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The fate of P22 DNA during lysogenization

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BOSTON UNIVERSITY

GRADUATE SCHOOL

Thesis

THE FATE OF P22 DNA DURING LYSOGENIZATION

by

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Abstract

Salmonella typhimurium LT2 was infected with tritiated thymidine labeled phage P22 at a multiplicity of infection of 0.133. Autoradiographic experiments exclude the possibility that P22 DNA replicates conservatively or semi conservatively during lysogenization of LT2. The model of phage DNA replication that fits the data is complete dispersion of phage DNA upon replication.

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Chapter I: Introduction

A cell when lysogenized by a temperate phage, carries the phage genetic determinants which are contained in the phage deoxyribonucleic acid (DNA) (Hershey and Chase, 1952). The lysogenized cell carries only the DNA of the phage and not its protein coat. Phage DNA which is carried by a lysogenic cell and which replicates along with it has been termed the prophage.

Morse, Lederberg, and Lederberg (1956) and Arber (1961) have shown that the lambda prophage has a specific location on the E. coli K12 chromosome, and that lambda phage can accomplish generalized as well as specific transduction. A lambdoid strain, lambda 434 has been shown by Jacob and Wollman (1958) to be less intimately associated with the K12 genome. The relationship between phage P22 and its host Salmonella typhimurium strain LT2 appears to be similar to that between 434 and K12 (Lederberg, Lederberg, Zinder, and Lively, 1951), but since there is no known sexual system for Salmonella typhimurium, one can not be sure.

This work concerns itself with the question of the fate of prophage DNA upon lysogenization of the host cell. The LT2 - P22 system was chosen for this study.

The macromolecule of practically all organisms which, under the proper conditions, directs cellular functions is deoxyribonucleic acid (DNA). DNA may be broken down into subunits called nucleotides. A nucleotide consists of a 5' phosphorylated monosaccharide, 2-deoxy-D-ribose, and one of four nitrogenous bases; adenine, thymine, guanine, or cytosine linked to deoxyribose carbon atom number one. Dinucleotides are formed by an ester linkage between the 5' phosphoryl group of one nucleotide and the 3' hydroxyl group of the next. Polynucleotides are formed by the same mechanism.

Recently, (Watson and Crick, 1953) the secondary structure of DNA has been determined. X-ray crystallographic data (Watson and Crick, 1953) and data obtained from base composition analyses (Chargaff, 1951) reveal that the DNA molecule consists of two dextro-plectonemic polynucleotide chains held together by specific hydrogen bonding. The hydrogen bonds are the result of base pairing. A purine must pair with a pyrimidine, the purines being adenine and guanine and the pyrimidines, thymine and cytosine. The most common tautomeric forms of the bases limit pairing to adenine with thymine and guanine with cytosine, in line with Chargaff's data (1951).

The Watson-Crick model can be used as a basis upon which to predict three general modes of replication of the DNA molecule: conservative, semi conservative, or dispersive.

CONSERVATIVE REPLICATION:

According to this model, a DNA molecule remains intact and directs the synthesis of an entirely new molecule. Stent (1958) has proposed a ribonucleic acid (RNA) intermediate, coded by the DNA molecule, which directs the synthesis of a new DNA molecule. The secondary structure of the DNA molecule permits the intimate pairing in the large groove of the double stranded DNA molecule with a single stranded RNA molecule.

SEMI-CONSERVATIVE REPLICATION:

According to this model, each strand of the DNA molecule serves as a template for the synthesis of a complementary strand. Thus an old molecule of DNA is equally partitioned between two new molecules upon replication.

Evidence supporting semi-conservative replication has been obtained by Meselson and Stahl (1958). E. coli cells were labeled with heavy nitrogen (N15) and grown in normal (N14) medium. The DNA of parental, first, second, etc. generation cells was subjected to cesium chloride density gradient centrifugation. A band of DNA with intermediate density corresponding to DNA molecules with one N15 strand and one N14 strand appeared upon centrifugation of first generation DNA. As replication proceeded this band became less pronounced and was superceded by a band of light density corresponding to DNA molecules with two N14 strands.

