The Potential of Gene Therapy

“The saddest aspect of life today is that science gathers knowledge faster than society gathers wisdom.”

-Isaac Asimov, master of hard science fiction writing

Content

1. Why genes are important in normal and disease biology
2. What is gene therapy?
3. Using silencing techniques to counter hepatitis B virus infection
4. Challenges
5. Relevance of gene therapy research

Genes are fundamental to biology

- Genes are carriers of information
- The information is transformed into function

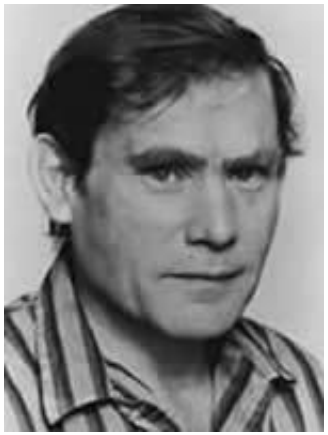
DNA

Protein

Conversion of information into function makes us what we are

The total explanation of all organisms resides within them (genes)…. Ultimately, the organism must be explicable in terms of its genes….

- Sydney Brenner, 1984



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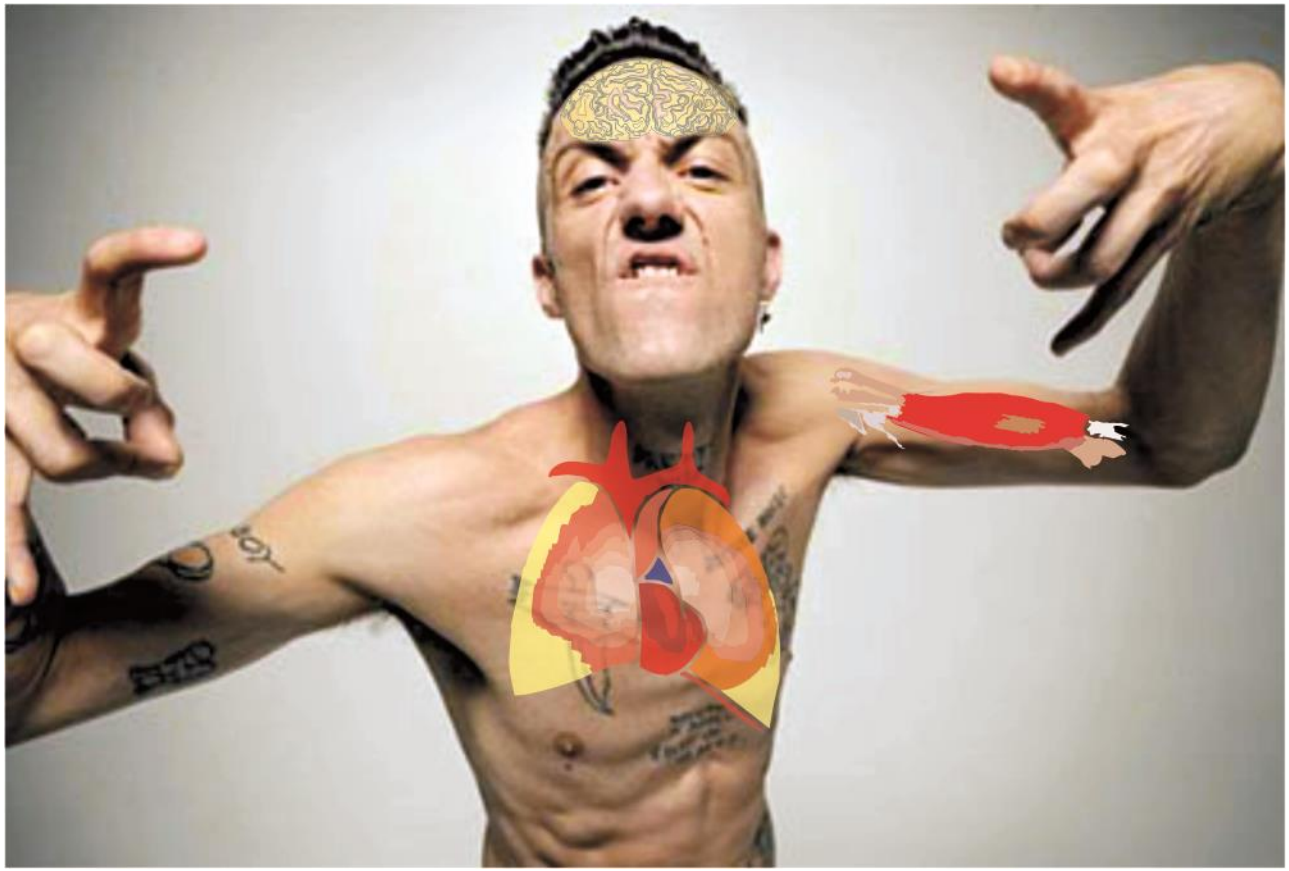
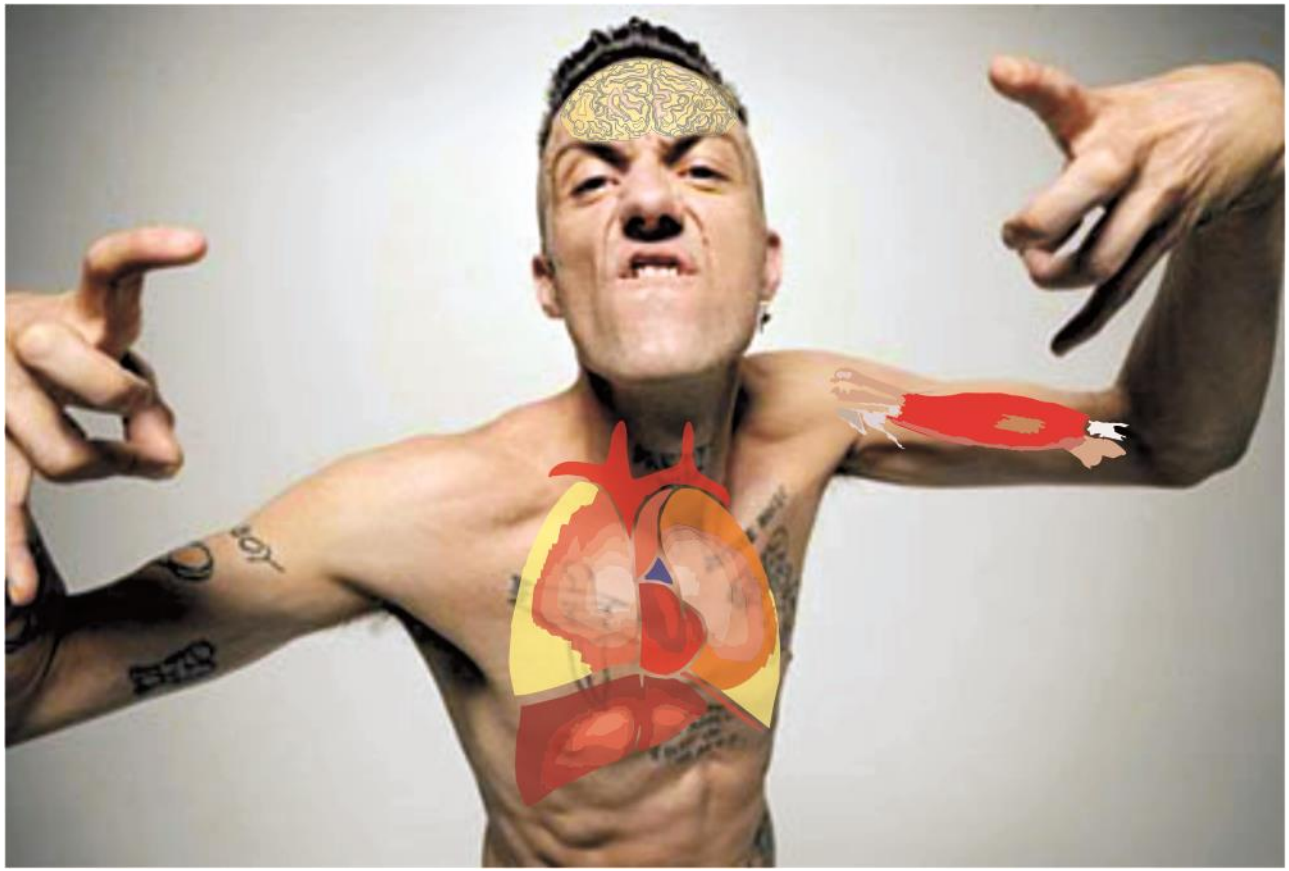
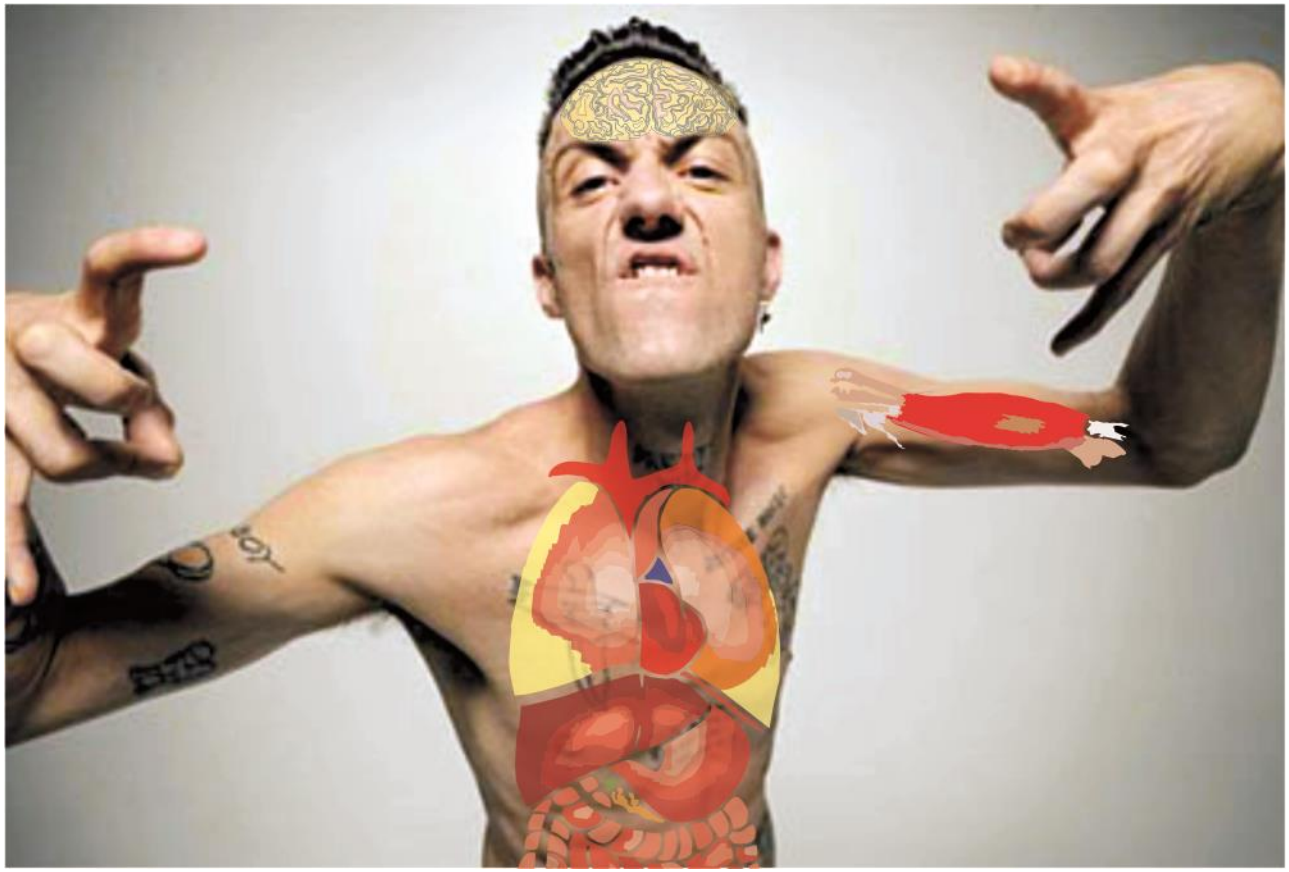
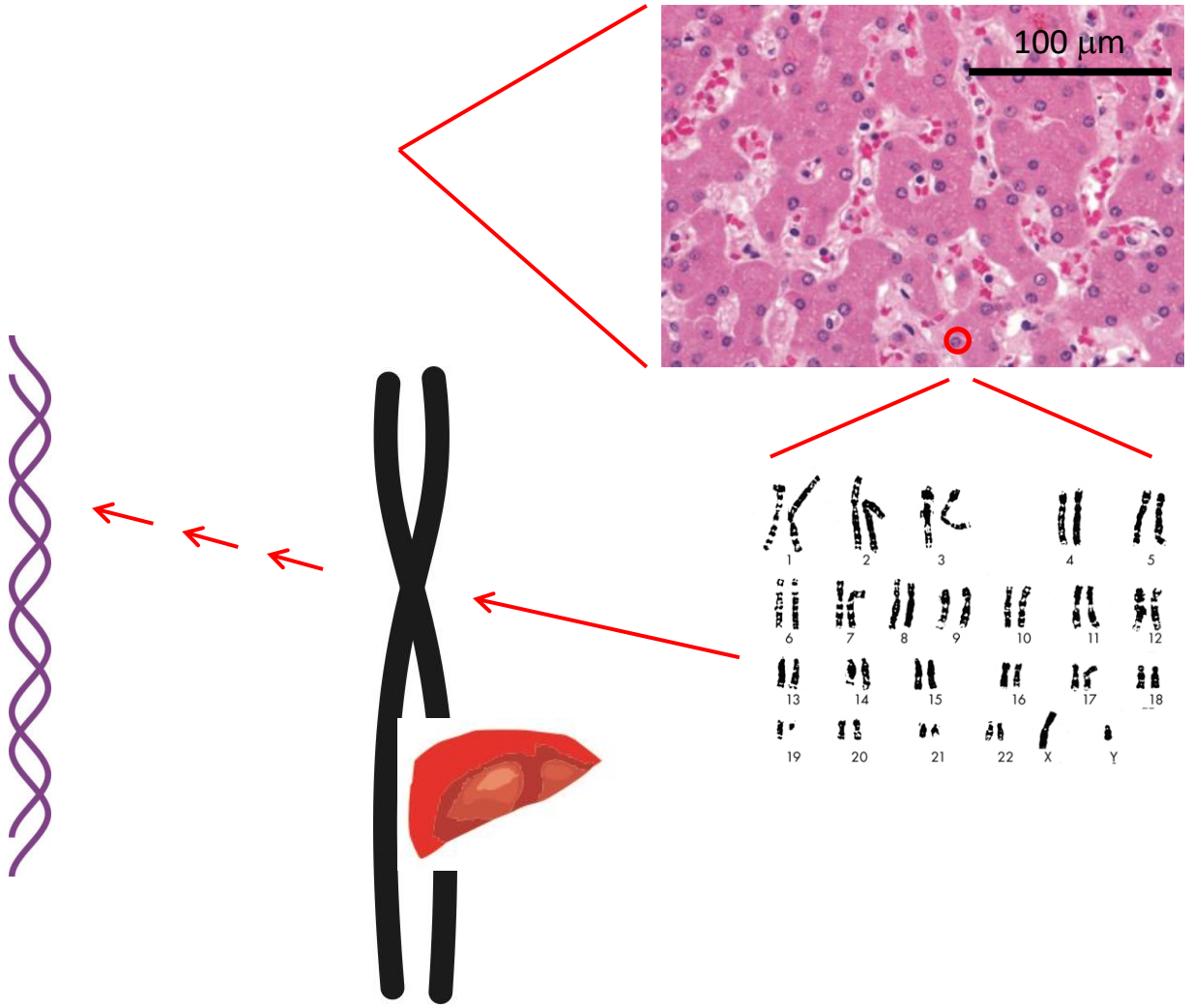






