AMERICAN COLLEGE OF EMERGENCY PHYSICIANS
SCIENTIFIC ASSEMBLY
1999

What Every Emergency Physician Should Know: Physical Examination

Making the diagnosis at the bedside---and doing it quickly---is the hallmark of emergency medicine. Physical examination skills are quick, inexpensive, sensitive, and specific for making diagnoses. The lecturer will highlight several specific, clever, and interesting aspects of the physical examination that are relevant to the day-to-day practice of emergency medicine.

- Describe three physical examination findings uniquely useful to the emergency physician.
- Demonstrate the performance of these physical examination findings.
- List the differential diagnosis of three physical examination findings.

MO-30
Monday, October 11, 1999
1:30 PM - 2:25 PM
Room # N247
Las Vegas Convention Center

FACULTY

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WHAT EVERY EMERGENCY PHYSICIAN SHOULD KNOW:

BASIC SCIENCE (MOLECULAR BIOLOGY)

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COURSE OBJECTIVES:

- Discuss two basic principles of molecular biology
- Describe the influence of basic science on emergency medicine clinical practice

This lecture will focus on some basic principles of molecular biology and their impact on the current and future clinical practice of emergency.

Two basic principles of molecular biology applicable to emergency medicine:
- polymerase chain reaction
- recombinant DNA technology

Influence of molecular biology on emergency medicine practice:
- diagnosis of infectious disease
- treatment of acute myocardial infarction
- management of cardiac arrhythmias

"The era when the physician will require a working knowledge of this discipline [molecular genetics] and an ability to apply its power is no longer approaching; it is here." Editorial, New England Journal of Medicine, 1982

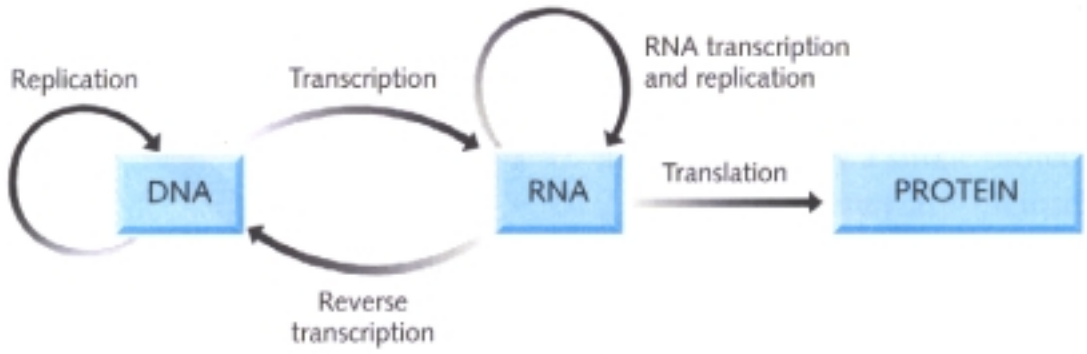
Despite the statement nearly two decades, the principles of molecular biology remain largely unknown to the emergency physician. Why the tardy acceptance?
- Emergency medicine is a clinical specialty even within the academic community. Clinical service burden limit free time to learn the basics of a "new" science discipline.
- The techniques of molecular biology are not a natural extension of basic medical medical education or our specialized interests.
- The nomenclature of molecular biology is a foreign language.
- The molecular biologist is not particularly interested in nonproliferating, terminally differentiated cells (ie, the medical patient).
- The application of molecular techniques to clinical medicine is recent.
- It is unclear whether medicine is trying to embrace molecular biology or whether the reverse is true.

The relevant history of molecular biology is comparatively brief:
- 1944: DNA identified as genetic material in bacterial cells
- 1951: DNA analysis reveals equal amounts of adenine-thymine and guanine-cytosine
- 1953: Double helical structure (Watson and Crick)
- 1960: RNA carries genetic info that codes for proteins
- 1966: Genetic code elucidated

- 1970: Discovery and application of specific restriction endonucleases
- 1970: Discovery of reverse transcriptase making it possible to develop a complimentary DNA (cDNA) for mRNA
- 1972: First recombinant DNA molecules are constructed
- 1973: Birth of cloning

- 1975-1977: Rapid sequencing of DNA

Basic principles and history can be summarized in a single diagram:



This sequence acts in both left to right and right to left directions. You can produce DNA if the protein sequence is known.

THE BASIC GLOSSARY OF MOLECULAR BIOLOGY

Gene components:
Codon—three consecutive base pairs of DNA or RNA that code for an individual amino acid.
Exon—the segments of a gene that remain after the splicing of the primary RNA transcript → mature RNA.
Intron—a segment of a gene that is transcribed into the pirmary nuclear RNA transcript but is excised during exon splicing. Also referred to as intervening sequences.
Coding region—portion of the gene or mRNA containing the nucleotide sequences that are translated into the corresponding protein.

Enzymes:
Ligase—enzyme involved in repairing the ends of DNA, resulting in the joining of two separate DNA fragments.
Polymerase—enzymes that synthesize sequences of RNA (RNA polymerase) or DNA (DNA polymerase) using the corresponding DNA template.
Reverse transcriptase—an enzyme that catalyzes the synthesis of DNA from a RNA template.
Restriction enzyme—enzymes that cut DNA at specific sequences in the double-stranded DNA molecule. Usually of bacterial origin.