Sueoka (1960) repeated the Meselson-Stahl experiment with the unicellular alga Chlamydomonas reinhardi and obtained similar results, indicating that DNA replication is similar for both the higher and lower organisms.

Kozinsky (1961) has shown that hybrid DNA fragments appear upon ultrasonication of phage T4 progeny DNA.

Autoradiographic experiments by Levinthal (1956) have shown that the DNA of a few phage T4 progeny contain half the phosphorus of the parental particle after

one and two cycles of growth in the host cell. These experiments may indicate that a large piece of the phage DNA replicates semi-conservatively and that the remaining piece is either nongenetic or technically lost. These experiments may also indicate a form of dispersive replication.

DISPERSIVE REPLICATION:

According to this model, a molecule of DNA is dispersed at replication and fragments are incorporated randomly into new DNA molecules.

Experiments by Stent and Jerne (1955) in which P³²-labeled phage were introduced to cells at high multiplicities of infection indicate that even the earliest progeny do not contain greater than two per cent of the parental phosphorus per phage particle. The results are made suspect by the discovery that homologous phage DNA introduced to an infected cell later than two minutes after the initial infection is degraded, a phenomenon referred to as super-infection breakdown. In subsequent experiments by Watson and Maaløe (1953) it was found that about half of the transferred parental DNA goes to the early formed phage progeny.

The problem of DNA replication is made more complex by the accompanying, if not dependent, process of genetic recombination. Recent experiments (Meselson and Weigle, 1961) indicate that one way of forming phage recombinants is by a fragmenting mechanism; actual physical exchanges between DNA molecules. Such a mechanism could certainly mask conservative as well as semi-conservative replication.

Phage heterozygotes, a class of phage particles which produce recombinants with high efficiency (Hershey and Chase, 1951), have not been studied extensively, but it seems apparent that their formation does not enhance the possibilities of determining the basic replicating mechanism.

A system which minimizes recombination is needed to delineate phage DNA replication. The system most closely approaching this requirement is found in the relationship between the temperate phage and the sensitive host cell. This situation obtains due to segregation of daughter phage genomes upon replication.

When a temperate phage infects a host cell, it has a choice of two alternate paths. It may either grow vegetatively and lyse its host or it may attach in some unknown way to the host cell genome and replicate with it, producing what has been termed a lysogenic clone (Lwoff and Gutman, 1950).

In essence, a phage particle is a nucleoprotein (Schlesinger, 1934). Hershey and Chase (1952) have shown that upon infection of a host cell, a phage injects its DNA and leaves its protein coat behind. The coat serves two purposes, protection of phage DNA and attachment to the host. The phage DNA directs the synthesis of mature phage progeny.

This paper describes experiments based upon an autoradiographic technique which has been adapted to answer the question, "What is the fate of phage DNA in lysogenized cells?"

High specific activity tritiated-thymidine labeled phage P22 were allowed to infect Salmonella typhimurium strain LT2 at a low multiplicity of infection. The complexes were grown on microscope slides in a non-radioactive agar medium. At intervals of time, the complexes were killed and a nuclear track emulsion was applied to the slides. The fate of phage DNA could then be followed by grains produced by the characteristic beta emission (a one micron track, maximum; Ferro and Wertheimer, 1960) seen above or within one micron of the extremities of the lysogenic cells.

Chapter II: Materials and Methods

(a) Bacteria

- (i) Salmonella typhimurium strain LT2, wild type, maintained in this laboratory since 1957.
- (ii) Salmonella typhimurium strain LT2 gal- (unable to ferment galactose), obtained by direct selection after UV irradiation.
- (iii) Salmonella typhimurium strain LT2 gal- Sr, a streptomycin resistant mutant of (a, ii) obtained by direct selection with 5 ug streptomycin per plate.
- (iv) Escherichia coli strain 15T- (unable to synthesize thymine), obtained from Barner, 1957.

