November 9, 2017

Fred Russell Kramer

Personal

Birth	July 7, 1942 – New York City
Family	Married forty years, widowed, two children, four grandchildren

Education

1956 - 1959	The Bronx High School of Science
1959 - 1964	University of Michigan – B.S. with Honors in Zoology
1964 - 1969	The Rockefeller University – Ph.D. (with Vincent Allfrey)
1969 - 1972	Columbia University – Postdoctoral training (with Sol Spiegelman)

Appointments

1962 - 1964 Laboratory Technician, Cytogenetics Laboratory
Carnegie Institution of Washington, Ann Arbor, Michigan

1969 - 1986 Department of Genetics and Development
and Institute of Cancer Research
College of Physicians and Surgeons
Columbia University

1969 - 1971	Fellow of the American Cancer Society
1971 - 1972	Research Associate
1972 - 1973	Instructor
1973 - 1980	Assistant Professor
1980 - 1983	Senior Research Associate
1983 - 1986	Research Scientist

1986 - present The Public Health Research Institute

1986 - present	Co-Director, Laboratory of Molecular Genetics
2000 - 2006	Director, PHRI Office of Technology Transfer
2006 - 2010	Associate Director of PHRI for Technology Transfer
2012 - 2017	Associate Director of PHRI for Business Development

1987 - 2014 Department of Microbiology
New York University School of Medicine

1987 - 2003	Research Professor
2003 - 2014	Adjunct Professor

2003 - present Professor of Microbiology, Biochemistry and Molecular Genetics
Public Health Research Institute, New Jersey Medical School

2003 - 2013	University of Medicine and Dentistry of New Jersey
2013 - present	Rutgers, The State University of New Jersey
2015 - present	Associate Member, Cancer Institute of New Jersey

Awards

2005 Jacob Heskel Gabbay Award in Biotechnology and Medicine

Professional groups

American Association of University Professors
American Society for Biochemistry and Molecular Biology
American Society for Microbiology
Association for Molecular Pathology
New York Academy of Sciences

Fred Russell Kramer

Laboratory of Molecular Genetics

Public Health Research Institute

RESEARCH SYNOPSIS

For the past forty-eight years, our laboratory has been exploring nucleic acid structure to understand the role that it plays in macromolecular interactions that control biological processes. The work has led to the design of novel nucleic acid molecules and the development of experimental techniques that enable the construction of extremely sensitive and specific molecular diagnostic assays. More than one hundred people have worked in the laboratory or participated as close collaborators. The following paragraphs provide a sketch of some of the significant research themes that the laboratory has pursued.

Mechanism of RNA replication

The laboratory studied the mechanism of RNA-directed RNA synthesis catalyzed by the bacteriophage polymerase, Qβ replicase. No one knew the mechanism by which the viral replicase selectively copies Qβ genomic RNA, while ignoring the vast number of other RNA molecules that are present in the bacterial host. Qβ RNA was too large to be studied with the techniques that were then available. However, we discovered a much smaller RNA (MDV-1 RNA) in Qβ-infected *Escherichia coli* that is replicated in the same manner as Qβ RNA (Kacian *et al*., 1972). Using classical enzymatic and electrophoretic techniques, we determined the complete nucleotide sequence of both complementary strands of MDV-1 RNA (Mills *et al*., 1973). This was the longest nucleic acid that had ever been sequenced. Knowledge of the sequence enabled experiments to be carried out that provided insights into the mechanism of RNA replication. We discovered that each complementary strand of MDV-1 RNA possessed extensive secondary structures (Klotz *et al*., 1980). We demonstrated that the rate of RNA synthesis was determined by pauses in polymerization that occur where secondary structures form in the nascent strand (Mills *et al*., 1978), and we showed that structural reorganizations occur during product strand elongation (Kramer & Mills, 1981). We also developed an electrophoretic technique for separating the complementary strands that enabled the elucidation of the overall mechanism of RNA-directed RNA synthesis (Dobkin *et al*., 1979), and we utilized chemical and enzymatic nucleic acid modification methods to identify the sequences and structures that are required for the selective recognition of the RNA by the replicase, and for the initiation of product strand synthesis (Mills *et al*., 1980; Bausch *et al*., 1983; Nishihara *et al*., 1983).