Things needed in synthesis:
cDNA—a single-stranded DNA fragment that is synthesized from the mRNA strand by reverse transcriptase; also refers to double-stranded DNA carried in a cloning vector that is complementary to a specifc mRNA.
Oligonucleotide—a short polymer of DNA or RNA that is usually synthetic in origin; used for several important DNA techniques (eg, isolation of gene products, determining nucleotide sequence in DNA or RNA)

Polymerase chain reaction—the amplification of DNA segments by using DNA polymerase and paired oligonucleotides subjected to repeated episodes of thermal cycling.
Primer—a stretch of oligonucleotides that are used to prime the synthesis of DNA
Promoter—the region of a gene that binds RNA polymerase and initiates gene transcription.
Template—the nucleic acid sequence that directs the synthesis of the cDNA or RNA strand.
Vector—a cloning vehicle that is used to propagate a DNA fragment in the appropriate host (eg, bacteriophage, plasmid, viral)

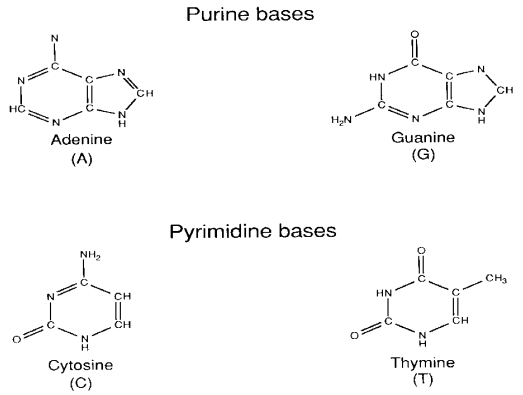
Isolation or identification techniques:

Electrophoresis—separation based on size

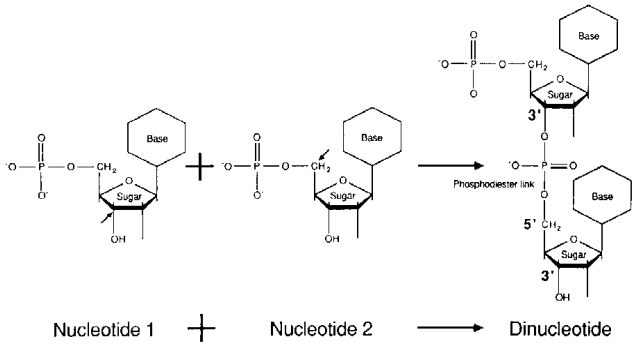
PUTTING OUR GLOSSARY INTO ACTION

Nucleic Acids

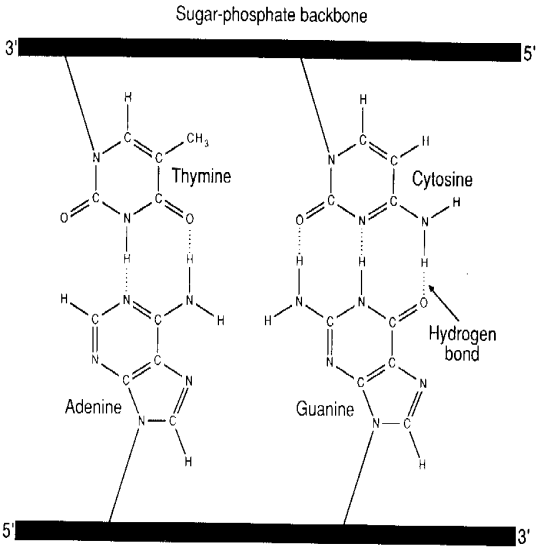
The structural formulas of the four nucleic acids contained in DNA. Chemically, these nucleic acids function as hydrogen-acceptors and are thus termed bases. The pyrimidine uracil substitutes for thymidine when complementary RNA is formed.



Add DNA polymerase = DNA Strand Components

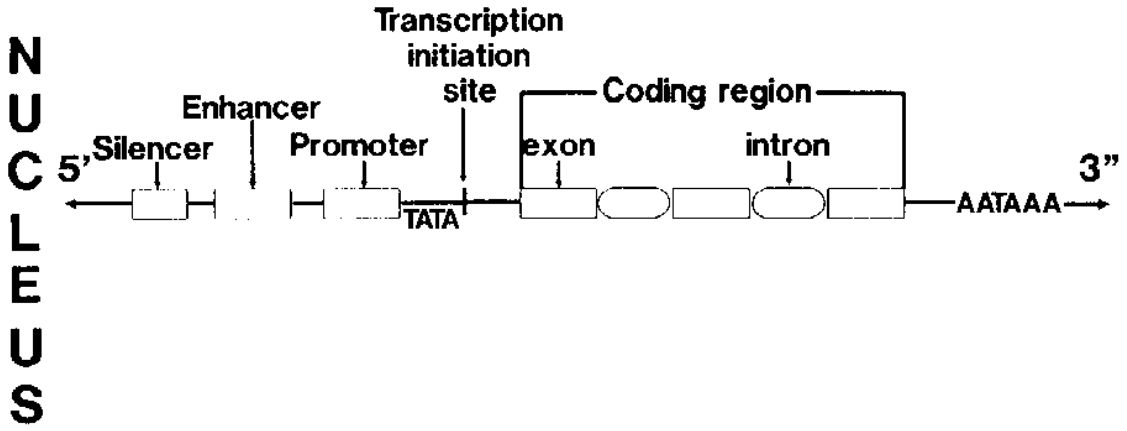


DNA strand components. Each strand of DNA is composed of nucleotides arranged in series and held together by phosphodiester organic bonds between the 5' and the 3' carbons of two adjacent ribose moities.