(b) Phage

- (i) P22, maintained in this laboratory since 1957.

LT2 was infected with phage P22, a temperate phage specific for

LT2, at a multiplicity of infection of 0.1 in tryptone broth (see c, i) and the adsorption mixture was incubated at 37° C with aeration for four to five hours. The adsorption mixture was then subjected to low speed centrifugation (5000 g) to sediment LT2. The supernatant was treated with a few drops of chloroform to insure against bacterial contamination. Phage assays were performed according to d'Herelle (1922).

(c) Media

- (i) Tryptone broth, used for dilutions, preparations of phage stocks, growth tubes, and growth of bacterial stocks and plating cells contained, per liter ion-free water, 10g bacto-tryptone, 5 g sodium chloride, and 1 g glucose.
- (ii) Eosin methylene blue galactose agar medium (EMB), prepared according to Ting (1960).
- (iii) T- broth (thymineless), used for preparation of tritiated thymidine labeled P22 (see b, i).

A small inoculum of E. coli 15T- (see a, iv) was grown for three and one half hours at 37° C with aeration in tryptone broth (see c, i) to stationary phase. The cells were centrifuged down and the supernatant was again inoculated with a small number of E. coli 15T- cells. Stationary phase of growth was this time far below the first and was reached within two hours, indicating that essentially no thymine remained in the medium. The cells were centrifuged down and the supernatant was autoclaved and stored at 4° C for use in labeling experiments.

- (iv) Agar medium, used for incubation of experimental slides, contained per liter ion-free water, 15 g agar.

(d) Abbreviations

- (i) (DNA) deoxyribonucleic acid, (RNA) ribonucleic acid, (m.o.i.) multiplicity of infection, (H^3 P22) tritiated thymidine labeled P22, (EMB) eosin methylene blue galactose agar medium, (AM) adsorption mixture, (UP) unadsorped phage, (LC) lysogenized cells, (VC) viable cell count.

(e) Labeling of Phage

- (i) An inoculum of 8.0×10^7 LT2 cells in 0.25 ml T- broth was added to 250 μ C tritiated thymidine (Schwartz BioResearch Inc.) in 0.5 ml water (specific activity 6.25 C/mM). The cells were incubated for one hour at 37° C and were infected with 1.0×10^7 P22 in a volume of 0.25 ml tryptone broth (approximate m.o.i., 0.1). The mixture was incubated for one hour at 37° C, the cells were centrifuged down, and chloroform was added to the supernatent.

(f) Efficiency of Lysogenization

- (i) LT2 at 1.0×10^8 cells per ml were infected with H^3 P22 to give an m.o.i. of 0.1. Seven minutes after adsorption, aliquots of the adsorption mixture (AM) were distributed to EMB plates seeded with an LT2 gal- Sr background to determine unadsorped phage (using chloroform to lyse cells) (UP) and the fraction of infected cells lysogenized (LC). Aliquots of the LT2 culture were spread on EMB plates to obtain a viable cell count (VC). From data obtained in this experiment, the efficiency of lysogenization was determined. UP and VC plates were incubated at 37° C for 24 hours. LC plates were incubated at 26° C to enhance lysogenization (Luria, 1958).

After 48 hours incubation, LT2 colonies appeared as green papillae against the white LT2 gal- Sr background. A lysogenic colony was characterized by a surrounding halo of lysis.

(ii) Colonies identified as lysogenic were tested. Thirty individual supposedly lysogenic colonies were sampled and the samples were suspended in five ml tryptone broth. A loopfull of each sample was spotted on an EMB plate supplemented with 5 ug streptomycin against an LT2 gal- Sr background. As a control, ten nonlysogenic colonies were tested. Plates were incubated for 24 hours at 37° C. All thirty lysogenic colonies produced areas of lysis on the LT2 gal- Sr background while none of the nonlysogenic colonies produced these areas.

