which are involved in assembly of the spliceosome and nonsense mediated decay (NMD) of pre-mRNA (Dogini et al. 2014). lncRNA are a novel class of functional RNAs that impinge on gene regulation involving recruitment of epigenetic modifier proteins, control of mRNA decay and DNA sequestration of transcription factors. The human genome appears to produce over 60,000 lncRNA, the majority expressed at low levels. Early examples of trans-acting lncRNAs are inhibitory polycomb repressive complex (PRC2) and the activating Trithorax/MLL chromatin modifiers HOTAIR and HOTTIP. The lncRNome contains cis-acting enhancer elements (eRNAs) which control the expression of

**Table 1.1** Expressed noncoding RNA

Name	Processing Mechanism	Function	Size
rRNA, ribosomal RNA	Exo- and endonuclease	mRNA translation	Large
tRNA, transfer RNA	RNaseP, RNaseZ, angiogenin	mRNA translation	90 nt
tRFs, tRNA fragments	Dicer, RNaseZ	Regulate translation initiation	13–30 nt
miRNA, micro RNA	Drosha and Dicer	Translation regulation	18-21 nt
siRNA, small interfering RNA	Dicer	RNA cleavage	21 nt
raRNA, repeat associated siRNA	Dicer	DNA/histone modifications	24–27 nt
piRNA, PIWI-interacting RNA	Ago3, aubergine, exo- and endonuclease	Regulate transposon activity	26–30 nt
snoRNA, small nucleolar RNA	5'- and 3'-exonuclease trimming	Pre-rRNA processing, modify tRNA, rRNA and snRNA bases	60–300 nt
sdRNA, sno-derived RNAs		Reulation of alternate splicing	
snRNA, small nuclear RNA	U1, U2, U4, U5: capping, U6-3'-end	Pre-mRNA splicing, nonsense mediated decay (NMD)	100-300 nt
lncRNA, long ncRNA	Transcription	Repression (PRC2), activation (HOTAIR), transcription enhancer (eRNA), alternate splicing (MALAT1), Staufen-mediated mRNA decay (SMD).	>200 nt
lincRNA, long intergenic ncRNA	Diverse	Regulation of splicing	>200 nt
vlincRNA, very long ncRNA	Transcription	Epigenetic regulation	>40 kb nt
Tel-sRNA, telomerase small RNA	Telomere transcript cleavage	Epigenetic regulation	
PARs, promoter-associated RNA	Transcription	Regulation of transcription	

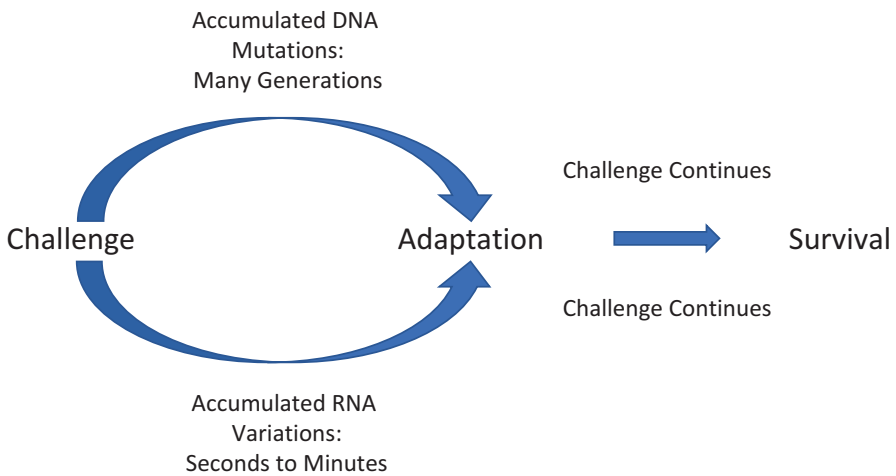
neighboring protein coding genes. A lncRNA mediating alternative splicing via assembly of serine/arginine splicing factors within subnuclear components called speckles, MALAT1, is retained in the nucleus. A lncRNA called TINCR binds to the 3'-UTR and elicits Staufen-mediated decay (SMD; (Kornfeld and Bruning 2014)).

Life is a battle of enthalpy over entropy. The membrane barriers and cell walls found in virtually every living thing separate the greater enthalpic state from the randomness of non-life. Each challenge toward entropy, lack of order, threatens the persistence of life. Changes in the ordered state are obligate to adapt to drivers of disorder. Biologists study living things and living systems making them experts in adaptations capable of repelling entropy.