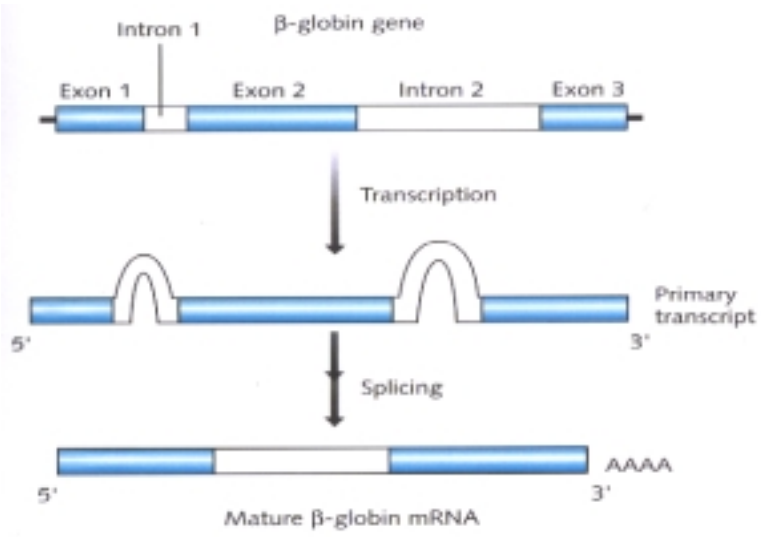
Double-stranded DNA. The DNA molecule consists of 2 nucleotide strands arranged in a 5' to 3' antiparallel orientation. The 5' to 3' antiparallel orientation creates the conformation changes necessary for the strong obligatory hydrogen bonding between complementary pyrimidine and purine base pairs.

A sequence of nucleic acids makes a gene

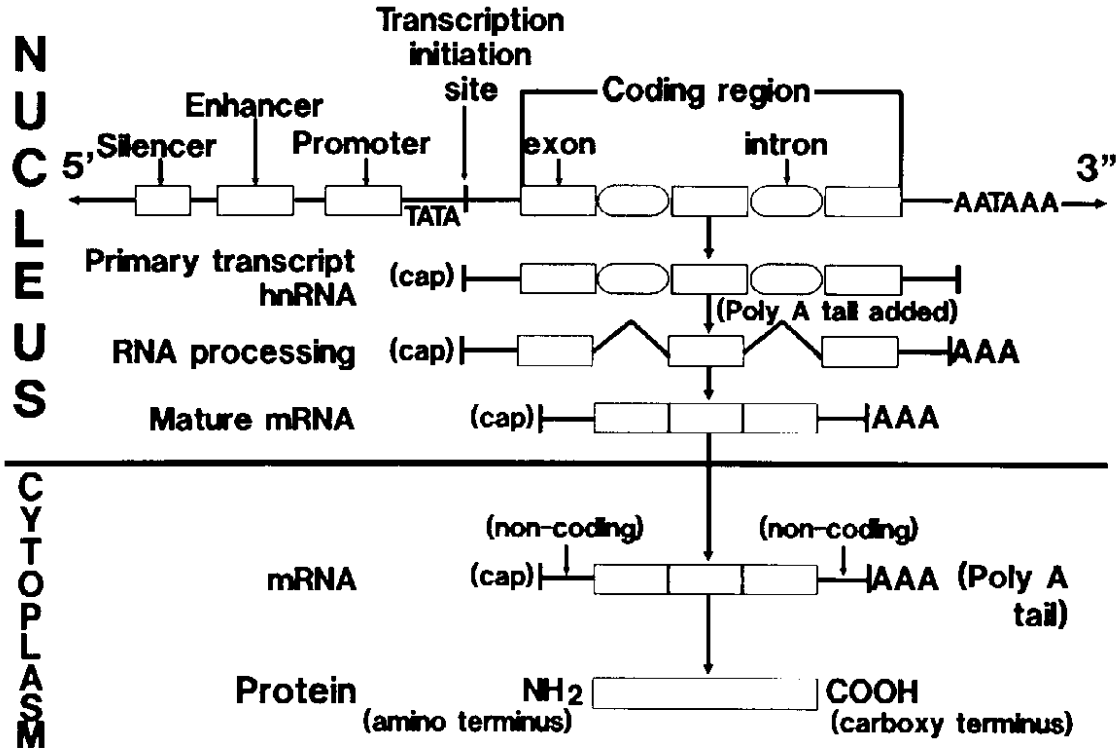


Gene structure. The majority of genes have a complex organization structure consisting of protein coding and noncoding regions. The coding region is comprised of sequences (exons) which are the blueprint components for protein synthesis. Within the coding region, intervening sequences (introns) separate the exons. RNA polymerase recognizes a specific initiation site for its attachment to commence transcription. Silencer, enhancer, and promoter sequences function as recognition sites for the binding of the silencer, enhancer, or promoter proteins which regulate gene expression. In the figure, T and A represent the nucleic acids thymine and adenine.