(g) Preparation of Microscope Slides for Autoradiography

(i) A 10^{-2} dilution was made from the adsorption mixture (see f, i) into tryptone broth and 0.1 ml samples were diluted into 10 ml soft agar medium (tryptone broth plus six grams agar per liter) at 46° C. Within twenty minutes, the mixtures were poured over microscope slides and incubated for zero, one, two, three, and four hours at 26° C on moist agar plates (see c, iv). At the appropriate times, the agar medium on the slides was dried down with warm air from a commercial hair dryer and the slides were held over chloroform vapors for one minute to kill cells. Control experiments showed that the efficiency of lysogenization was not affected by brief exposure of infected cells to a 46° C temperature under the conditions outlined above.

Dried, chloroform-treated slides were dipped in a dilute parloidian solution to prevent agar solvation and subsequent diffusion of bacterial cells

of a colony. Slides were then immersed in Kodak nuclear track emulsion NTB3 in the dark at 46° C and stored for two weeks at -15° C in light-tight slide boxes with anhydrous calcium sulfate as a dessicant. Slides were developed for a minute and a half in full strength Kodak D-11 developer, rinsed for two minutes in ion-free water, fixed for ten minutes in a one to four dilution of Kodak F-5 fixure, and rinsed in ion-free water for fifteen minutes.

Microscopic observations were taken with an A0 Spencer phase under oil immersion (magnification 1940) and with a Koehler illuminator.

Chapter III: Experimental

EFFICIENCY OF LYSOGENIZATION

Table I reports the results of four independent experiments with H^3 P22 to determine the efficiency of lysogenization. It may be seen that the efficiency of lysogenization is high enough to perform autoradiographic experiments.

AUTORADIOGRAPHIC DATA

The data reported in Table II are for four and eight celled colonies, since these two classes contained the largest number of representatives. A radioactive center is defined as one grain either over a cell or within approximately one micron of a cell's extremities, since most centers consisted of one grain (for background corrections, see appendix sections (a) and (b)).

The effective activity per phage was obtained from single cell data (time zero slides). At an m.o.i. of 0.133 the activity is 1.30 grains per phage (for derivation see appendix sections (a), (b), and (c)).

Table I

Efficiency of Lysogenization

<u>Adsorption Time</u> <u>(minutes)</u>	<u>m.o.i.</u>	<u>Efficiency of Lysogenization (%)</u>
10	0.130	14.60
5	0.072	26.60
7	0.110	19.90
*7	0.133	12.90

*Autoradiographic procedures performed on this sample.

Cultures of LT2 were grown with aeration to approximately 1.0×10^8 cells per ml and infected with H₃ P22 at an approximate m.o.i. of 0.1. After adsorption, aliquots of the adsorption mixture were plated to determine UP and LC. Plates were incubated at 26° C. Aliquots of uninfected LT2 culture were plated to determine VC. Plates were incubated at 37° C.

Table II

Distribution of One, Two, Three, and Four Centered Colonies

<u>Cells per colony</u>	<u>Centers</u>				<u>Total</u>
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	
4 U	23	27	9	5	64
4 C	23.90	23.35	8.10	4.00	59.35
8 U	40	39	12	5	96
8 C	40.25	29.10	9.11	3.20	81.95

U: uncorrected for background

C: corrected for background

Conservative Replication of Phage DNA

The distribution of one, two, three, and four centers for four and eight celled colonies (Table II) rules out the possibility that P22 DNA is conserved upon infection of LT2 at an m.o.i. of 0.133. At this multiplicity, essentially none of the infected colonies contain more than two phage (Table III) particles and it is impossible to account for the presence of multi-centered colonies by complete conservation of P22 DNA (see Table IV for expected distribution).