Arrangement of DNA within a cell



Discovery of double helical structure of DNA was crucial

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

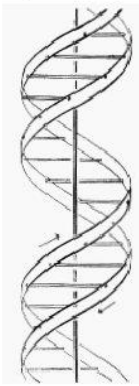
We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons:

(1) We believe that the material which gives the X
the salt, not the free acid. Without the acidic hydr
not clear what forces would hold the structure tog
as the negatively charged phosphates near the axi
other. (2) Some of the van der Waals distance
small.

Another three-chain structure has also been sug
(in the press). In his model the phosphates are on
the bases on the inside, linked together by hydro
structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining ß-D-deoxyribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow righthanded helices, but owing to the inverse sequences of the atoms in the two chains run in opposite directions.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Each chain loose
model No. 1; that
inside of the heli
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and the atoms nea
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base. There is a
every 3·4 A. in t
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residues in the s
structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain, does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then

guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be ...ed, it follows that if the sequence of bases o...

but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in time following, communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereo-chemical arguments.

It has not escaped our notice that the specific pairing we have

...ough not entirely on published exp...
...stereo-chemical arguments.

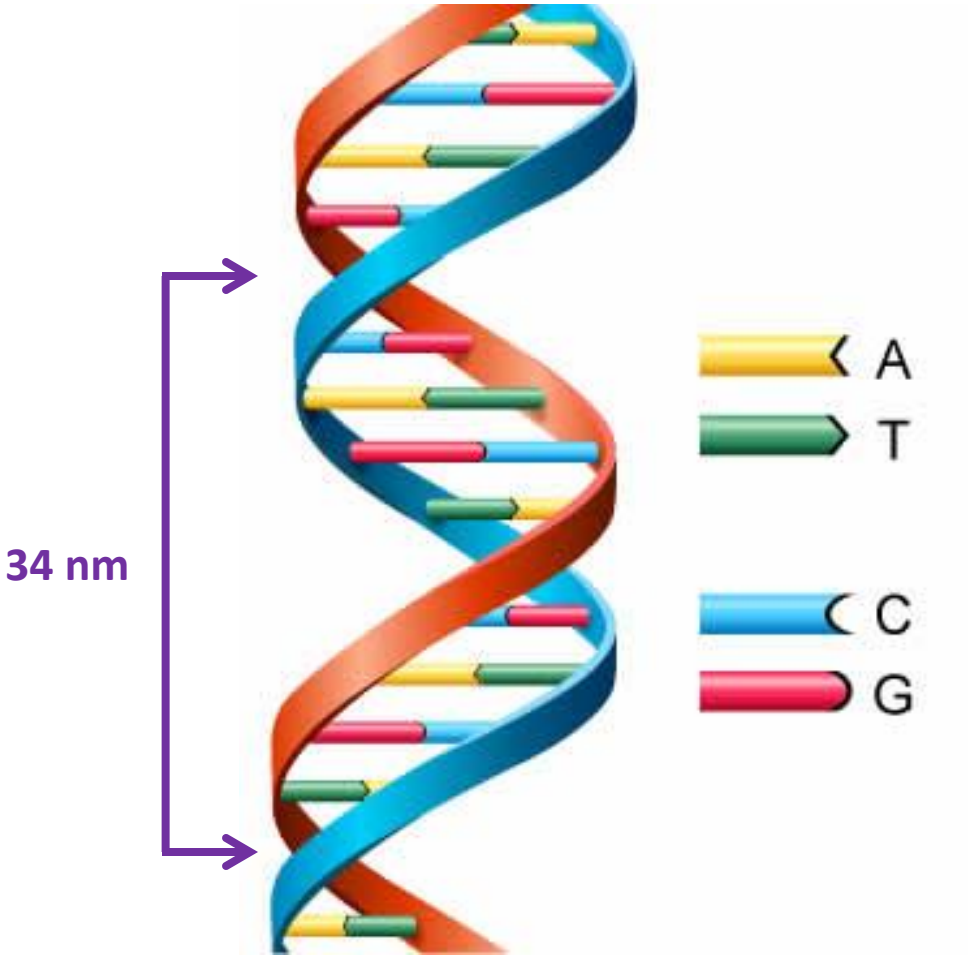
It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assum... together with a set of co-ordinates ...

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

[1] Pauling, L., and Corey, R. B. nature, 171, 346 (1953); Proc. U.S. Nat Acad. Sci., 39, 84 (1953).
[2] Furberg, S., Acta Chem. Scand., 6, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., Biochim. et Biophys. Acta, 9 402 (1952).
[4] Wyatt, G.R. J. Gen. Physiol., 36 201 (1952).
[5] Astbury, W.T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947)
[6] Wilkins, M. H. F. and Randall, J. T. Biochim. et. Biophys. Acta, 10, 102 (1953).

The sequence of nitrogen bases encodes information in genes



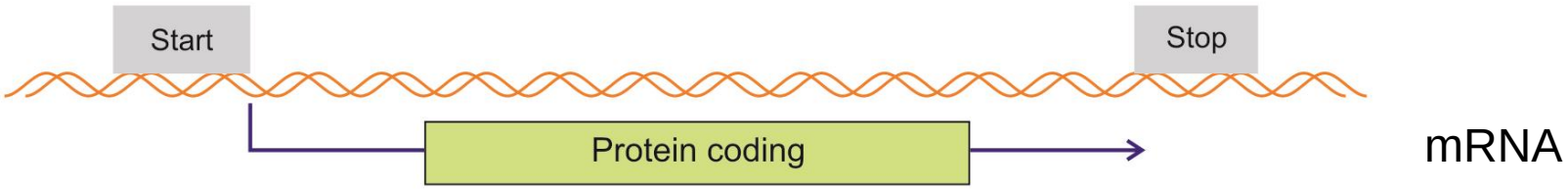
The human genome comprises:

- 46 chromosomes
- Just over 3 billion base pairs (haploid)
  - A adenine
  - G guanine
  - C cytosine
  - T thymine
- Approximately 23,000 protein coding genes

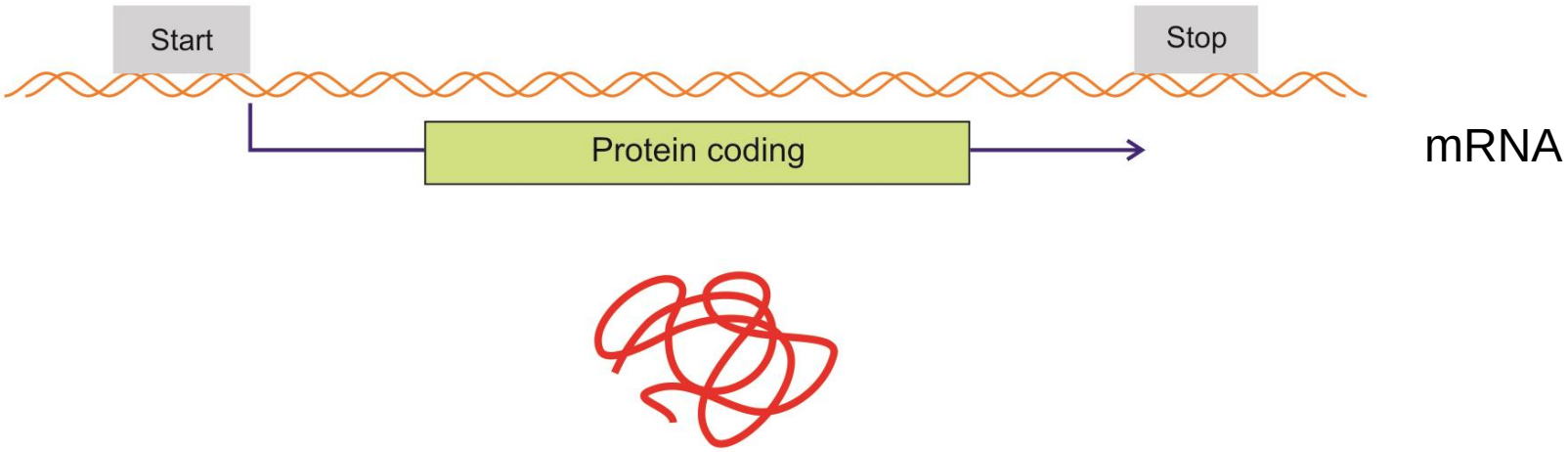
What is a gene?

What is a gene?

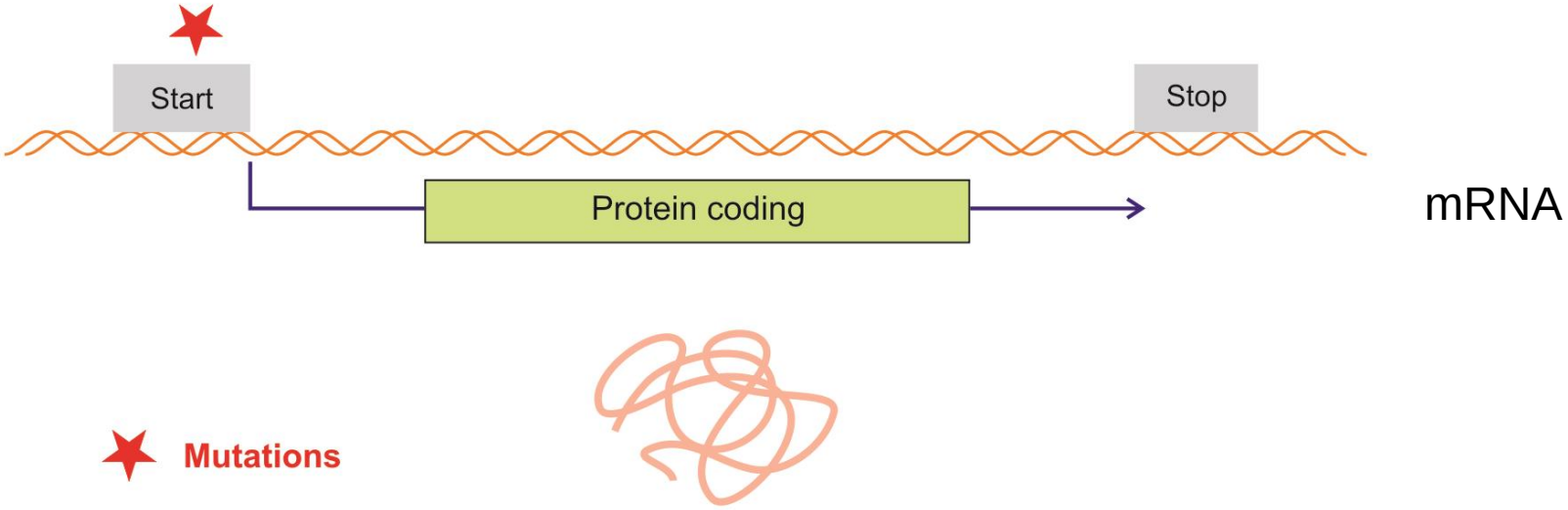
What is a gene?

