Lecture 1. Columbia University. April 6, 1964

Principles Derived from Study of Bacterial and Phage Genetic Systems

I. The Organization of the Nucleus in the Bacteria:

1. DNA is not bound with protein as in higher organizims.
2. No true nucleus as in higher organisms; a "nuclear vacuole" containing the DNA.
3. No nucleolus; no nuclear membrane. Slides 1, 2, 3, 4
4. DNA in form of a ring. E. coli, K-12; DNA 1,100 to 1,400 microns long. 30-40 Angstroms wide.
5. Replication of bacterial chromosome: Semi conservative. Starts at one position and continues along chromosome: Cairns Diagram Slide 5

   Autoradiograph Study: Photograph: Slide 6
6. Position of start of replication process: Examined in E. coli and B. subtilis.

   (a) B. subtilis: One strain: always starts at one point and goes in one direction:

   Begins → ade — thr — tyr — ind his — leu — iluval — phe — lys met ileu To end

   (b) Other strain: No discovered set position. starts at different positions

   (c). E. coli, K-12. Relation of start of replication to the sex factor, when present: Always starts at F factor: Slide 7

   (d). No mitotic apparatus; cell membrane; particular position; Mode of growth of bacterium during division. Slide 8

   When F episome incorporated into bacterial chromosome - its replication system dominates bacterial system, as shown by Nagata. Significance important for sex behavior. Will return to this.

II. Types of genes recognized in bacteria: Classes:

Class A: (1) "Structural" genes; related to production of enzymes:

m RNA - transcription from DNA. Protein - translation from mRNA

Mutant sites in the structural genes: Diagram

Change in base; = change in amino acid in protein. transfer

leader from ???. An end to transcription.

Class B: Only RNA produced:

(2) Ribosomal genes - 2 % of genome

(3) Transfer RNA - soluble RNA one or more for each amino acid # known; how acts. One or more for each amino acid. amino acid → code

(4) Regulator genes -- product not yet known. Possibly RNA attached to protein

Special classes: C.

(5) The super-suppressors: Types DNA + RNA phage Imp.

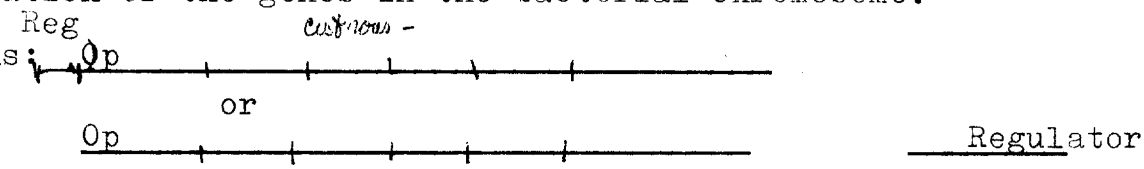
(a) Sup. Garen et al. general - bacteria phage.

(b) Sm^r certain mutants requiring streptomycin. Ribosomal modification Imp. 5 or 6 locus

(6) The "operators" At initiation point of reading (transcriptional) of DNA of gene or operon.

III. The organization of the genes in the bacterial chromosome.

1. The operons:



Examples: Histidine: Genes -enzymes for biosynthetic pathway to production of histidine: Slide 9
Order of genes and enzymes in pathway:
Position of first enzyme and the operator. Coordination.

2. Biosynthetic pathway - genes not together: Example: Arginine genes Slide 10 . The Regulator - position; Coordination not as above

3. Salmonella and E. coli: related. Order of genes the same in both organisms.

4. Special type of gene organization and control: The H_1 and H_2 genes producing flagella antigen:
Duplicate genes; only one acts at a time. Control mechanisms -- will consider along with maize control mechanisms.

IV. Transformation and transduction: significance for relating bacteria to higher organisms.

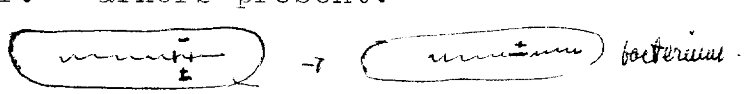
1. Transformation: DNA molecules extracted from one strain: placed in medium with another; markers present; uptake of DNA molecules; replacement of DNA in bacterium by introduced DNA molecule.
Extraordinary process: Synapsis on the molecular level; occurs with great rapidity.

2. Transduction: occurs through participation of episomes. Introduce DNA from one bacterium to another through being carried by the episome.

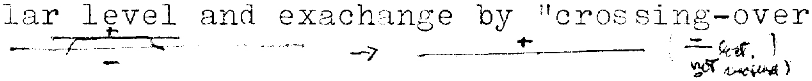
3. The bacterial viruses: DNA viruses. Different types. Different sizes.