The purpose of this book is to explore adaptive mechanisms from a molecular biology perspective. Evolution is a subject that embraces changes in DNA leading to survival and expansion in the number of species. The challenges involve the interplay of features from living things to changes in the environment, which captures the study of Ecology. While separate, evolution and ecology overlap.

Evolution requires reproduction for the vertical transmission of traits. Changes or mutations in the genome sequence lead to separations between individuals regarding which is “fittest” in the face of a selection pressure, or driver of entropy. The accumulation of fit traits leads to an organism best suited to live in a specific ecology. In the case of humans, the accumulation will take numerous generations, which are roughly 20 years in duration. In the case of a virus, the accumulation will take hours. In this regard, viruses are hundreds of thousands of times more adaptable than humans. The co-existence of humans and viruses might suggest viruses will always adapt and given the race to repel entropy, viruses always win. Bacteria, fungi, and protozoa will follow quickly leaving humans to a short pulse of history in life on Earth.

Look at this premise more closely. Humans have a shorter time of existence but given the adaptability gap, we should already be gone. This points to an alternative, rapidly adaptive survival source in humans running in parallel with evolution (Fig. 1.1). That human adaptive mechanism relies of the exceptional variability in RNA. The purpose of this book is to reveal the origins of this highly tuned adaptive system.



**Fig. 1.1** RNA Resilience Hypothesis. DNA mutations accumulate over generations and are associated with adaption over multiple generations. RNA variations are expressed in seconds to minutes and are well suited to rapid adaptation, a plausible mechanism for resilience

When I was a graduate student only 35 years ago, we thought 2–3% of our genome made RNA. Over the past decade, we have observed an explosion in RNA information. While nearly all of our genome makes RNA, we do not know the function of all the diverse forms of RNA. Some have referred to this phenomenon as the “Dark Matter” of the genome. The potential for these non-coding RNAs to play a role in regulating transcriptome plasticity and resilience came to mind.

The approach to this question takes elaboration of RNA mechanisms of adaptability, beginning with single-stranded RNA viruses. As life becomes more complex, the mechanisms of adaptability become more elaborate as in bacteria. Mammalian complexity takes adaptability to the edge of chaos. While an RNA virus relies on RNA to adapt, humans integrate both DNA and RNA mechanisms to adapt. Multicellular organisms elaborated enthalpy in immune defenses and recognition of small molecules in the environment. The goal of this book is to build a logical bridge to the development of genomic enthalpy and explore integration of adaption to exposure to small molecules.

My time as a bench scientists appears to be behind me. The exponential gains in scientific information force us to focus our attention to more refined subject matter. I have taken time at this late stage of my career to reflect and study a bigger research picture. The larger the subject net, the more information gems will be missed. A book that pushes thought boundaries should be more interesting to read. In addition, by laying down a few paving stones of thought, the hope is for future scientists to push these boundaries of understanding into workable models of adaption. These models may one day prove insightful in solving pressing problems or even a rationale to leave some problems alone.

## Overview

The first six chapters elaborate threats to human health. The Chap. 2 looks at how human populations spontaneously expand into niche boundaries exposing us to threats that drive the resilience process. The inevitable expansion beyond boundaries is termed “social entropy” since seeking life beyond boundaries is a fact of life. Resilience means life is often threatened but it is not fragile. Chapter 3 focuses on infectious disease, particularly emerging infectious single-stranded RNA viruses, as the most significant threat to human health. I review antiviral drugs including personal experiences with antiviral drug discovery and development. Viral genomes are sensitive to environmental conditions and have become resilience experts and evolution is mediated by RNA sequence plasticity. Chapter 4 describes “nonlinear anomalies” to highlight limitations in predicting the human outcome based on research studies with cells in culture and animal models. A personal experience with development of medical countermeasures for Ebola and Marburg viruses and interactions with the US FDA exploring the “Animal Rule” for drug approval. Chapter 5 focuses on bacterial infections and their diverse threat to human health. I include a brief review of antibacterial drugs and personal experience with bacterial resilience mediated by horizontal gene transfer. Chapter 6 shifts focus to cancer as a threat to

human health. Personal experience in discovery of novel therapeutics for the treatment of leukemia forms the body of the chapter. The spontaneous resolution of AML in children with Down syndrome highlights human resilience and the foundation for optimism in finding a cure to leukemia. Chapter 7 is a review of chemicals in the environment as a threat to human health. Brief examples of chemical carcinogenesis are included to illustrate how chemicals disrupt genomes. Historic research ignored RNA damage as a transient and irrelevant consequence of chemically induced nucleic acid damage. The emergence of important forms of RNA and their possible role in resilience is proposed.