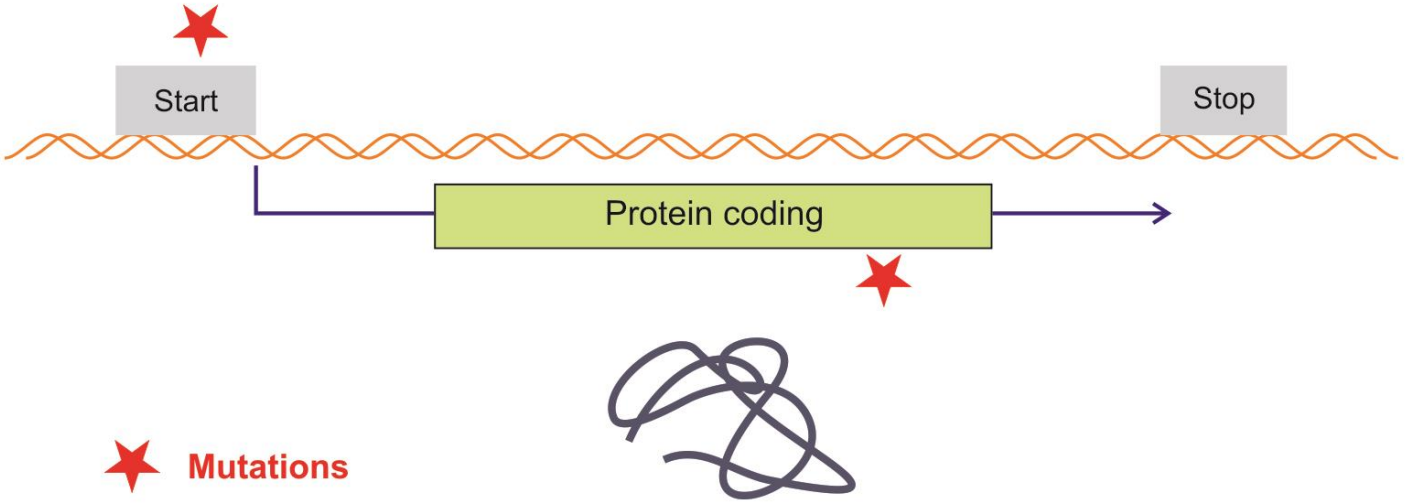


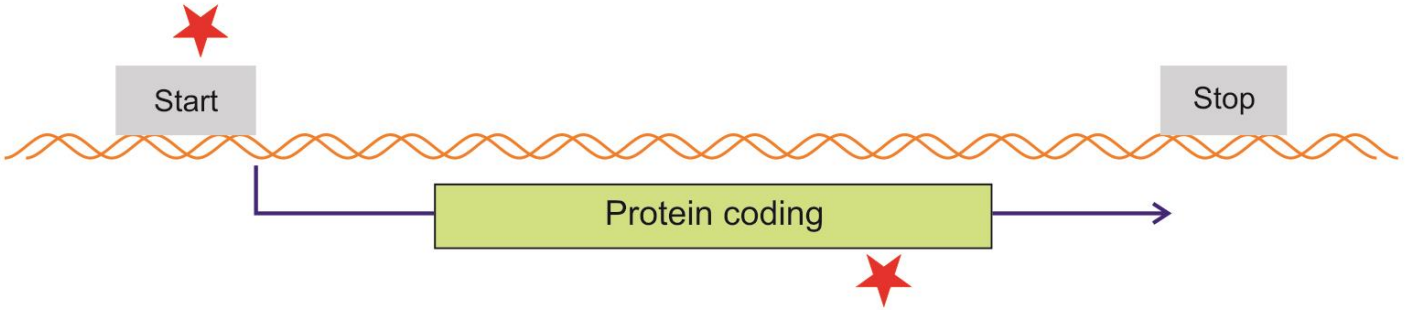
What is a gene?



What is a gene?




Start
Stop
Protein coding
mRNA
Mutations



mRNA

Mutations

'Rogue' genes

What is gene Therapy?

- A therapy that alters gene function?
  - Not really

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- Using genes to treat diseases?
  - Now generally accepted that gene therapy may also include use of RNA (not only DNA)

Gene Therapy is a procedure that employs nucleic acids to achieve a therapeutic effect

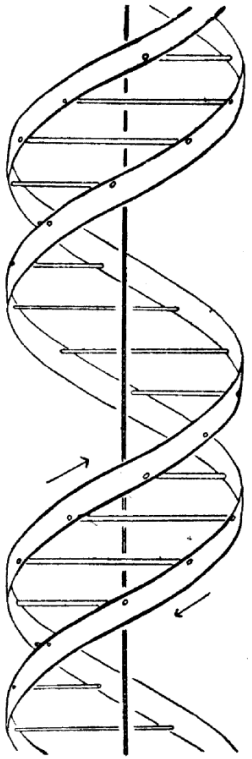
A rational approach to drug design

Gene Therapy Applications

- Used to correct gene expression (e.g. inherited diseases such as haemophilia)
- Induce killing of cells (e.g. cancer treatment)
- Suppress gene expression (e.g. treatment of virus infection)

So far not very useful for bacterial and parasitic infections

DNA is negatively charged



Delivery of nucleic acids remains the greatest challenge

Cell membranes provide a barrier to charged DNA/RNA – they do not mix

… just like olive oil and balsamic vinegar

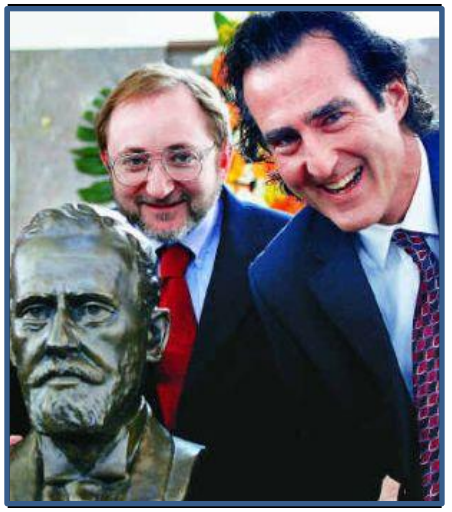
Types of carriers (vectors) for gene therapy

- Engineered viruses
- Chemically synthesised carriers
- Something in between
  - virus-like particles that may be modified chemically

Viruses are considerably more efficient than manmade vectors

Discovery of RNA interference has been important to the field of gene therapy

Nobel prize for Medicine or Physiology in 2006 awarded to Andrew Fire and Craig Mello for discovering the RNA interference pathway



Nobel Prize committee cited the following

RNAi provides

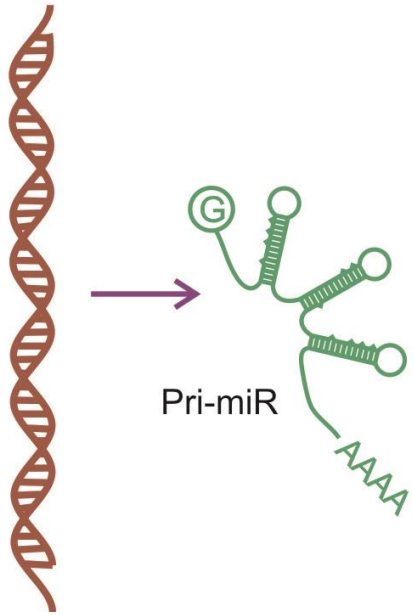
1. Regulation of protein synthesis
2. Protection from viral replication
3. Silencing of mobile genetic elements
4. Transcription modulation
5. **Potential for therapy**

