June 1975

NOTES ON GENETIC ENGINEERING SEMINAR

1) What are the aims?

A) Reasonable chance of success

1) Correction of single gene defect diseases; recessive inborn errors of metabolism; occur 1/1000 births; over 100 diseases known.

2) Production of valuable products such as hormones in bacteria.

3) Production of better species by cloning and by introducing nitrogen fixing genes into non-leguminous plants.

B) Unlikely to succeed

1) Dominant gene defect diseases such as familial hypercholesterolemia

2) Multigenic defect diseases such as diabetes

3) Diseases with extra chromosomes such as Down's syndrome

2) What are the techniques available?

A) Transformation - use either DNA or chromosomes; either directly or by cell fusion.

1) Bacteria - well characterized

2) Eukaryotes -

a) Drosophila - produce stable mosaic flies when treat embryos with DNA; propose "exosome" model since donor DNA maps at homologous locus but recipient DNA is still present.

b) Mice - induction of melanin synthesis in albino mice skin by DNA from pigmented mice; transfer of IUDR and azaguanine resistance in lymphoma cells.

c) Deer - incorporation of poly dA poly dT in chromosomes of Montjak deer determined by "C band" staining for repetitive sequences.

d) Plants - thiamineless mutants of Arabidopsis were corrected by treating seeds with DNA from E. Coli.

B) Transduction

1) Bacteria - well characterized

2) Eukaryotes

a) Nondefective viruses

1) Herpesvirus - thymidine kinase in TK^- mouse cells

2) Shope papilloma virus - lowered serum arginase levels in humans; now think virus induces host cell enzyme rather than coding for arginase

so not useful. Dubious also on grounds of inoculating tumor virus into children.

3) Friend leukemia virus - 60S genome RNA associated with 9S hemoglobin m - RNA.

b) Defective viruses

~~1)~~ λ gal - gal operon in human galactosemic cells

2) λ lac - lac operon acquired by lac⁻ plants

c) Pseudoviruses

1) Host cell DNA only - random selection of DNA; particle penetrates, uncoats and enters nucleus, but is degraded rapidly and radio-label is incorporated into DNA; could construct an artifical pseudovirion using pure DNA with capsid protein to protect DNA and provide specificity of cells infected.

2) Host cell DNA integrated in viral DNA - SV40 supercoils contain mostly unique sequences of host cell DNA when virus is passed at high MOI.

C) Construction of hybrid DNA molecule

1) Cleave purified, closed/circular recipient viral or plasmid DNA with restriction endonuclease, example: R_I endonuclease makes one scission in SV40 DNA.

2) If donor DNA is also closed circular DNA then cleavage with same endonuclease will produce cohesive ends which will reanneal to produce a "nicked" hybrid DNA. If donor DNA is not susceptible to restriction endonucleases, use terminal transferase to add complementary bases to both 3' ends of the donor and recipient DNA's which will then reanneal to form a "nicked" hybrid DNA.

3) DNA ligase produces the covalently closed circular hybrid DNA.

D) Function of hybrid DNA's

1) Hybrid DNA composed of two resistance factor plasmids (TET and STR) caused resistance to both antibiotics in E. Coli; isolated the hybrid plasmid from a resistant colony; conclude that the hybrid replicates and codes properly for the enzymes which govern resistance. (PNAS 70, 3240, 73 and PNAS 71, 1030, 74).

2) Hybrid DNA composed of Xenopus ribosomal DNA and p SC101 DNA replicates in E. Coli and RNA complementary to the Xenopus DNA is synthesized (PNAS 71, 1743, 74).

3) How to obtain single gene for transformation

A) Chemical synthesis - Example: ALA t-RNA

B) Isolation - Example: Lac gene from *E. Coli*

C) Reverse Transcriptase - isolate m -RNA and transcribe into DNA. Use this directly or as a probe to recover cell sequences

D) Restriction Endonuclease - cleave DNA into random population of gene size pieces then employ selection techniques in culture to obtain cell with correct gene. Example: Select for cell with HGPRTase gene in HAT medium. The R_1 endonuclease cleaves at a sequence which, on a random basis would occur once every 4,000 to 16,000 nucleotide pairs so fragment ~~would~~ might contain one or more intact genes.

4) What is the sequence of events at the cellular level using transduction or purified DNA for gene therapy? What are the associated problems?

A) Uptake ?Efficiency ?Specificity

B) Uncoating and transport to nucleus ?degradation by DNase in lysosomes

C) Stabilization eg., either integration or independent replication ?integration site ? acquiring a replicon. Could use viral DNA to provide integration site or replicon.

D) Transcription of DNA - ? promotor; ? correct processing

E) Translation - ? correct start and stop signals; ? correct reading frame

F) Functional protein - ? correct base sequence; ? correct amount; ? correct cell type to use the protein

5) What are the hazards in the introduction of foreign DNA?

A) Mutagenesis during preparation of DNA

B) Introduction of cellular or viral genes for malignancy

C) Damage to existing synthetic regulatory processes

6) Special problems in whole animal or person

A) Differentiation of cells - see deficient enzyme only in specific organ, eg., 1) PHE hydroxylase (deficient in PKU) is only in liver so if put gene into another type of cell, it may not function 2) gene for hormones have to be put into cells which respond to inducer 3) blood-brain barrier may prevent enzyme from getting to brain so may have to modify brain cells directly.

B) Immune system may recognize the new enzymes as foreign.

7) Research criteria which should be met before gene therapy

A) Adequate biochemical characterization of disorder - if mutant DNA results in no enzyme then gene therapy is okay, but if normal DNA results in normal enzyme which is rapidly inactivated then gene therapy is not okay.

B) Sufficient prior experience with the disease and its alternative therapies

1) Some normal adults have high PHE so can't treat blood chemistry only

2) Some newborns have high PHE and normal PHE later.

C) Adequate characterization of DNA and its vector.

D) Successful animal studies - requires animal model.

E) Test cells in tissue culture - to assess side effects such as chromosome damage or malignancy.

8) Protection against abuse

A) Informed consent

B) Clearance by human experimentation committee

C) Funding of grants

9) Alternative to gene therapy for disease

A) Supply missing metabolite

B) Supply missing protein

Genetic counselling

C) Limit intake of precusor

D) Metabolic inhibitors

E) Induction of degradative enzymes

F) Organ transplant

10) Answers to questions

A) Question 1:
a) SV40 DNA may cause malignancy
b) Diabetes is a multigene gene disease which is not cured by insulin alone. The vascular changes continue despite adequate insulin.
c) Passage of cells in culture predispose to malignant change

Question 2: See part 5 of these notes.

Question 3: 4×10^5 daltons of DNA make 2×10^5 daltons of RNA which represents about 6×10^2 bases which code for 2×10^2 amino acids which weigh 2×10^4 daltons. Since there are approximately 2×10^{12} daltons of DNA per cell, one gene represents $4 \times 10^5/2 \times 10^{12}$ or $1/5 \times 10^6$ of the DNA of the cell.

Question 4: See part 3 of these notes.

Question 5: See part 4 of these notes.

Question 6: Relatively good in Lesch-Nyhan and galatosemia since they are recessive enzyme deficiencies. Perhaps Lesch-Nyhan has an edge since you can purify X-chromosome and recover gene more easily than galactosemia. Relatively poor in dominant enzyme defects, multigene diseases and diseases with extra-chromosomes.

Question 7: See part 1 of these notes.

Question 8: See parts 7, 8 and 9 of these notes.

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