The next section including Chaps. 8–10 discuss threat recognition and defense systems responding to improve resilience. Chapter 8 describes a key mediator of resilience, the immune response, as a threat recognition system and response via diverse RNA expression. Research into oligonucleotides designed to suppress specific RNA to manipulate the immune response including strategies to induce exon-skipping are described. Chapter 9 describes a parallel threat recognition and response system in the human cytochrome P450 (CYP) family of enzymes. The interesting observation that infections modulate CYP expression points to a role parallel to the immune response. CYP expression includes diverse splice variants that are largely uncharacterized. I propose metabolic clearance of small molecules as a metabolic companion to the immune system. Chapter 10 highlights the diversity in RNA expressed from a single gene. This represents a list of mechanisms for RNA transcriptome plasticity that provide the basis for resilience at the molecular level.

Chapter 11 is a personal account of the recently approved drug for the treatment of Duchenne muscular dystrophy, eteplirsen (Exondys 51). Based on observations of “spontaneous revertant muscle fibers” it was clear that we could design a drug to induce skipping of the dystrophin exon 51 to restore reading frame that would lead to expression of functional dystrophin. Controversy in the approval process highlights resistance to paradigm shifting technologies.

Chapter 12 is the summary. I address the question “what informs molecular mechanisms of resilience” that drives the limits to the adaptive response oscillation and provides boundaries for molecular resilience. I speculate that radical oxygen, epigenetic modifications, and ligands to nuclear hormone receptors play critical roles in regulating molecular resilience.

## References

- Annalora AJ, Marcus CB, Iversen PL. Alternative splicing in the cytochrome P 450 superfamily expands protein Diversity to Augment gene function and redirect human drug metabolism. *Drug Metab Dispos.* 2017;45:375–89.
- Arora V, Knapp DC, Smith BL, Stadtfield ML, Stein DA, Reddy MT, Weller DD, Iversen PL. C-Myc antisense limits rat liver regeneration and indicates role for c-myc in regulating cytochrome P-450 3A activity. *J Pharmacol Exp Ther.* 2000;292:921–8.