Inhibiting gene expression using RNA interference

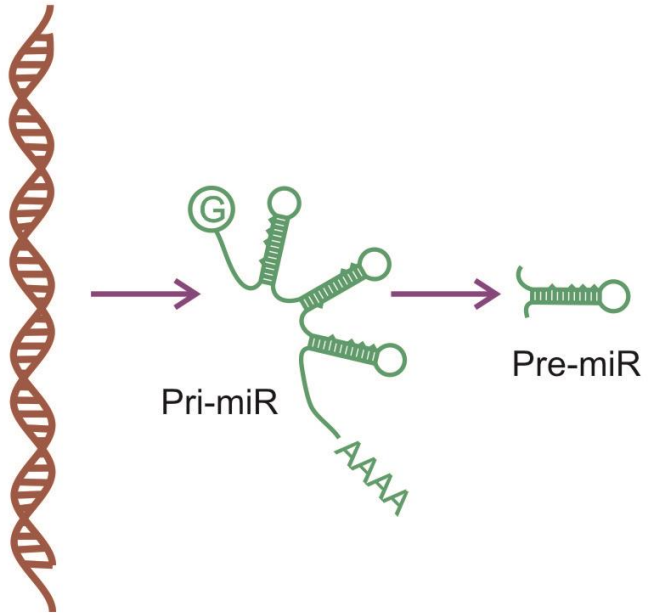
- A small fraction of disease-associated genes are suitable targets for small drugs
- RNAi enables rationale drug design
- Powerful & specific gene silencing (antagonism only)
- Rapid and fairly easy to develop lead gene silencers
- Quickly reached a stage of advanced clinical trial

The RNAi pathway and how to design a gene silencer

The RNAi pathway and how to design a gene silencer



The RNAi pathway and how to design a gene silencer



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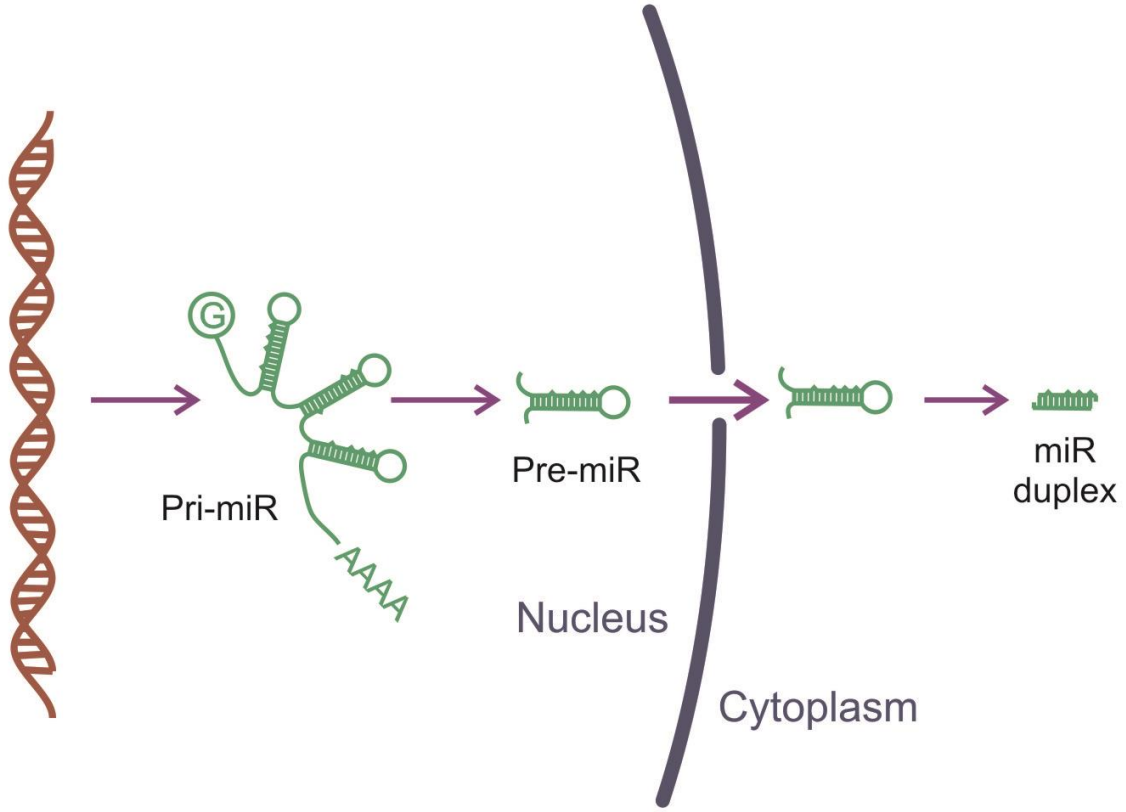

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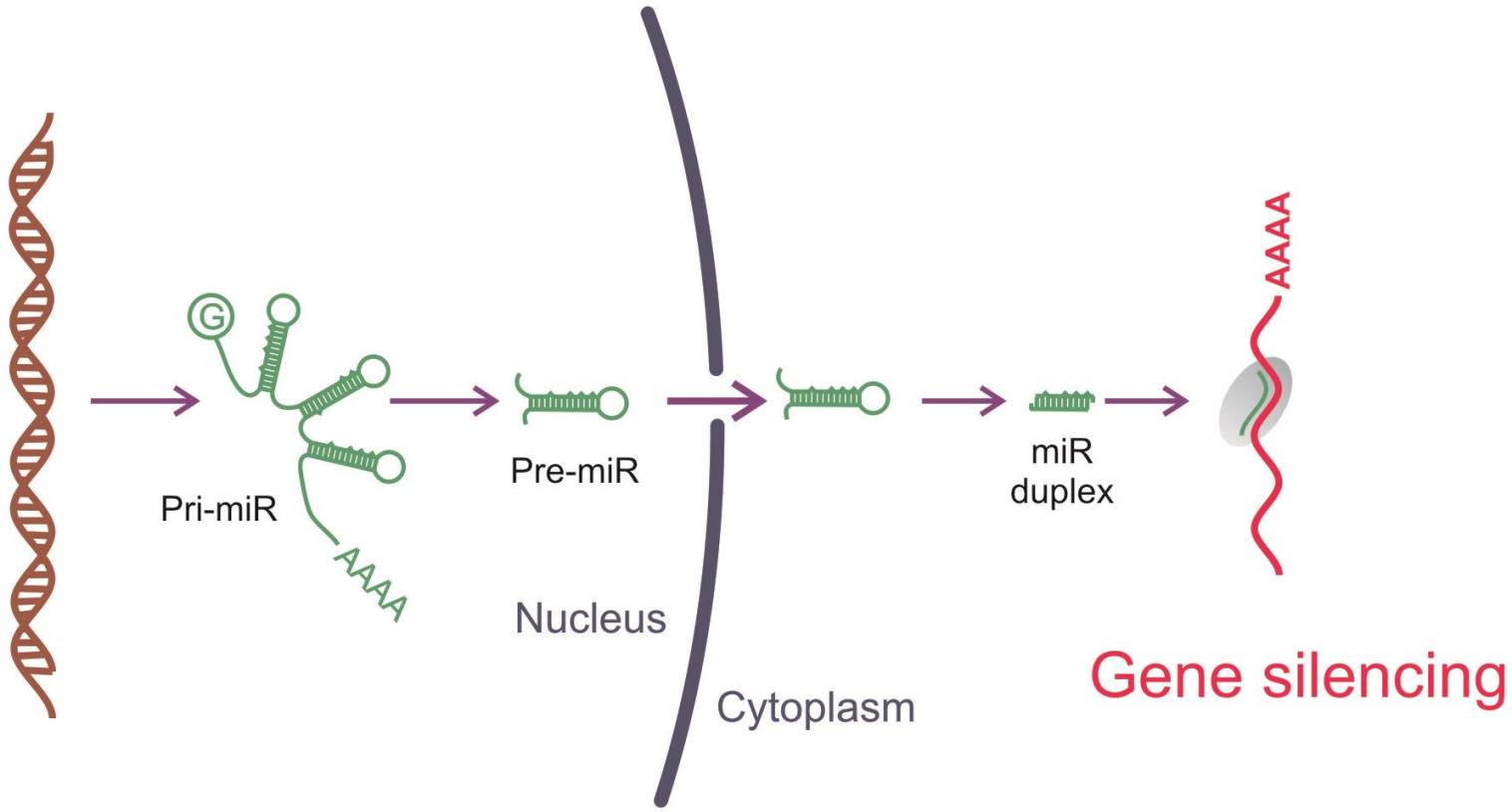