Introns must be removed in order to generate a mature RNA. These are removed by splicing, resulting in a mature RNA containing contiguous exonic sequences.



SYNOPSIS OF PROTEIN SYNTHESIS



Protein synthesis is a dynamic process beginning in the nucleus with a DNA blueprint and ending in the cytoplasm after translation. A primary mRNA transcript is formed from the gene by RNA polymerase. Mature nuclear mRNA results after the transcript undergoes processing by removing intron sequences. The processed mature mRNA traverses the nuclear membrane into the cytoplasm where it binds to ribosomal RNA (rRNA) for translation into the amino acid sequence of the polypeptide.

TECHNIQUES IN MOLECULAR BIOLOGY

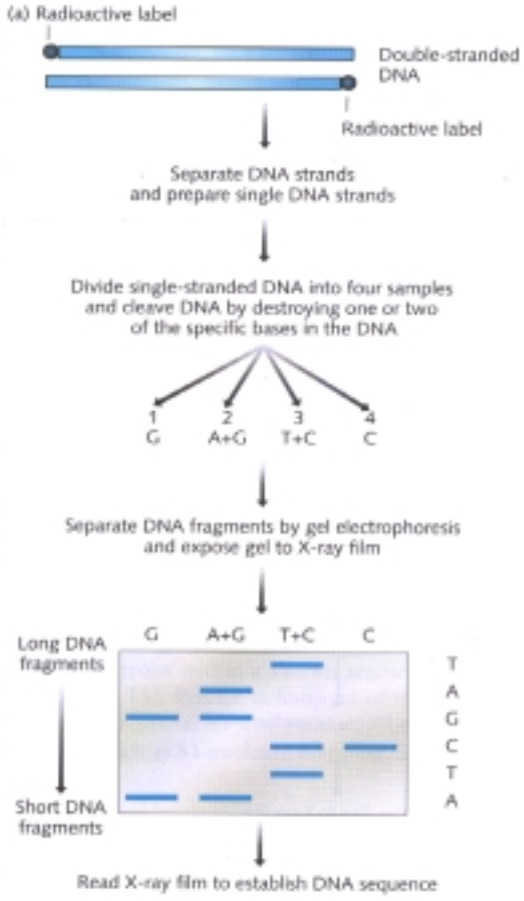
Fundamental to molecular "genetics" and its techniques:

- Complementary pairing between nucleotides (guanine will always pair with cytosine and adenine with thymidine.
- DNA can be denatured and separated into its two complementary strands and, under appropriate conditions, reanneal and again become double-stranded (hybridization).

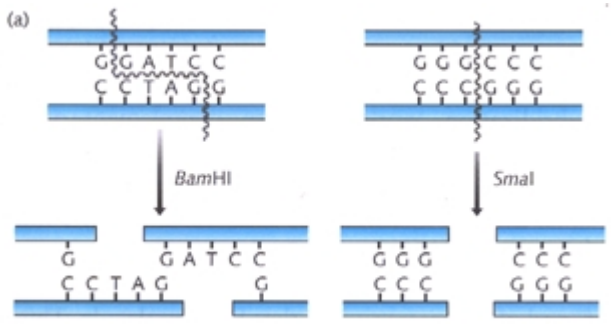
The complementary base pairing and ability to hybridize underlies all the essential techniques of molecular genetics, especially the generation and application of probes to identify specific genes, DNA fragments and mRNA, the construction of DNA and cDNA libraries, and the polymerase chain reaction (PCR) used to amplify single copy sequences by a million-fold. Lastly, the DNA to protein process is bidirectional, ie, DNA $\rightarrow$ protein $\rightarrow$ cDNA.

DNA Sequencing

One end of the DNA to be sequenced is labeled with ^{32}P. This labelled DNA DNA is then divided into 4 samples and each is treated with a set of chemicals that degrades one or two of the 4 DNA bases in each sample in such a way that only a few sites in any one DNA molecule are degraded. This results in a set of DNA molecules of varying length. Samples separated by electrophoresis. Gel exposed to Xray film. Pattern of the labelled DNA fragments can then be used to determine