Example: The phage particle; its parts. Slides 11, 12
Attachment of phage to bacterium: Slide 13
Insertion of phage chromosome: Slide 14
Behavior of bacterium during phage reporduction: Slide 17
The phage chromoso e: Lambda: Slides 15, 16 16.3 microns long
Small phage. attachments end-to-end
The order of the genes in phage T-4: Slide 18

4. Transduction: Phage picks up piece from bacterial chromosome: Infects bacterium. Does not contain its own genes. No phage reproduction. Piece from one bacterium to another. Markers present:



5. Importance: Synapsis on molecular level and exachange by "crossing-over"



6. Abortive transduction: piece of bacterial chromosome introduced by phage does not become incorporated. Has not right parts for crossing over? Example of action:

Flagella genes: Bacterium - - for flagella gene.
introduced gene - + for " " , not incorporated.

non-motile bacteria - divides in location
single motile bacterium - continues to move: Result - a trail of non-motile bacteria left behind.

Illustration: Slide 19

Importance: Naked DNA = able to produce mRNA. Not incorporated into nuclear vacuole; passed from cell to cell; cant replicate!

V. Comparison of above with higher organisms: Organized nucleus; nuclear membrane, nucleolus; mitotic apparatus.

1. Chromosome constitutions in higher organisms: DNA associated with protein. Strands thus much wider: Slides.
Protein present Slide 20 Size of strand:
Protein removed Size of strand, chromatids

(b). Same, monolayer - low power Slide 21

(c). Triturus - Slide 22

(d) " Slide 23 Histone removed in parts.

2. Activity of DNA: Appears that histone must be removed from gene before it can produce mRNA. Some controll mechanism must be present for this. Evidence (will be) reviewed by Dr. Moore.

3. Types of genes: Same kinds found in the higher organisms. Expect more sophisticated control "genes" to be present in higher organisms.

4. Organization of the genes in higher organisms: Same synthetic pathways in higher organisms as in E. coli and Salmonella. Genes not organized in long operons. More like the arginine genes in bacteria. Not even on same chromosome.

5. Synapsis of homologous "molecules" in higher organisms in somatic cells -- when diploid.

a). Synapsis of homologous chromosomes in diptera - standard.

b). Mode of synapsis: not clear that it is molecule for molecule.

c). Synapsis at meiosis: not altogether a homologous act: Only initiated by homology.

d). Under yet unknown conditions, synapsis must occur in some cells in higher organisms as shown by somatic crossing over (see 6, below).

[illegible]

6. Somatic exchange between homologous parts of chromoso es in diploids:
   a). In fungi - Aspergillus; [illegible] Yeast; [illegible] etc. Somatic crossing-over in <u>diploid</u> cells is not uncommon.

   b). In higher organisms: occurs on occasions: Drosophila: Stern and students. Maize: How tested:
      (a) Cytological exam: Knob exchanges

      (b) <u>Rare</u> crossover: cluster on ear: from somatic event.

   c). Rarity - suggests some special conditions of chromosomes: Naked DNA? When gene active with histone removed -- like bacteria?

   d). Difficulty of observation in higher plants and animals: characters.

[illegible]

7. Activity of fragment chromosomes: Comparison with abortive transduction.
   a). Fragment of only two or three chromomeres in maize: Active as long as it is within the nucleus. (<u>Sh Bz</u> or just <u>Bz</u> fragment in case of Fragment Chromosome 9.) Demonstration: <u>Ear of maize</u>.

   b). Chromoso e in separate nuclei - will be active if they have a nuclear membrane. If no nuclear membrane formed, genes not active.

      Evidence for function of genes when nuclear membrane present: Frances Clark, recessive gene for divergent spindles at meiotic divisions.

      Slides: Anaphase I (slide 24); Telophase I (slide 25); Normal tetrad (slide 26); Divergent spindle - spore prophase through first division in spore (slides 27, 28, 29)

   c). Fragment of chromoso e - left in cytoplasm: Not active if no nucle r membrane formed:
      (1) Appe rance of fragment in cytoplasm: pycnotic • <u>Slide 30</u>

      (2) Proof of inactivity of fragment:

(a) Break:

Behavior of nucleus.

Use: (b) (Ring chr. bm_1; death of cells).

Illustrations: Ring chr. paper.

VI. Summary of important evidence derived from bacterial genetics: to present discussion:

VII. Lysogeny: incorporation of DNA of episome into bacterial chromosome.

1. When phage enters a bacterium, one of two things may happen:

(a) Commences vegetative multiplication, as described above. Or,

(b) Phage DNA becomes incorporated into bacterial chromosome. Slide 31

(1) Phage now multiplies along with bacterial multiplication: Replication with bacterial DNA replication as part of bact. chromosome.

(2) Presence of phage in bacterial chromosome determined in several ways:

(a) Will not allow phage of same type to multiply in cell. Incorporated phage produces a repressor substance that represses first stages of phage multiplication.

(b). Phage sometimes released from bacterial chromosome and multiplies vegetatively as above: bacterium lysed and phage particles released. Occurs spontaneously or induced by U.V. or chemical treatment.

Lysogeny: name associated with potential for lysing bact.

2. Position where phage is incorporated into bacterial chromosome: Two types

(a) Can enter any location in the bacterial chromosome: Used for producing phage for transduction of different bacterial characters: Grow bacteria carrying prophage (phage DNA in bacterial chromosome). Treat with U.V. light to release phage.