- Bayever E, Haines K, Iversen PL, Ruddon RW, Pirruccello SJ, Mountjoy CP, Arneson MA, Smith LJ. Selective cytotoxicity of human leukemic Myeloblasts produced by Oligodeoxyribonucleic Phosphorothioate complementary to p 53 nucleotide sequences. *Leuk Lymphoma*. 1993a;12:223–31.
- Bayever E, Iversen PL, Bishop M, Sharp JG, Tewary HK, Arneson MA, Pirruccello SJ, Ruddon RW, Kessinger A, Zon G, Armitage JO. Systemic administration of a Phosphorothioate oligonucleotide with a sequence complementary to p 53 for acute myelogenous leukemia and myelodysplastic syndrome: initial results of a phase I trial. *Antisense Res Dev*. 1993b;3:383–90.
- Copple BL, Gmeiner WM, Iversen PL. Reaction between metabolically activated acetaminophen and Phosphorothioate oligonucleotides. *Toxicol Appl Pharmacol*. 1995;133:53–63.
- Cornish KG, Iversen PL, Smith LJ, Arneson MA, Bayever E. Cardiovascular effects of a phosphorothioate oligonucleotide with sequence antisense to p 53 in the conscious rhesus monkey. *Pharmacol Commun*. 1993;3:239–47.
- Deas TS, Binduga-Gajewska I, Tilgner M, Ren P, Stein DA, Moulton HM, Iversen PL, Kauffman EB, Kramer LD, Shi P-Y. Inhibition of Flavivirus infections by antisense oligomers specifically suppressing viral translation and RNA replication. *J Virology*. 2005;79(8):4599–609.
- Desjardins JP, Iversen PL. Inhibition of the rat cytochrome P 450 3A2 by an antisense Phosphorothioate Oligodeoxynucleotide *in vivo*. *J Pharm Exptl Ther*. 1995;275(3):1608–13.
- Desjardins J, Mata J, Brown T, Zon G, Iversen PL. Cholesteryl-conjugated phosphorothioate oligonucleotides modulate CYP2B1 expression *in vivo*. *J Drug Target*. 1995;2:477–85.
- Desjardins JP, Sproat BS, Beijer B, Blaschke M, Dunkel M, Gerdes W, Ludwig J, Reither V, Rupp T, Iversen PL. Pharmacokinetics of a synthetic chemically modified hammerhead ribozyme against the rat cytochrome P-450 3A2 mRNA after single intravenous injection. *J Pharm Exptl Ther*. 1996;278(3):1–9.
- Dogini DB, Pascoal VDAB, Avansiini SH, Viera AS, Pereira TC, Lopes-Cendes I. The new world of RNAs. *Genet Mol Biol*. 2014;37:285–93.
- Egan W, Boal J, Iyer R, Strom C, Wilson S, Meyer A, Iversen P. Abasic oligodeoxyribonucleotide phosphorothioates as inhibitors of the human immunodeficiency virus-1 (HIV-1): phosphorothioate inhibition of HIV-1 reverse transcriptase and interactions with Syrian hamster fibroblast (V79) cells. *Nucleosides Nucleotides*. 1991;10(1–3):457–60.
- Ge Q, Pастey M, Kobasa D, Puthavathana P, Lupfer C, Bestwick RK, Iversen PL, Chen J, Stein DA. Inhibition of multiple subtypes of influenza A virus in cell cultures with morpholino oligomers. *Antimicrob Agents Chemother*. 2006;50(11):3724–33.
- Geller BL, Deere JD, Stein DA, Kroeker AD, Moulton HM, Iversen PL. Inhibition of gene expression in *Escherichia coli* by antisense Phosphorodiamidate Morpholino oligomers. *Antimicrob Agents Chemother*. 2003;47:3233–9.
- Hanson GR, Partlow LM, Iversen PL. Neuronal stimulation of non-neuronal (glial) cell proliferation: lack of specificity between different regions of the nervous system. *Dev Brain Res*. 1982;3:547–55.
- Heald AE, Iversen PL, Saoid JB, Sazani P, Charleston JS, Axtelle T, Wong M, Smith WB, Vutikullird A, Kaye E. Safety and pharmacokinetic profiles of Phosphorodiamidate Morpholino oligomers with activity against Ebola virus and Marburg virus: results of two single ascending dose studies. *Antimicrob Agents Chemother*. 2014;58(11):6639–47. [25155593](#)
- Heald AE, Charleston JS, Iversen PL, Warren T, Saoud JB, Al-Ibrahim M, Wells J, Warfield KL, Swenson DL, Welch L, Sazani P, Wong M, Berry D, Kaye E, Bavari S. AVI-7288 for Marburg virus in nonhuman Primates and humans. *N Engl J Med*. 2015;373(4):339–48. [26200980](#)
- Hines RN, Levy JB, Conrad RD, Iversen PL, Shen ML, Bresnick E. Gene structure and nucleotide sequence for rat cytochrome P-450c. *Arch Biochem Biophys*. 1985;237:467–76.
- Hudziak RM, Summerton J, Weller DD, Iversen PL. Antiproliferative effects of steric blocking Phosphorodiamidate Morpholino antisense agents directed against c-myc. *Antisense Nucleic Acid Drug Dev*. 2000;10:163–76.
- Iversen PL. *In vivo* studies with Phosphorothioate oligonucleotides: pharmacokinetic Prologue. *Anticancer Drug Des*. 1991;6:531–8.