Restriction enzymes and DNA isolation



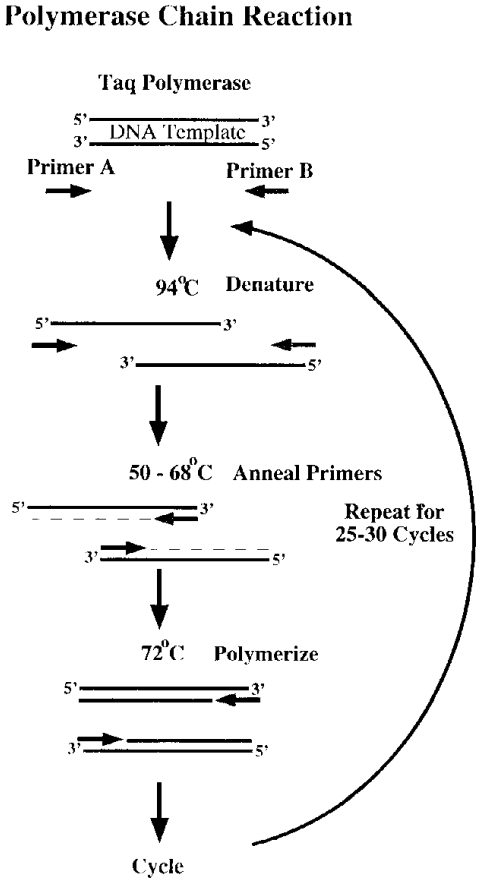
Restriction enzymes, typically derived from microbial sources, recognize defined DNA sequences and cut out a defined DNA sequence. These sequences can then be identified via “blotting” techniques or used in identifying other products.

SUMMARY OF BLOTTING PROCEDURES

Procedure	Substance Detected	Probe	Major Application

Southern blot	DNA	Nucleic acid	Gene structure
Northern blot	RNA	Nucleic acid	Gene structure
Western blot	Protein	Antibody	Protein levels
Southwestern blot	Protein	DNA	DNA-protein interactions
Farwestern blot	Protein	Protein	Protein-protein interactions

POLYMERASE CHAIN REACTION



PCR generates large quantities of a specific DNA sequence from a complex of DNA within a short period of time and without cloning. The procedure uses a thermostable DNA polymerase (in the example above, Taq polymerase from the bacterium *Thermophilus aquaticus*) and two synthetic oligonucleotides as primers (these oligonucleotides are available commercially). The DNA sequence of the gene to be amplified needs to be partially known, so that two oligonucleotide primers corresponding to the ends of the target DNA can be synthesized. The procedure then consists of repeated cycles of three steps: 1. denaturation of DNA, 2. primer annealing to DNA, and 3. primer extension. During denaturation, heat separates the DNA strands into single strands. These strands then hybridize to the matching oligonucleotides during the annealing step if the temperature is lowered. Once the primer has annealed, DNA polymerase can copy the remainder of the single strand if the required deoxynucleotide

triphosphates are provided. The amount of DNA is doubled with each cycle. After 25-30 cycles, the desired sequence can be enriched over a millionfold. A complete PCR typically takes 1-3 hrs. Newer technology may decrease this to seven minutes. Limitations: 1. misincorporation of nucleotides by the Taq polymerase (Taq errors), 2. inability to amplify relatively large lengths of DNA. Ongoing revision of the technology may limit these limitations for eventual large scale use in medical diagnostics. One of the major uses of PCR is to amplify genomic DNA for diagnostic studies.

Practical Diagnostic Applications of PCR

- HIV, HTLV-1
- Herpes simplex (meningitis)
- Enterotoxigenic *E Coli*
- Respiratory pathogens (including Tb)
- STDs, particularly *Chlamydia trachomatis*
- Infectious disease epidemiology, antibiotic resistance
- Early diagnosis of septicemia (sepsis syndrome)

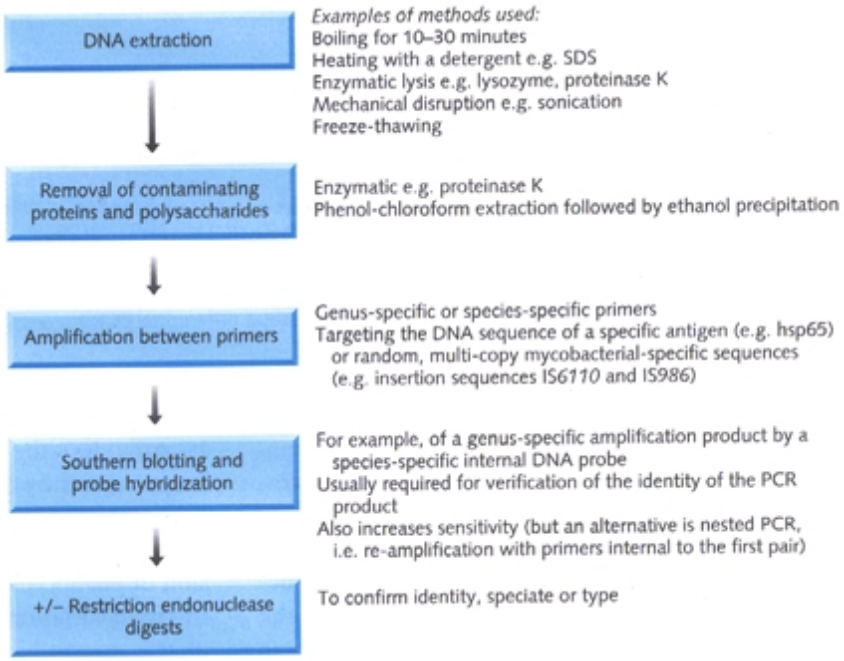
Chlamydia PCR

Specimen	Test	Sensitivity	Specificity
Urethra or endocx	Culture	45-85%	100%
	EIA	80%	100%
	PCR	>90%*	100%
Urine	PCR	100%	100%