Novel nucleic acid sequencing techniques

Rapid nucleic acid sequence analysis was essential to further studies of replication. We developed a chain-termination method for RNA sequence analysis (Kramer & Mills, 1978) at the same time that Fred Sanger developed a chain-termination method for DNA sequence analysis. Knowledge of the extensive secondary structure of MDV-1 RNA led us to realize that both of these sequencing techniques were compromised by the persistence of strong secondary structures during electrophoretic separation of the partially synthesized strands. We introduced a widely adopted solution to this problem, which was based on the use of modified nucleosides, such as inosine, that form weaker secondary structures (Mills & Kramer, 1979). Years later, we conceived novel techniques that enable entire genomes to be sequenced in a concerted manner by hybridization to oligonucleotide arrays (Chetverin & Kramer, 1993; 1994). These techniques were licensed exclusively to the Affymetrix Corporation (U.S. Patents 6,103,463 and 6,322,971).

In vitro evolution of replicating RNA populations

The *in vitro* replication of RNA by Qβ replicase provides a model system for studying precellular evolution. When MDV-1 RNA is replicated *in vitro*, the number of RNA molecules doubles every 20 seconds, resulting in an exponential increase in the number of RNA strands. Occasionally, errors occur during replication, producing RNA molecules with a mutated nucleotide sequence. When replication is carried out in the presence of an inhibitor of replication, mutant molecules that resist the inhibitor have a selective advantage, and if allowed to replicate for hundreds of generations, these mutants become predominant in the RNA population. Since phenotype and genotype reside in the same molecule, sequence analysis of the selected RNAs provided insights into the mechanism of Darwinian evolution.

Our laboratory carried out extensive studies on the *in vitro* evolution of replicating populations of MDV-1 RNA. Utilizing serial transfer techniques, hundreds of replicative generations could be completed in a day. By imposing different selective pressures, different variants emerged. Sequence analysis of the replicating RNA populations at different times during their evolution elucidated how the nucleotide changes that occurred conferred resistance to the particular inhibitor that was used (Kramer *et al*., 1974). Parallel molecular evolution experiments carried out in the presence of the chain elongation inhibitor, ethidium bromide, confirmed that many different genotypic pathways lead to the same phenotypic result, just as in the evolution of organisms. These experiments laid the foundation for modern *in vitro* selection techniques that are used to isolate nucleic acid molecules possessing predetermined catalytic activities.

The results of the *in vitro* evolution experiments also provided useful insights into the structural constraints that are required for an RNA to be replicatable. Though mutations occur everywhere in an RNA, the only mutations selected during the evolution of MDV-1 RNA occurred in single-stranded regions of the molecule, indicating that double-stranded structures are essential to the replicative process. When ribonuclease T1 was used as a selective agent, the mutants that arose were significantly resistant to the nuclease. The macromolecular dimensions of both the nuclease and the RNA limited cleavage to only a few sites on the exterior of the RNA molecule. The selected RNAs possessed non-cleavable nucleotide substitutions at just those exposed sites. These experiments elucidated the tertiary structure of MDV-1 RNA, enabling us to design exponentially amplifiable recombinant RNAs.

Recombinant RNAs

Many investigators wished to use the exponential amplification of RNA by Qβ replicase to synthesize large amounts of any mRNA or any genomic RNA. However, Qβ replicase is highly specific for Qβ phage RNA. We devised a scheme that enabled the replication of any heterologous RNA. Novel RNA templates were constructed by covalently inserting heterologous RNA sequences within the MDV-1 sequence at a single-stranded site that occurs on the exterior of the MDV-1 RNA molecule (Miele *et al*., 1983). The resulting recombinant RNAs possessed all of the secondary and tertiary structures that are required for replication, and the presence of the inserted sequence on the exterior of the molecule did not interfere with access to the structures required for replication. Consequently, Qβ replicase was able to catalyze the exponential synthesis of the entire recombinant RNA. Moreover, the recombinant RNAs were bifunctional, in that they retained the biological activity of the inserted sequence, as well as the replicatability of the MDV-1 RNA.

We constructed recombinant RNAs that contained the entire mRNA sequence encoding chloramphenicol acetyltransferase. These recombinant molecules were amplified exponentially *in vitro* by incubation with Qβ replicase, and the replicated RNA served as template for the cell-free synthesis of enzymatically active chloramphenicol acetyltransferase (Wu *et al*., 1992). We demonstrated that these recombinant mRNAs could be continuously synthesized and that large quantities of biologically active protein could be produced in a coupled replication-translation system that contained both Qβ replicase and bacterial ribosomes (Ryabova *et al*., 1994). We also constructed amplifiable recombinant RNAs that contained entire viroid genomes (U.S. Patent 5,871,976), and the recombinant, by itself, was infectious when placed on the leaves of tomato plants.