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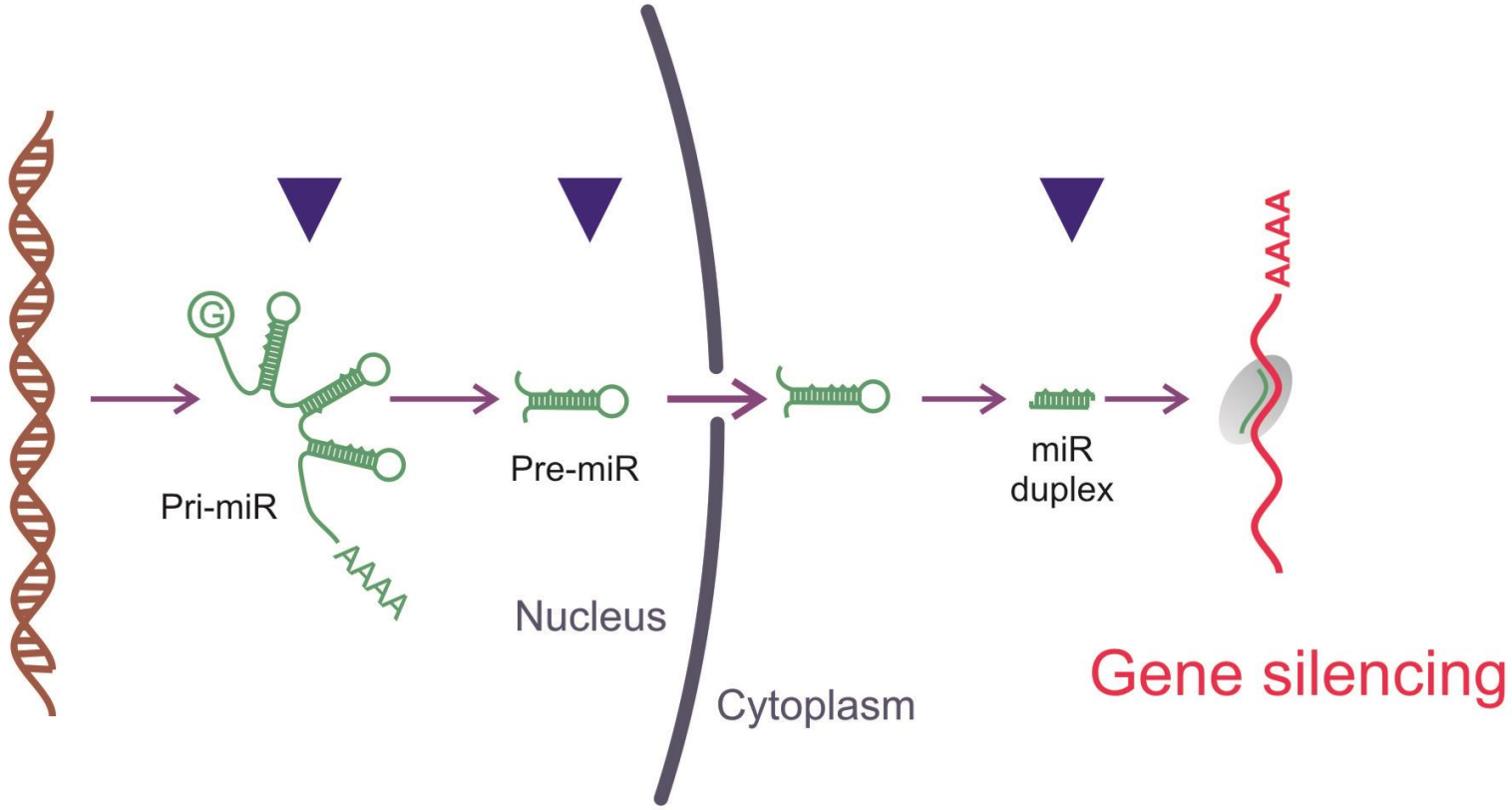

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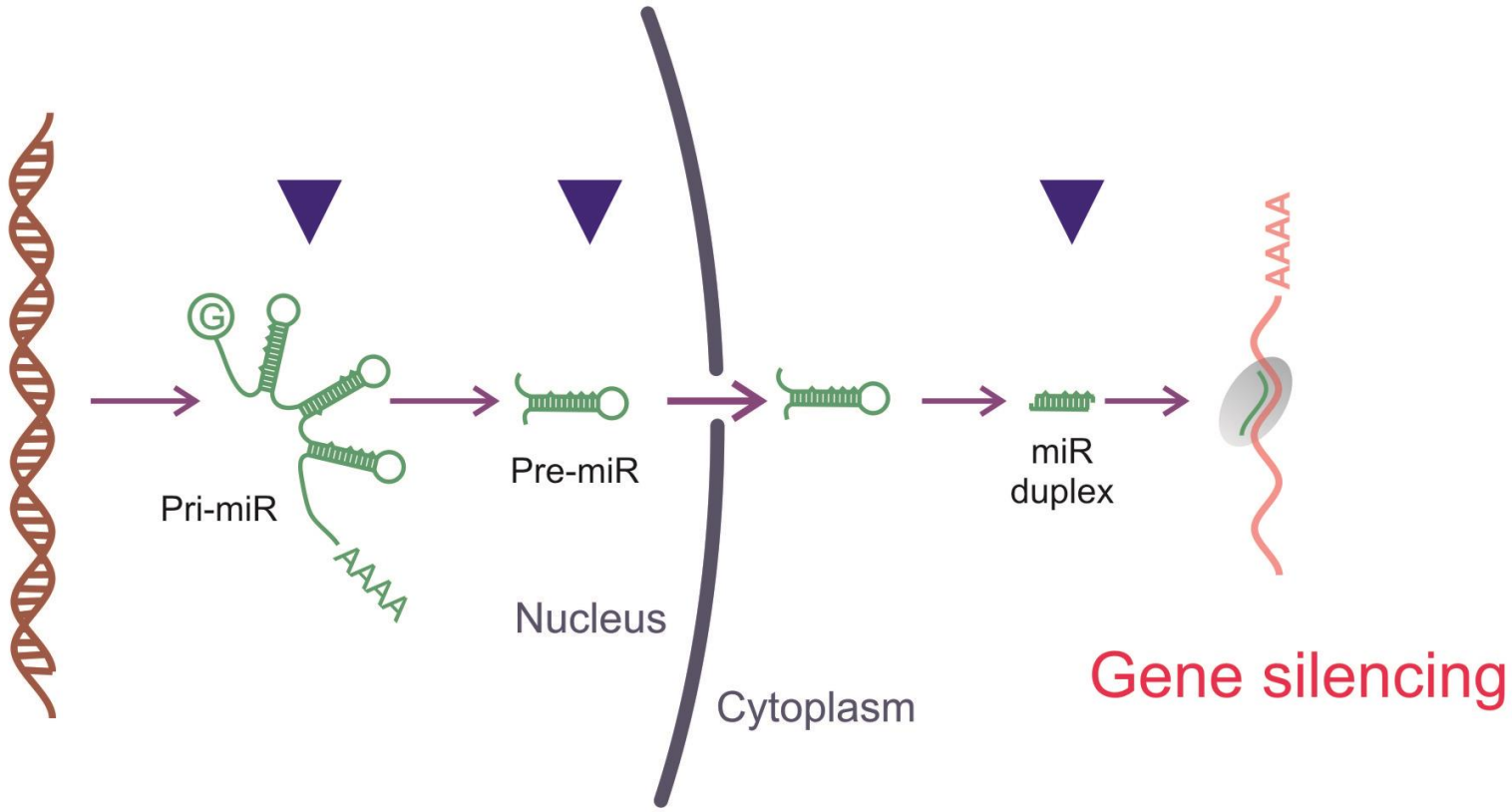
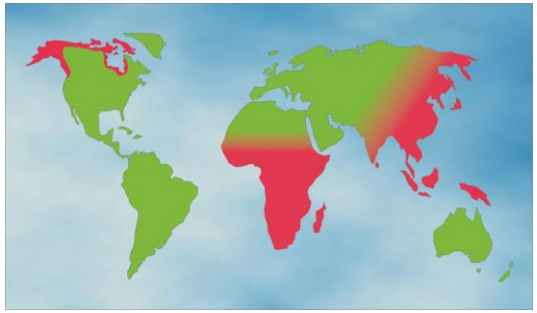


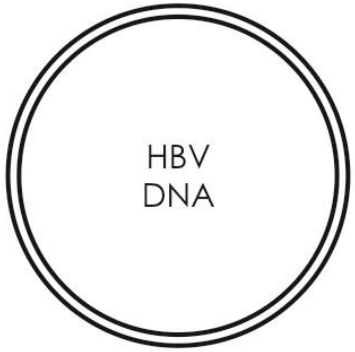
HBV infection

- 387 million chronic carriers in the world
- Childhood infection commonly associated with long term disease
- 25% of HBV carriers will develop liver cancer
- HCC has a very poor prognosis (annual incidence=annual mortality)
- Vaccine prevents HBV infection effectively