EIA = enzyme immunoassay

*depends on whether transport medium is used and the type of medium

PCR in the Diagnosis of TB



PCR IN SEPSIS

There is currently no “gold standard” to diagnose blood stream infection. Accepted criteria: positive blood culture and clinical manifestations of sepsis. In most large scale sepsis studies, >15% of enrolled patients had no documented infection despite clinical manifestations and <50% had positive blood cultures with presumed infection on clinical grounds. The lack of a gold standard leads to the use of unnecessary antibiotics in patients with noninfectious causes of the systemic inflammatory response syndrome and to withholding antibiotics in patients with true bacteremia and false negative blood cultures. It has been stated that the number of false-positive blood cultures can be as high as the number of true-positive cultures.
Additionally, as many as 5% of patients die before positive blood cultures are reported. Antibiotic coverage based upon presumed source has been “appropriate” in only about 60%. (Pedersen et al, Scand J Infect Dis, 1997)

PCR can amplify and identify a specific DNA sequence in 4-8 hrs using current methodology. This may be shortened to <1 hr with evolving technology. The same 16S ribosomal RNA is shared by a number of gram + and gram – bacteria and other specific genetic products can be used to specifically detect candida, E Coli, and anaerobes. PCR is not dependent on the viability or growth of bacteria.

Kane et al, Ann Surg, 1998

- Bacterial DNA detected in all patients who eventually had a +blood cluture
- 64% of 40 critical patients had +PCR, only 3 (14%) had + cultures
- PCR useful in patients already receiving antibiotics

Cursons et al, Crit Care Med, 1999

- 84 pts with SIRS, severe sepsis, or septic shock

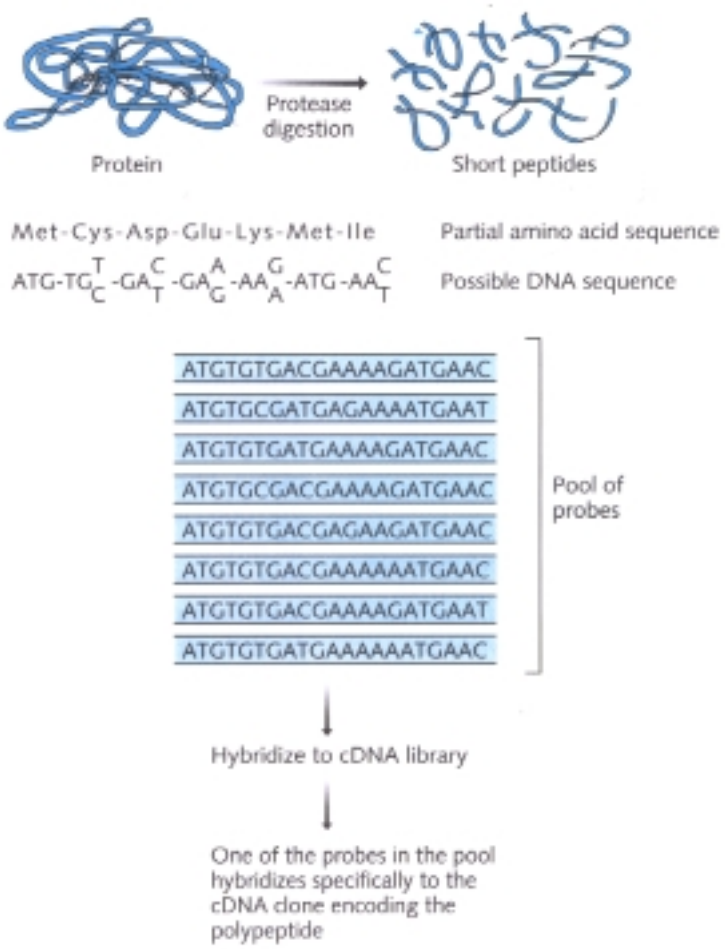
- blood samples for PCR of bacterial 16S RNA in leukocytes and plasma
- two extraction techniques
- PCR sensitivity = 68%; specificity = 87%
- problems detecting candida and gram+ species

RECOMBINANT TECHNOLOGY

Unique features of recombinant DNA:

- In vivo structure-function analysis
- Ability to generate large quantities of proteins present in trace amounts and to genetically design specific drugs
- Diagnostic in situ hybridization
- Molecular genetic applications
- Ability to determine molecular basis for disease