Extremely sensitive gene detection assays

With the advent of the AIDS crisis, it became imperative that very sensitive assays be developed for the detection of pathogenic retroviruses. We realized that an attractive strategy for detecting rare targets is to link a nucleic acid probe to a replicatable reporter that can be amplified exponentially after hybridization to reveal the presence of the target (Chu *et al*., 1986). We therefore covalently linked MDV-1 RNA to an oligonucleotide probe that was complementary to a predetermined genetic target. The resulting molecules were used in assays in which the probes bind specifically to target sequences, unbound probes are washed away, and the probe-target hybrids are incubated with Qβ replicase to generate a large number of easily detected reporter molecules. Since as little as a single molecule of MDV-1 RNA can serve as template for the exponential synthesis of millions of RNA copies by Qβ replicase, these assays were extremely sensitive.

We also realized that it was simpler to perform these assays with recombinant MDV-1 RNA molecules in which a probe sequence is embedded within the MDV-1 RNA, rather than being attached to the RNA by a linker. We constructed recombinant-RNA probes and demonstrated that they were bifunctional, in that they bound specifically to their targets, and after they were bound they served as templates for their own exponential amplification (Lizardi *et al*., 1988). We demonstrated that recombinant-RNA hybridization probes could be used in sensitive gene detection assays (Lomeli *et al*., 1989; Kramer *et al*., 1992). The inclusion of intercalating fluorescent dyes, such as ethidium bromide, in the reaction mixtures to detect the reporter RNA enabled the assays to be carried out in real-time under homogeneous conditions in sealed tubes (Kramer & Lizardi, 1989; Lomeli *et al*., 1989). We also demonstrated that the time required to synthesize a given quantity of reporter RNA is inversely proportional to the logarithm of the number of target molecules originally present in a sample, thus enabling quantitative determinations over an extremely wide range of target concentrations (U.S. Patent 5,503,979). This quantitative analytical technique has found wide application in real-time clinical assays that utilize polymerase chain reactions.

The sensitivity of Qβ replicase assays employing recombinant RNAs was limited by the inability to wash away every unbound probe. Persistent nonhybridized probes were amplified along with hybridized probes, generating a background signal that obscured the presence of rare targets. We investigated a number of different ways to eliminate this background (Kramer & Lizardi, 1989; Blok *et al*., 1997; U.S. Patents 5,118,801 and 5,312,728). Rather than trying to improve existing washing techniques (which were already quite efficient), we altered the design of the probes so that they could not be replicated unless they were hybridized to their target. We divided the recombinant-RNA probes into two separate molecules, neither of which could be amplified by itself, because neither contained all of the elements of sequence and structure that are required for replication by Qβ replicase. The division site was located in the middle of the embedded probe sequence. When these "binary probes" were hybridized to adjacent positions on their target sequence, they could be joined to each other by incubation with an appropriate ligase, generating a replicatable reporter RNA, which was then exponentially amplified by incubation with Qβ replicase. Nonhybridized probes, on the other hand, because they were not aligned on a target, could not be ligated, and signal generation was strictly dependent on the presence of target molecules. Because there were no background signals, the resulting assays were extraordinarily sensitive. As little as a single HIV-1 infected cell could be detected in samples containing 100,000 uninfected lymphocytes (Tyagi *et al*., 1996). This technique was licensed to Abbott Laboratories (U.S. Patents 5,759,773 and 5,807,674) and has been used in automated assays that detect the genes of many different infectious agents in human clinical samples.