Semi-conservative Replication of Phage DNA

According to the semi-conservative hypothesis, three and four centered colonies are expected only for multicomplexes, never for monocomplexes. As many as three nucleoids per cell have been observed. Figure I demonstrates one possible mode of segregation of label with multiplicities of one or two phage assuming three nucleoids per cell and a semi-conservative model. The other assumptions implicit in Figure I are that nucleoid segregation is essentially complete at the eight celled stage and that lysogenization occurs before the first nucleoid division. Deviation from the pattern in Figure I tends to lengthen segregation time and therefore decrease the frequencies of three and four centered colonies. The predicted distribution of label on the semi-conservative hypothesis has been restricted to eight celled clones (Table IV) (for derivations, see appendix section (e)).

Dispersive Replication of Phage DNA

It is clear that P22 DNA may be dispersed if one or many phage particles infects a cell. The predicted distribution of one, two, three, and four centered colonies, based on a completely dispersive model is shown in Table IV.

Table III

Distribution of Number of Phage Infecting Cells at a Multiplicity of
Infection of 0.133

<u>Cells Infected With</u>	<u>% of Infected Cells</u> <u>m.o.i. 0.133</u>
1 phage	93.80
2 phage	6.20
3 phage	0.00
> 3 phage	0.00

Calculations are made on the basis of a Poissan distribution.

Table IV

Predicted vs. Observed Distribution of One, Two, Three, and Four
Centered Colonies for All Models

	<u>Centers</u>				<u>*Level of Significance</u>
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	
<u>Observed Distribution</u>					
8 celled colonies (corrected for background)	40.3	29.1	9.1	3.2	
<u>Calculated Distribution</u>					
D	41.8	27.0	10.2	2.7	(90-95)%
SC	53.7	26.1	1.6	0.3	< .01%
C	78.1	3.6	0.0	0.0	< .01%
<u>Observed Distribution</u>					
4 celled colonies (corrected for background)	23.9	23.4	8.1	4.0	
<u>Calculated Distribution</u>					
D	32.6	20.3	5.8	0.7	< .01%

* The level of significance was determined by Chi square tests

Abbreviations:

(D) dispersive model, (SC) semi-conservative model, (C) conservative model
 (see appendix for derivations)

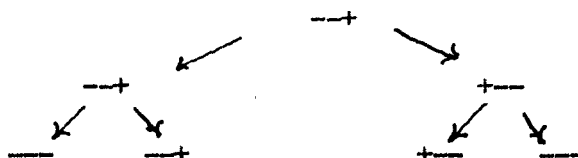
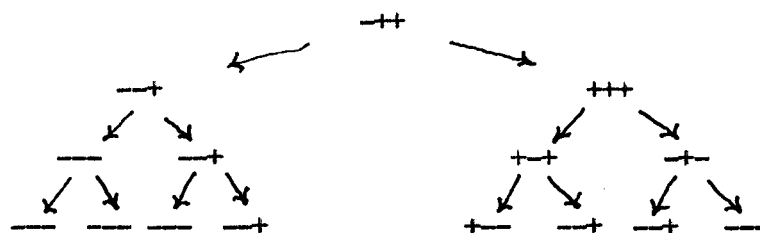
Legend to Figure I

Figure I assumes three nucleoids per cell. For further assumptions, see text.

(+), indicates a nucleoid carrying phage label.

(-), indicates a nucleoid not carrying phage label.

Figure I

Segregation of Nucleoids in Cells Infected with One and TwoPhage on Semi Conservative HypothesisCell Infected with One Phage ParticleCell Infected with Two Phage Particles

Chapter IV; Discussion

The distribution of centers for eight celled colonies (Table IV) clearly indicates dispersive replication of phage DNA during lysogenization of LT2. The distribution for four celled colonies is not nearly as clear.