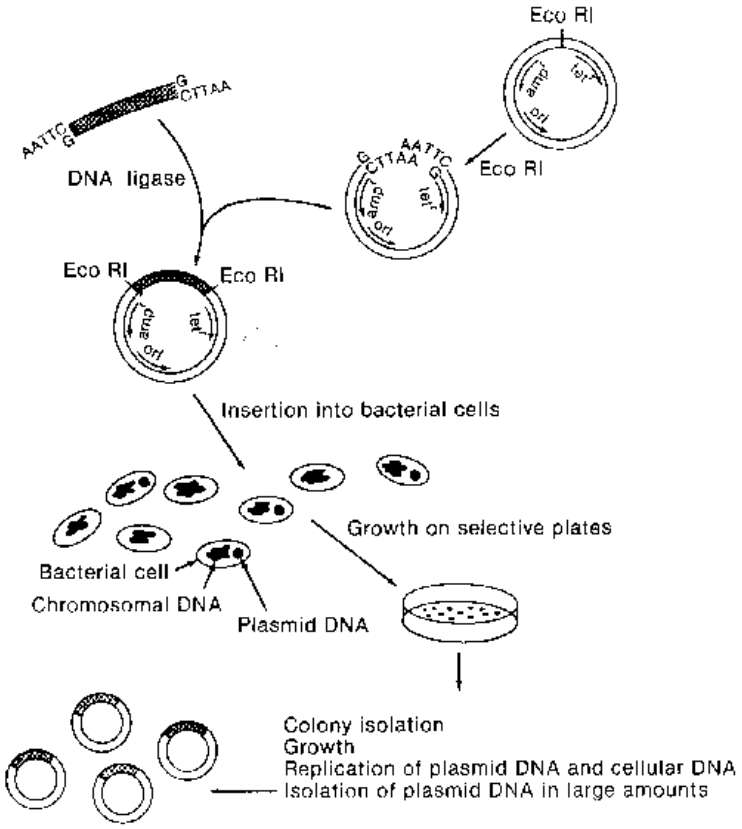
Gene Cloning from a Protein Product



Microsequencing of an isolated protein can result in the amino acid sequence of small polypeptides within the protein. Based on the peptide sequence, small synthetic

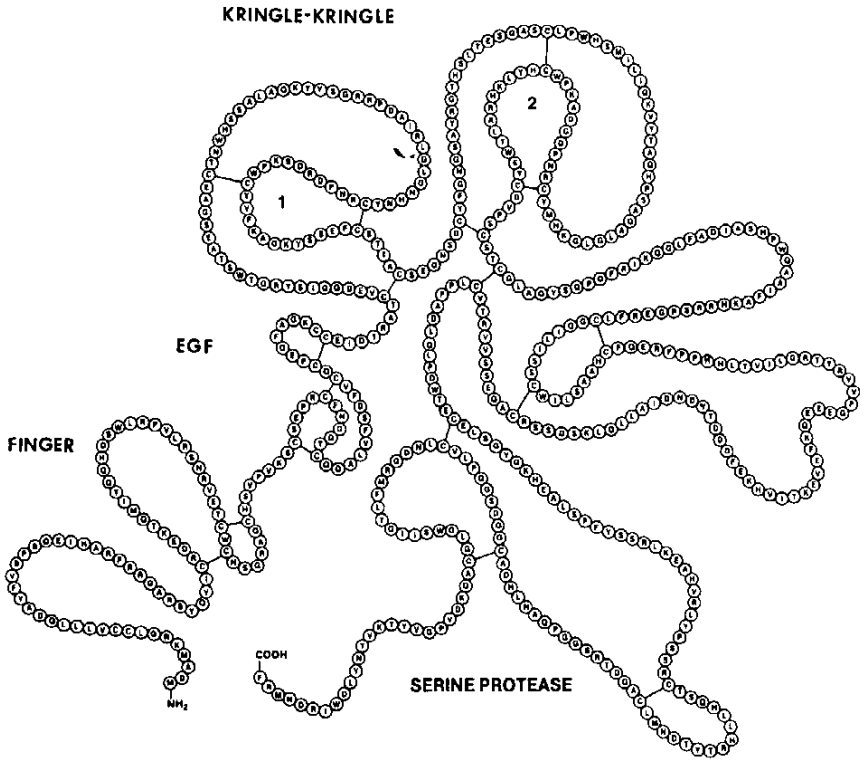
oligonucleotides can be synthesized which encode the corresponding amino acids of the polypeptide sequence.

Cloning a DNA Fragment in a Vector



In 1972, Berg and colleagues inserted a fragment of DNA that had been cut with the E Coli restriction enzyme Eco RI into a plasmid vector that had also been cut with Eco RI. Plasmid vectors are circular DNA molecules that can replicate autonomously in bacterial cells. After ligation of cohesive ends of the two molecules generated by Eco RI with DNA ligase, the new plasmid molecule was produced that contained a piece of foreign DNA. After this recombinant plasmid DNA was reintroduced into bacterial cells, to could be grown and purified in large and homogenous quantities. This seemingly simple cloning process, in essence the same that is used in 1999, ushered in the era of recombinant DNA.