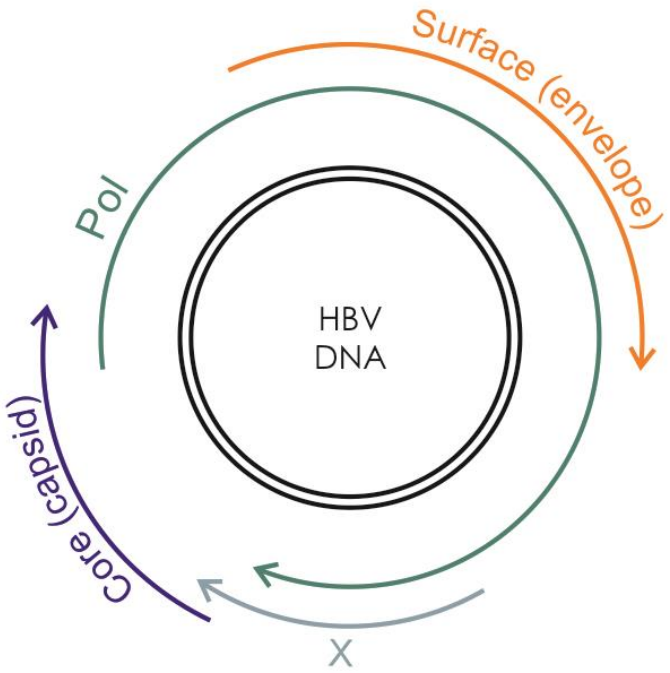


- RNAi-based approaches have shown promise: both expressed and synthetic RNAi-inducers

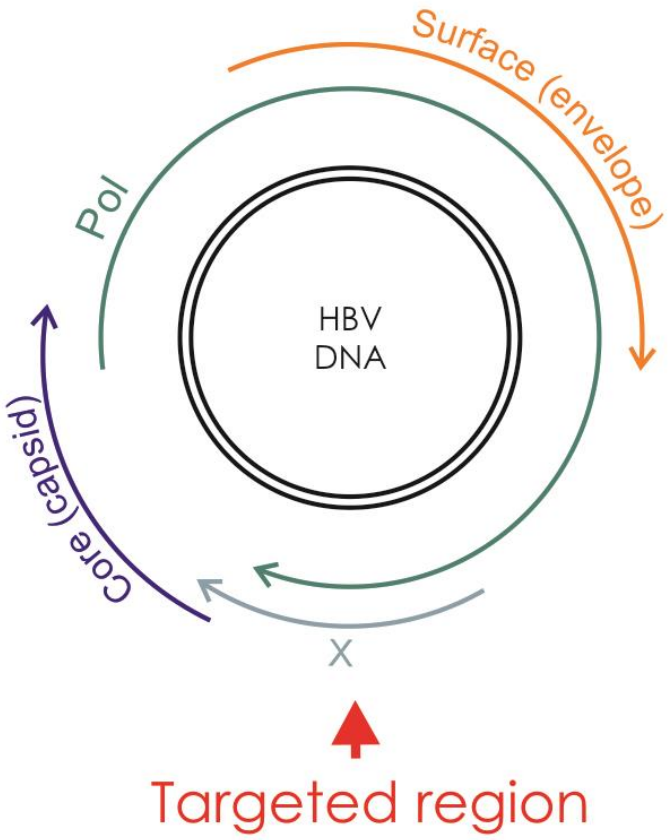
Anti HBV gene silencing strategy



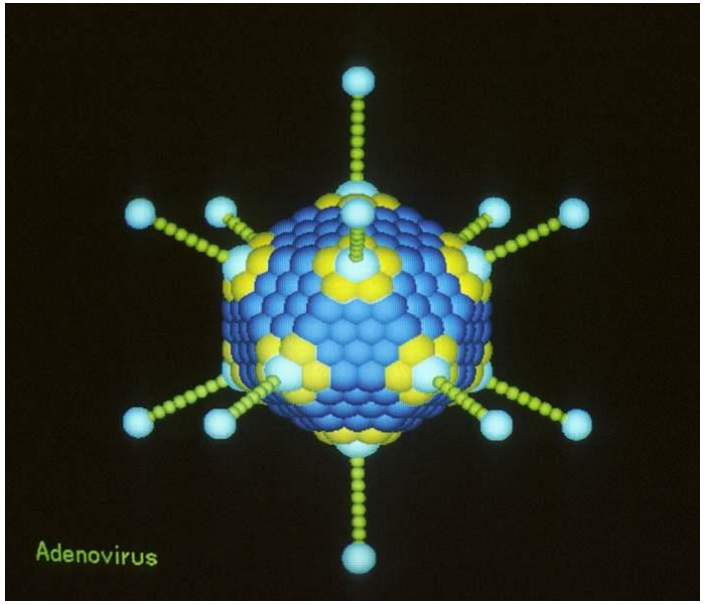
Anti HBV gene silencing strategy



Anti HBV gene silencing strategy



Engineering Adenoviruses to silence HBV



Engineering a virus to silence HBV

Engineering a virus to silence HBV

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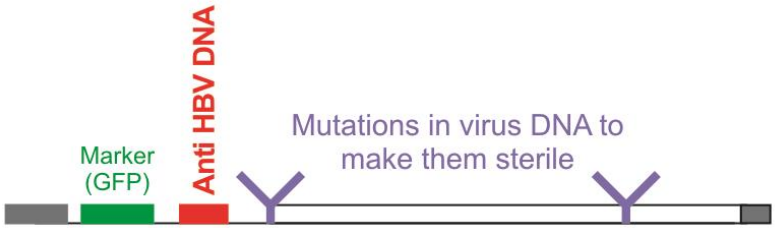
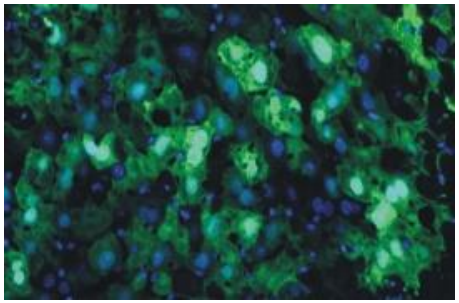
Engineering a virus to silence HBV



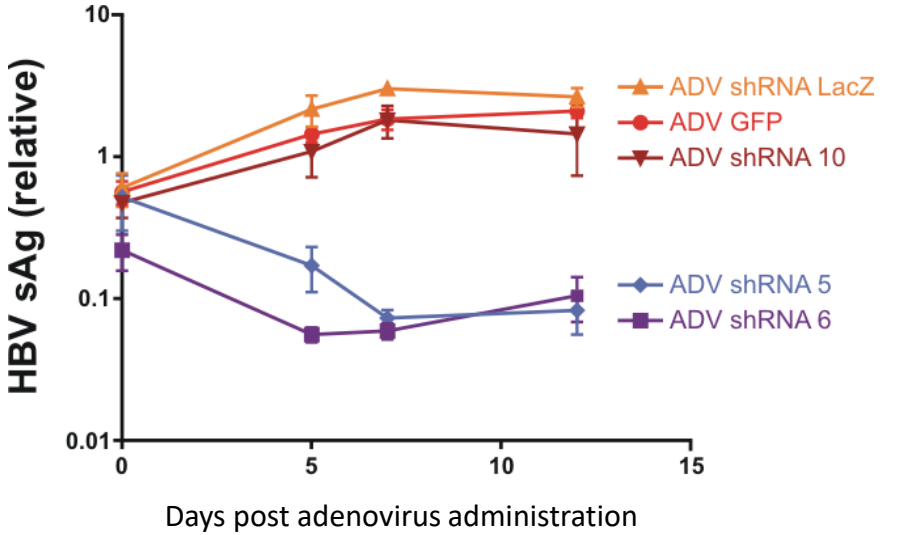
Using engineered adenoviruses to silence HBV



60-80% marker gene delivery to hepatocytes

Serum HBsAg concentrations

HBV transgenic mice treated with recombinant adenoviruses



Days post adenovirus administration

Gene Therapy for HBV Infection

- Proof of principle has been established
- Challenge is to convert a complex biological formulations into feasible therapies
- Convenient and safe large scale preparation of efficient vectors remains paramount

Defining the HBV delivery difficulties

- Delivery to enough liver cells
- Targeting the liver
- Sustained and complete ‘knocking out’ of the virus
- Complex drugs
  - side effects
  - immune responses
  - predictable distribution
  - good dose control
- Expense