Molecular beacons

We invented novel hybridization probes called "molecular beacons," which enable the direct detection of specific nucleic acids in living cells and in diagnostic assays (Tyagi & Kramer, 1996). These probes are hairpin-shaped oligonucleotides with a fluorophore at one end and a nonfluorescent quencher at the other end. When they are not bound to a target nucleic acid, the fluorophore is in contact with the quencher and the probes are dark. When these probes bind to their targets, they undergo a conformational reorganization that separates the fluorophore from the quencher, resulting in a bright fluorescent signal that indicates the presence of the target. Because these probes only fluoresce when they are bound to target sequences, there is no need to isolate the probe-target hybrids

We showed that the mechanism of fluorescence quenching involves the transient formation of a nonfluorescent fluorophore-quencher complex, thus any desired fluorophore can be used as a label (Tyagi *et al*., 1998; Marras *et al*., 2002). When a set of molecular beacons are prepared, each specific for a different target sequence, and each labeled with a differently colored fluorophore, different nucleic acid targets can be detected simultaneously in the same assay tube or in the same cell. Moreover, by taking their thermodynamic behavior into consideration (Bonnet *et al*., 1999), molecular beacons can be designed so that they are significantly more specific than corresponding conventional linear hybridization probes. Molecular beacons can be designed in such a manner that the presence of even a single nucleotide substitution in a target sequence prevents the formation of a probe-target hybrid (Tyagi *et al*., 1998; Marras *et al*., 1999).

Our laboratory demonstrated the advantages of using molecular beacons as amplicon detector probes in quantitative, real-time, exponential amplification assays. We designed extremely sensitive, multiplex, clinical PCR assays that simultaneously detect four different pathogenic retroviruses in blood (Vet *et al*., 1999); and we designed "wavelength-shifting" molecular beacons (Tyagi *et al*., 2000) that enable many different genetic targets to be detected simultaneously in the same sample, utilizing simple instruments that possess a monochromatic light source. We also pioneered the use of molecular beacons for high-throughput "spectral genotyping" (Kostrikis *et al*., 1998); and we demonstrated the ease with which molecular beacons can distinguish single-nucleotide polymorphisms in PCR assays (Marras *et al*., 1999). We showed that molecular beacons work well in NASBA assays (Van Beuningen *et al*., 2001), as well as in PCR assays; and we demonstrated how molecular beacons can be used to monitor *in vitro* transcription in real time (Marras *et al*., 2004).

Our laboratory also designed a panel of assays that identify mutations in potential parents that cause Tay-Sachs disease and cystic fibrosis in the children of Ashkenazi Jews; and we developed a single-tube, multiplex assay that utilizes molecular beacons for the detection of bacteria that can be used as agents of bioterror: *Yersinia pestis*, *Bacillus anthracis*, *Burkholderia mallei*, and *Francisella tularensis*. We also developed a single-tube version of a PCR assay that rapidly identifies multidrug-resistant *Mycobacterium tuberculosis* in sputum samples (El-Hajj *et al*., 2001). This assay underwent clinical trials (Varma-Basil *et al*., 2004), was developed for commercial distribution, was endorsed by the World Health Organization, and is now the principal assay for the direct detection of tuberculosis utilized throughout the world. We have also contributed to the development of assays that detect hospital-acquired infections caused by pathogenic fungi and by methicillin-resistant and vancomycin-resistant *Staphylococcus aureus*.

Highly multiplex screening assays

Our laboratory has developed multiplex screening assays that utilize color-coded molecular beacons in single-tube gene amplification reactions that identify which infectious agent, if any, is present in a clinical sample (U.S. Patents 7,385,043 and 7,771,949). The first assay of this type is able to identify the 15 most prevalent bacterial species that are found in blood samples taken from febrile patients (Marras *et al*., 2017). Unlike classical blood cultures, which take many days to yield results, these “molecular blood cultures” require only two hours to complete. Each of the 15 species-specific molecular beacons is labeled with a unique combination of two differently colored fluorophores selected from a palette of six differently colored fluorophores. The two-color fluorescence signal that arises during the course of a PCR assay that amplifies a segment of the bacterial 16S ribosomal RNA gene uniquely identifies the species that is present. Future assays will utilize three differently colored fluorophores (selected from a palette of seven colors) to uniquely label each of 35 species-specific molecular beacons. This will enable simultaneous screening for the presence of both common species and rarely seen species, such as agents of bioterror. Widespread use of these assays will enable the rapid identification of common infectious agents, while at the same time providing an early warning system that will help contain the spread of major epidemics.

We have also designed highly multiplex screening assays based on a different principle. In these assays, only four differently colored molecular beacons are present during the amplification of a segment of the bacterial 16S ribosomal RNA gene. Unlike the assays described above, these molecular beacons contain relatively long probe sequences, enabling them to bind to amplified 16S ribosomal RNA gene

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.