t-PA Structure and Function

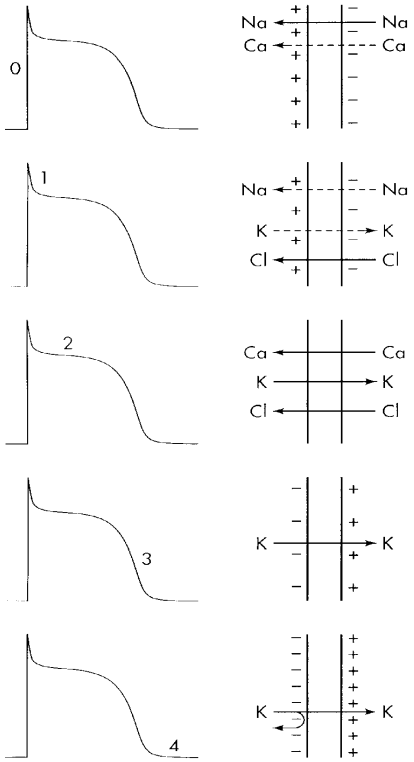


Tissue plasminogen activator (t-PA) is a serine protease composed of a single polypeptide containing 527 amino acids which catalyzes the conversion of plasminogen to plasmin. The principle site of synthesis is the endothelial cell. Release can be elicited with various mechanical and pharmacologic stimuli, including vascular occlusion, physical exertion, thrombin production, and IV norepinephrine administration. Identification and purification of t-PA from a melanoma cell line and eventual cloning of cDNA in E Coli was accomplished in 1983. A small stretch of purified t-PA protein was determined. Because the genetic code allowed the prediction of the possible DNA sequence that code for this protein, a series of short, synthetic DNA molecules (oligonuceotide probes) were designed to hybridize specifically the t-PA gene. Copies of cDNA were synthesized and inserted into bacteria and t-PA specific cDNA subsequently isolated.

Currently, t-PA is produced through recombinant DNA technology (rt-PA) as either a single chain or double chain. The single chain is converted to double chain in vivo. The protein has five “domains”: 1. Fibronectin finger domain, 2. An epidermal growth factor domain, 3. Two kringle domans, and 4. A serine protease inhibitor domain. The finger and kringle domains are essential for fibrin binding. Changes in these sites can result in a molecule with greater fibrin affinity. Other structural changes can alter pharmacokinetics. TNK t-PA is a new recombinant product undergoing testing for these properties.

STRUCTURE-FUNCTION RELATIONSHIPS OF ANTIARRHYTHMICS AND IONIC CHANNELS: THE FUTURE

The Action Potential

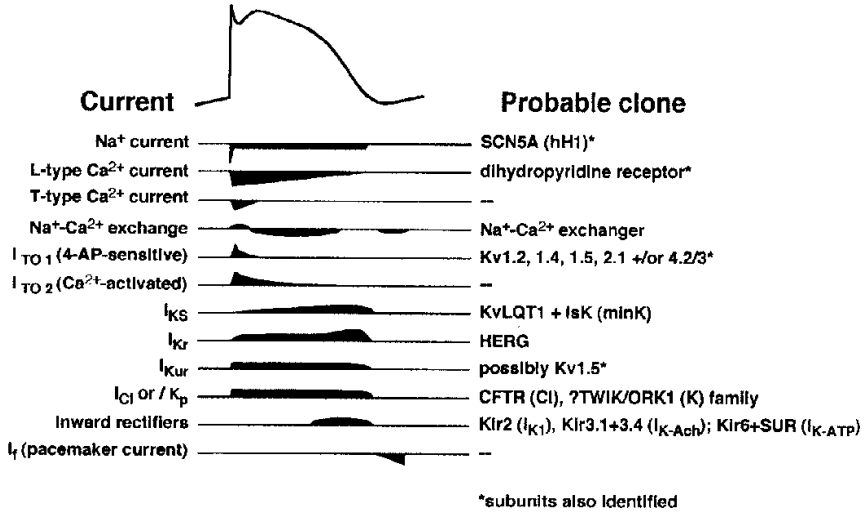


The action potential of cardiac myocytes is typically presented in a fashion similar to that shown in the figure. Although trans-membrance conductance of Na^{+}, K^{+}, and Ca^{+2} play a role in different phases of depolarization and repolarization, ionic currents are substantially more complex than initially thought and the current classification of antiarrhythmic drugs may be far to simplistic to allow tailored therapy in a given patient for supraventricular and ventricular arrhythmias.

Class	Electrocardiographic Effect	Membrane Effect	Examples of Drugs
Vaughan Williams			
IA	↑ QRS and ↑ Q-T intervals	Sodium channel block; intermediate kinetics potassium channel block	Quinidine Procainamide hydrochloride Disopyramide
IB	↓ Q-T interval	Sodium channel block; rapid kinetics	Lidocaine Tocainide Mexiletine hydrochloride
IC	↑↑ QRS interval	Sodium channel block; slow kinetics	Flecainide acetate Propafenone hydrochloride Moricizine
II	↓ Heart rate; ↑ P-R interval	β-adrenergic receptor inhibition	Propranolol hydrochloride and others
III	↑ Q-T interval	Potassium channel block; slow sodium channel facilitator	*N*-acetyl-procainamide Sotalol hydrochloride Amiodarone Ibutilide fumarate
IV	↓ Heart rate; ↑ P-R interval	Calcium channel block	Verapamil Diltiazem hydrochloride
Digitalis	↑ P-R interval; ↓ Q-T interval	Na^{+}, K^{+}-ATPase inhibition	Digoxin Digitoxin
Adenosine	↓ Heart rate; ↑P-R interval	Purinergic receptor agonist	Adenosine

*↑ *indicates increased;* ↓, *decreased. Adapted with permission from Siddoway.*[3]

As a byproduct of the work that lead to the isolation of the gene responsible for the inherited form of the prolonged QT interval syndrome, the proteins responsible for ionic pore channels of cardiac myocytes have been identified.



The availability of the protein constitutes of the ionic pores will allow direct assessment of drug-protein interaction and mechanism of action of a given antiarrhythmic as well help define specific differences in a patient's response to two drugs in the same class.

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