(b) Phage is incorporated into one particular position in bacterial chromosome. Example, Lambda phage at locus of gal genes in bacteria.

(c) Induce lysis of bacteria carrying lambda. Occasional phage particle that picks up part of gal locus; loses part of its own DNA. Will transduce the gale locus at very high frequency as a consequence. Called high frequency transducing phages.

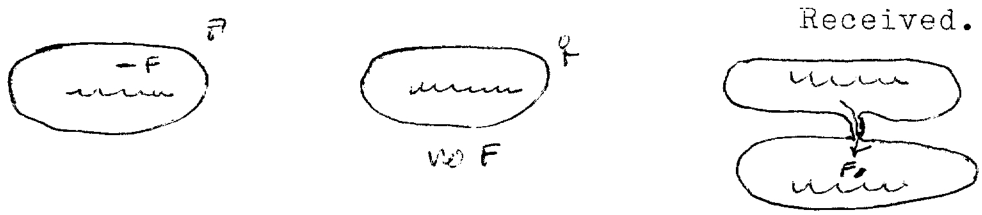
VIII. The sex-factor. A DNA carrying episome. Does not lyse the bacterium;

1. F factor exists in two different states: In cytoplasm of the bacterium or incorporated into the bacterial chromosome.

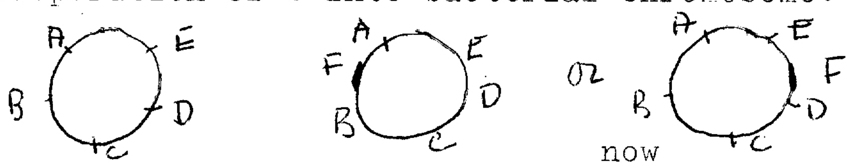
2. When in cytoplasm; divides along with the bacterial chromosome: Slide 32 Has its own replication system; associated with cell membrane.

3. Membrane association of F related to conjugation between bacteria carrying F and those with no F: Transfers the F factor from male to female through the tube: Slide 33

Male | Female | Female becomes a male when F Received.



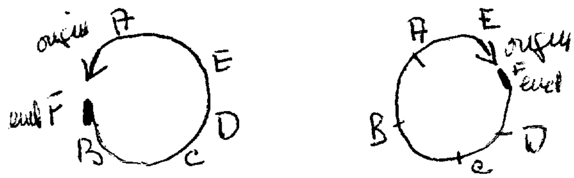
4. Incorporation of F into bacterial chromosome: Positions



now

5. F factor: its replication system takes over control of initiating position of replication of the bacterial chromosome: "Dominant".

6. When incorporated into bacterial chromosome: carries bacterial chromosome into female during conjugation:



7. Position of incorporation of F: Varies. Any one position gives high frequency of transfer of those genes near the origin. Chromosome entering bacterium takes 120 minutes at normal temperature

\# Chromosome can be broken off during process; part that has entered can under go recombination "crossing-over" with chromosome of female.

8. Factor -- controlling element -- on F factor that controls enterance of F into female,(with or without chromosome of bacteria attached.) If within bacterial chromosome, will carry bacterial chromosome along with it. Resembles the controlling factor in Sciara X.

IX. The sing-stranded DNA phages: Single strand in phage particle.
During replication, a double strand and a ring.shape.
Double strand required for replication.

X. Importance of double-strand: Required for reduplication of DNA.
Required for gene action although onely one strand is used for transcription process.

XI: Summary to present of contribution of bacteria and phage to genetics:

Slides

1. Bacterium - parts - From Stent
2. " DNA rounds " Bragg — Salmonella typhimurium cocci - Tetra coccus
~~4~~ 3 " " " and attachment to membrane - Jacob
~~5~~ 4. Replication - Diagram - Cairns
~~6~~ 5 " - Bact. chromosome - Cairns
~~7~~ 6. " - E. coli K-12 HFR - Nagata
8 Division of bacterium - Diagram. Jacob
9 Histidine operon - Ames, et al
10 Arginine genes - Jones
11 phage particle - elect. microscope + diagram - Stent.
12 " " " " - Stent
13 " particles attached to bacterium - elect. micr. Stent
14 " DNA from one burst out " " - Stent - full page
15. Phage chromosome - λ - small phage - 16.3 μ long. Ris
16 " " end to end λ - Ris.
17 ~~Growing~~ Effect of phage on bacterium of ten unit intervals - series of steps.
18 Growing genes in " T_4 in phage chromosome - Epstein -
19. Abortive transduction - illustration - Stent
20 Calf-thymus - DNA - Ris
21 " " " - mono-layer. low power. Ris
22 Tubules " " Ris 2 parts
23 " " " Ris - histone removed
24. Divergent spindle A I
25 ~~Four spindles at T II~~ Divergent spindles - T I
26 Four normal spindles T II

27 - Divergent Spindle - Spores (figs 14-16) Pro
28 " " " (" 20-22) metaphase to telo.
29 " " " (23-25) later stage.
30 - [illegible] - [illegible]
31 " [illegible] - [illegible]
32 F [illegible] - [illegible] together - [illegible]
33 Conjugation of [illegible] ? 2