The frequencies of three and four centered four celled colonies are more easily explained on the basis of dispersion than by either semi-conservative or conservative replication. Maximally 6.2 percent of the four celled colonies should be three and four centered if semi-conservative replication takes place and 0.0 percent if replication occurs conservatively (m.o.i. 0.133).

The expected dispersive distribution of four celled colonies may be obtained from the raw data corrected for 267 background grains per field.

The expected semi-conservative and conservative four celled distributions can not be obtained from the raw data by background correction since background would have been so high that all colonies would have been at least two centered.

The observed distribution of four celled colonies may deviate from the expected because of the small number of these colonies observed (64 four celled colonies as opposed to 96 eight celled colonies).

These experiments do not preclude the possibility that replication of phage DNA prior to lysogenization occurs. For any further work to be meaningful, this possibility must be investigated.

It has been shown (Luria, Fraser, Adams, and Burrows, 1958) that when P22 infects LT2 at an m.o.i. of 10 and the adsorption mixture is incubated at 26° C, most cell progeny are lysogenic. Experiments of this type are planned at a low m.o.i. Since there are three nucleoids per log phase bacterium, replication of phage DNA prior to lysogenization should be exemplified by lysogenicity of all cell progeny.

Chapter V: Appendix

(a) Background

- (i) The diameter of the microscope field under oil immersion (1940x) is approximately 138 microns (u).
- (ii) The area of the field is $3.14(69)^2 = 1.49 \times 10^4 \text{ u}^2$.
- (iii) The actual two dimensional area per cell is $2 \text{ u} \times 5 \text{ u} = 10 \text{ u}^2$.
- (iv) The effective two dimensional area per cell is $4 \text{ u} \times 7 \text{ u} = 28 \text{ u}^2$ since the maximum beta track given off by tritiated thymidine is 1 u.
- (v) The average background per field is approximately 30 grains.
- (vi) The area per grain is approximately 1/10 the area per cell, approximately 1 u^2 . There are therefore $30/1.49 \times 10^4 = 2.01 \times 10^{-3}$ grains per unit area field.
- (vii) From vi and iv there are $2.01 \times 28 \times 10^{-3} = 5.63 \times 10^{-2}$ grains per cell in the field or $5.63n \times 10^{-2}$ grains per n cells background.

(viii) Since background occurs at random, colonies of n cells should have background grains according to a Poisson distribution.

The frequency of n celled colonies with no background grains will be:

$$P_0 = e^{-5.63n} \times 10^{-2}$$

In general, the frequency of n celled colonies with r background grains (centers) will be:

$$P_r = \frac{(5.63n \times 10^{-2})^r e^{-5.63n \times 10^{-2}}}{r!}$$

(b) Correction of Data for Background

(see Table II)

If N'_x is the background-corrected number of clones with x centers and N_x is the total observed number of clones with x centers, since the greatest number of centers observed for any one clone was four, then for n celled clones where P_x is the appropriate term of the Poisson distribution:

$$N'_1 = N_1 - P_1N_1 + P_1N_2 + P_2N_3 + P_3N_4$$

$$N'_2 = N_2 - P_1N_2 - P_2N_2 + P_1N_3 + P_2N_4$$

$$N'_3 = N_3 - P_1N_3 - P_2N_3 - P_3N_3 + P_1N_4$$

$$N'_4 = N_4 - P_1N_4 - P_2N_4 - P_3N_4 - P_4N_4$$

(c) Autoradiographic Specific Activity per Phage

The autoradiographic specific activity per phage was 1.28 grains as calculated below. Of 646 cells observed on zero time slides, 58.5 (corrected for background) showed grains. At an m.o.i. of 0.133, 81 labeled cells are expected. The frequency of labeled cells showing no grains should therefore be 0.278 (0.28 if corrections are made for multiple infection). The mean number of grains per phage infected cell, assuming a Poisson distribution is thus 1.28.