Current Focus on Delivery of antiHBV sequences

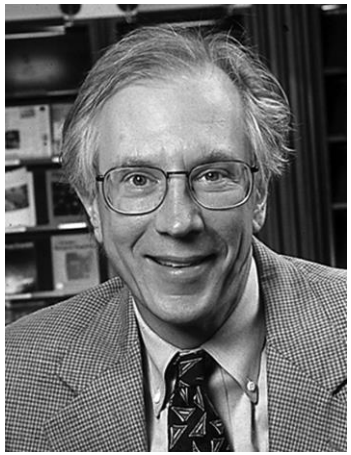
- A key will be development of technology to enable large scale preparation
- Valuable lessons may be learned from viruses
- Semi synthetic viruses

- Need to be selective in virus harnessing properties

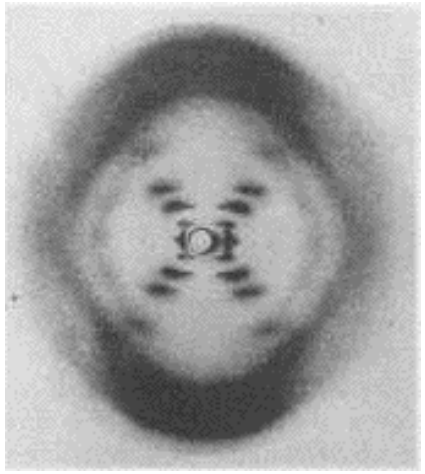
The Appeal of Gene Therapy

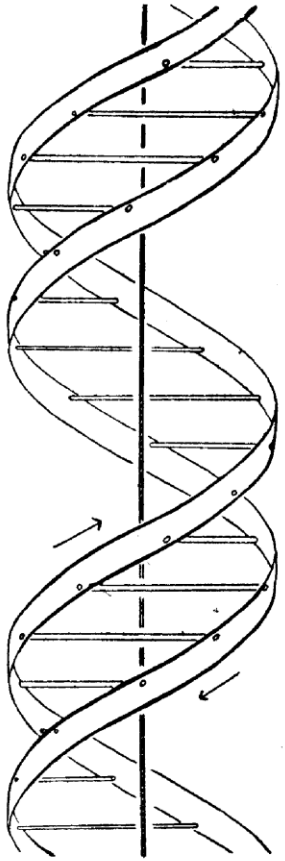
…. Science is knowing nature. This implies that there is a fixed set of facts that everyone wants to know. ….. I suspect that artists are not striving for commonality, but rather, that the content as well as the form of their work is unique.

Thomas Cech, Nobel laureate in Chemistry in 1989

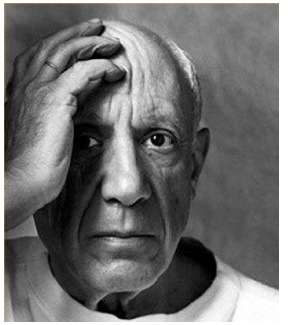


Discovery of the double helical nature of DNA





Picasso's Guernica

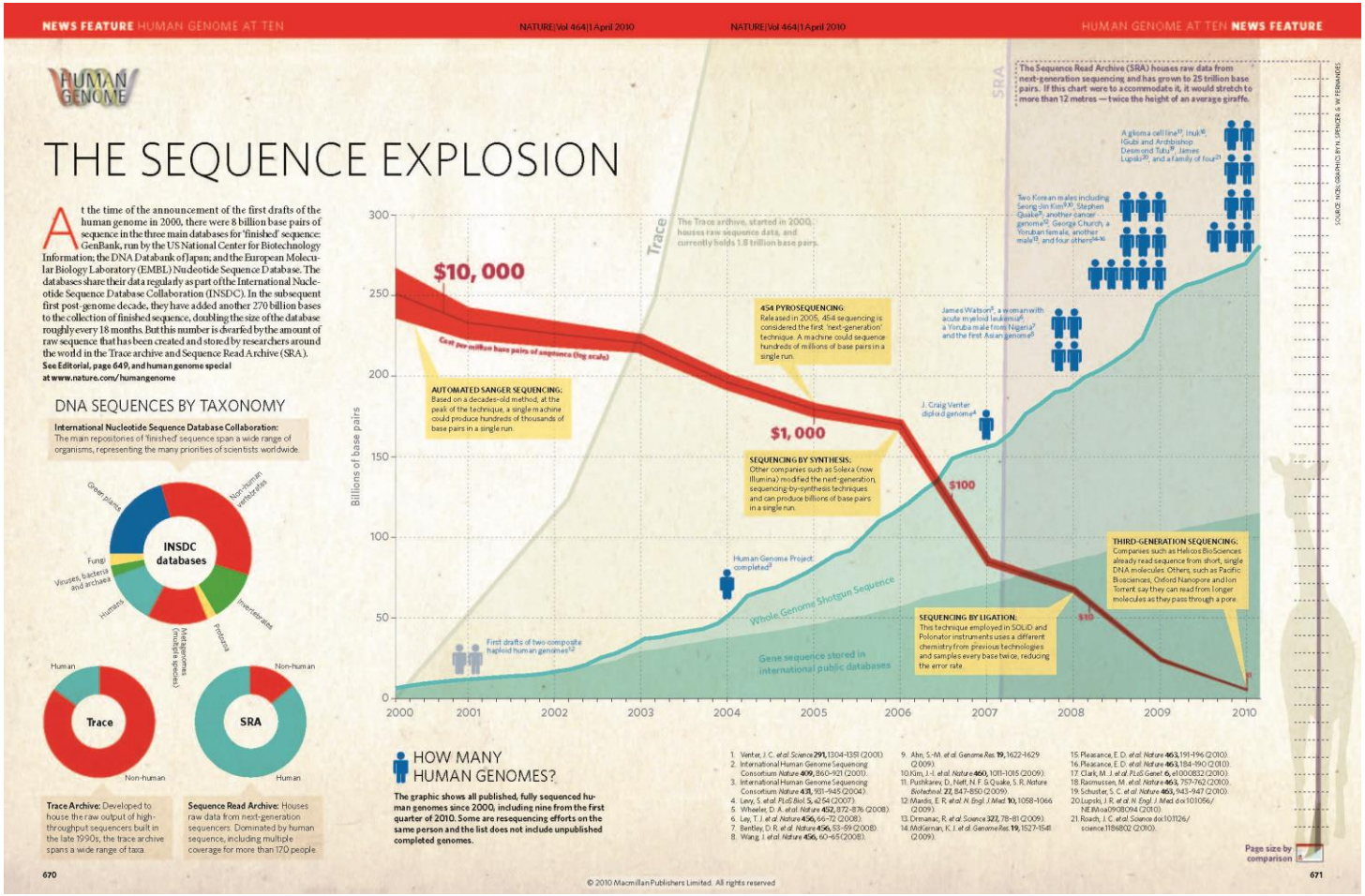


Pablo
Picasso

Gene Therapy: Prospects and Relevance

- Progress is rapid and the field has considerable momentum
- New technologies continuously being developed
- Successive waves of innovation will come and they will advance gene therapy
- Impressive developments in DNA sequencing technology will have an impact on the future applications of gene therapy

Large scale sequencing



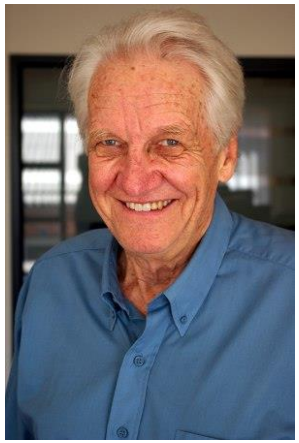
Nature, April 2010

Gene Therapy: Prospects and Conclusions 2

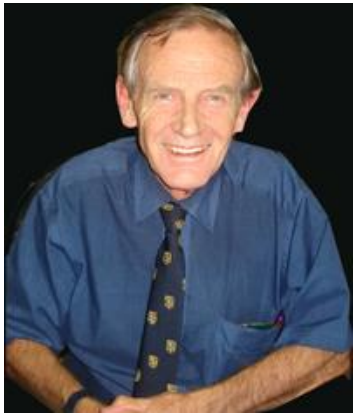
- Blockbuster drugs that will lose their patent exclusivity will be greater than the number of drugs that will be approved
- Gene therapy will enable rapid rationale drug design for many diseases
- Important that scientists in South Africa participate fully in development of these new technologies

Research partners and influential people

Wyn Fitschen



Michael Kew



Many international and South African research collaborators
Nicolas Ferry
Christian Bréchot
Bill Robinson
Pat Marion
Mark Feitelson
Piet Herdewijn
Joachim Engels

AGTRU Team

AGTRU
antiviral gene therapy research unit

Marc Weinberg

Abdullah Ely