- Iversen PL, Partlow LM, Stensaas LJ, Motamed F. Characterization of a variety of standard collagen substrates: ultrastructure, uniformity, and the capacity to bind and promote growth of neurons. *In Vitro*. 1981;17(5):540–52.
- Iversen PL, Liu Z, Franklin MR. Selective changes in cytochrome P-450 and UDP-glucuronosyl transferase subpopulations following partial hepatectomy in rats. *Toxicol Appl Pharmacol*. 1985;78:10–8.
- Iversen PL, Heiger WJ, Bresnick E, Hines RN. Structural details of the human cytochrome P-450c gene. *Arch Biochem Biophys*. 1987;256:397.
- Iversen PL, Mata JE, Tracewell WG, Zon G. Pharmacokinetics of an antisense Phosphorothioate Oligodeoxynucleotide against *rev* from human immunodeficiency virus type 1 in the adult male rat following single injections and continuous infusion. *Antisense Res Dev*. 1994;4:43–52.
- Iversen PL, Warren TK, Wells JB, Garza NL, Mourich DV, Welch LS, Panchal RG, Ad Bavari S. Discovery and early development of AVI-7537 and AVI-7288 for the treatment of Ebola virus and Marburg virus infections. *Viruses*. 2012;4(11):280–2830. [23202506](#)
- Kessel SR. Wildland inventories and fire modeling by gradient analysis in glacier National Park. In: *Gradient modeling: resource and fire Management*: Springer; 1976. p. 115–62.
- Kinney RM, Huang CL-H, Rose BC, Kroeker AD, Dreher TW, Iversen PL, Stein DA. Inhibition of dengue virus serotypes 1 to 4 in cell culture with Morpholino oligomers. *J Virology*. 2005;79(8):5116–28.
- Kipshidze NN, Kim H-S, Iversen PL, Yazdi HA, Bhargava B, Mehran R, Haundenschild C, Dangas G, Stone GW, Roubin GS, Leon MB, Moses JW. Intramural delivery of advanced antisense oligonucleotides with infiltrator Catheter inhibits c-myc expression and intimal hyperplasia in the porcine. *J Am Col Cardiology*. 2002;39(10):1686–91.
- Kipshidze N, Iversen P, Overlie P, Dunlap T, Titus B, Lee D, Moses J, O’Hanley P, Lauer M, Leon MB. First human experience with local delivery of novel antisense AVI-4126 with infiltrator catheter in de novo native and restenotic coronary arteries: 6-month clinical and angiographic follow-up from AVAIL study. *Cardiovas Revasc Med*. 2007;8(4):230–5.
- Kornfeld JW, Bruning JC. Regulation of metabolism by long, non-coding RNAs. *Front Genet*. 2014;5:1–8.
- Lai SH, Stein DA, Liao SL, Guerrero-Plata A, Hong C, Iversen PL, Casola A, Garofalo RP. Inhibition of respiratory Syncytial virus infections in cell cultures and in mice with Morpholino oligomers. *Mol Ther*. 2008;16(6):1120–8.
- Neuman BW, Stein DA, Kroeker AD, Paulino AD, Moulton HM, Iversen PL, Buchmeier MJ. Antisense Morpholino oligomers directed against the 5’-end of the genome inhibit coronavirus proliferation and growth. *J Virol*. 2004;78(11):5891–9.
- Neuman BW, Stein DA, Kroeker AD, Churchill MJ, Kim AM, Dawson P, Moulton HM, Bestwick RK, Iversen PL, Buchmeier MJ. Inhibition, escape and attenuation of SARS coronavirus treated with antisense morpholino oligomers. *J Virology*. 2005;79:9665–76.
- Paessler S, Rijnbrand R, Stein DA, Ni H, Yun NE, Dziuba N, Borisevich V, Seregin A, Ma Y, Blouch R, Iversen PL, Zacks MA. Inhibition of alphavirus infection in cell culture and in mice with antisense morpholino oligomers. *Virology*. 2008;5(2):357–70.
- Schlegel RA, Iversen PL, Rechsteiner MC. The turnover of tRNAs microinjected into animal cells. *Nucleic Acids Res*. 1978;5(10):3715–29.
- Skorski T, Nieborowska-Skorska M, Nicolaidis NC, Szczylik C, Iversen P, Isso RV, Zon G, Calabretta B. Suppression of Philadelphia leukemia cell growth in mice by bcr-abl antisense oligodeoxynucleotides. *Proc Natl Acad Sci U S A*. 1994;91:4504–8.
- St. Laurent G, Vyatkiin Y, Kapranov P. Dark matter RNA illuminates the puzzle of genome-wide association studies. *BMC Med*. 2014;12:97–105.
- Stein DA, Skilling DE, Iversen PL, Smith AW. Inhibition of Vesivirus Infections in mammalian tissue culture with antisense Morpholino oligomers. *Antisense Nucleic Acid Drug Dev*. 2001;11:317–25.
- Stevenson M, Iversen PL. Inhibition of HIV mediated Cytopathicity by poly-L-lysine conjugated synthetic antisense Oligodeoxyribonucleotides. *J Gen Virol*. 1989;70:2673–82.

- Stone J, Stein DA, Rijnbrand R, Iversen PL, Andino R. Inhibition of multiple species of picornavirus using a morpholino oligomer targeting highly conserved IRES sequence. *Antimicrob Agents Chemother.* 2008;52(6):1970–81.
- Warren TK, Warfield KL, Wells J, Swenson DL, Donner KS, Van Tongeren SA, Garza NL, Dong L, Mourich DV, Crumley S, Nichols DK, Iversen PL, Bavari S. Advanced antisense therapies for postexposure protection against lethal filovirus infections. *Nat Med.* 2010;16:991–4.
- Worley C. et al. Ecological planning for a safe environment: studies of man's impact on the Utah Strawberry reservoir. Westminster College, Salt Lake City, Utah June 1, 1976-August 31, 1976. 1977.