The validity of the assumption of a multiplicity of infection of 0.133 on the slides is strengthened by the fact that the distribution of single cells with 1, 2, 3, and 4 grains follows a Poisson where $P_0 = 0.278$.

(d) Derivation of Results Predicted for
Dispersion

If (A) is the effective activity per phage, dispersion would lead to an effective activity of (A/n) in an n celled colony. Decay occurs at random and therefore the probability of a cell not having a center over it is:

$$P_0 = e^{-A/n}$$

and the probability of a cell having at least one center is:

$$P_{\geq 1} = 1 - e^{-A/n}$$

The frequencies of one, two, three, and four centered colonies for colonies of n cells are terms of the binomial expansion:

$$(P_0 + P_{\geq 1})^n$$

For one centered colonies the frequency is:

$$nP_0^{n-1} P_{\geq 1} = a$$

For two centered colonies, the frequency is:

$$n(n-1)P_0^{n-2} P_{\geq 1}^2/2 = b$$

For three centered colonies, the frequency is:

$$n(n-1)(n-2)P_0^{n-3} P_{\geq 1}^3/6 = c$$

For four centered colonies, the frequency is:

$$n(n-1)(n-2)(n-3)P_0^{n-4} P_{\geq 1}^4/24 = d$$

This derivation applies to cells infected with x phage, where $x > 0$. Cells infected with two phage which replicate dispersively would lead to an effective

activity per cell of an n celled colony of $(2A/n)$.

If the frequencies of cells infected with one and two phage respectively are E and F .

$$E + F = 1$$

then the frequency of one, two, three, and four centered, n celled colonies is:

One Center

$$E(a_1) + F(a_2) = i$$

Two Centers

$$E(b_1) + F(b_2) = j$$

Three Centers

$$E(c_1) + F(c_2) = k$$

Four Centers

$$E(d_1) + F(d_2) = 1$$

where the subscript refers to the number of phage causing the initial infection.

The number of one, two, three, and four centered colonies predicted is:

One Center

$$\frac{i}{i + j + k + 1} \times N$$

Two Centers

$$\frac{j}{i + j + k + 1} \times N$$

Three Centers

$$\frac{k}{i + j + k + 1} \times N$$

Four Centers

$$\frac{1}{i + j + k + 1} \times N$$

where $N = N^1 + N^2 + N^3 + N^4$ (see appendix section (b)).

(e) Derivation of Results Predicted for

Semi Conservative Replication

If (A) is the effective activity per phage, semi conservative replication would lead to two cells of an n celled colony with (A/2) activity per cell (if the initial infection was caused by one phage). Cells infected with two phage would have four cells eventually with activity (A/2) per cell etc.

If x is the number of cells containing label, e.g. x = 2 when the initial infection is caused by one phage, then the frequency of cells containing label showing no centers is:

$$P_0 = e^{-A/x}$$

while the frequency of cells containing label showing at least one center is:

$$P_{\geq 1} = 1 - e^{-A/x}$$

Each class of phage infected cells follows a binomial distribution for one, two, three, and four centered colonies, i.e.

$$(P_0 + P_{\geq 1})^x$$

where x is the number of cells of an n celled clone containing label.

From this point on, calculations are made as in section (d).

(f) Derivation of Results Predicted for Conservative

Replication

Cells infected with one phage

One cell would have an activity of A and therefore:

$$P_0 = e^{-A}$$

or the probability of a one centered colony would be:

$$P_{\geq 1} = 1 - e^{-A}$$

Cells infected with two phage

In this case two cells of an eight celled colony would have an activity of Λ .

$$P_0 = e^{-\Lambda} \text{ and } P_{\frac{1}{2}} = 1 - e^{-\Lambda}$$

In this case, the binomial distribution is:

$$(P_0 + P_{\frac{1}{2}})^2$$

From this point, the distribution of eight celled colonies is calculated as in section (d).

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