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THE EFFECT OF STARVATION ON RECOMBINATION IN PHAGE T4

by

Beth Hillary Minzter

A Thesis Submitted to the Faculty of the

COMMITTEE ON GENETICS (GRADUATE)

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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Harris Bernstein

HARRIS BERNSTEIN, Ph.D.
Professor of Microbiology and Immunology

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Date

To Mom and Dad, Ron and Sue, whose love and health are what it's all about. And to Arnold, who's been there for me as long as I can remember.

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ABSTRACT

Repair of deoxyribonucleic acid (DNA) damage is a fundamental process in biology which, when inaccurate or incomplete, may ultimately cause cancer, aging, and birth defects. Furthermore, DNA damage in germ-line cells probably has more serious consequences for genetic survival than somatic cell DNA damage. Indeed, recombinational repair of germ-line DNA may be the major function of meiosis and sexual reproduction. This study examined the ability of a specific natural stress which likely increases DNA damage to stimulate the type of DNA repair associated with meiosis and sexual reproduction. The effect of starvation in bacterium *Escherichia coli* and its bacteriophage T4 was determined after assaying at various times for total progeny and wild-type recombinant progeny. After two hours of infection under starvation conditions there was a dramatic, nearly two-fold increase in recombination of phage T4. These results suggest that depleting the energy reserves of the cells causes DNA damage which stimulates recombination as a repair mechanism.

Efforts to elucidate the molecular pathway of this recombination and the possible involvement of the *E. coli recA* protein in the mechanism have thus far proved inconclusive.

INTRODUCTION

Repair of DNA damage is a fundamental process in biology. DNA damages that are not repaired accurately are thought to be the ultimate cause of cancer, aging, and birth defects. Repair of DNA damages in germ line cells needs to be more reliable than repair in somatic cells, as damage in germ line DNA has more serious consequences for genetic survival than damage in somatic cell DNA. The primary evolutionary advantage of meiosis, and hence sexual reproduction, may be the opportunity it provides for recombinational repair of germ-line DNA (Bernstein, Byers and Michod, 1981). The work described here shows that natural stressful conditions which may increase DNA damage stimulate the type of DNA repair, recombinational repair, associated with meiosis and sexual reproduction.

Certain microorganisms when grown under unfavorable environmental conditions such as crowding or starvation convert from asexual to sexual reproduction (Bell, 1982, p.370-371). The work reported here was undertaken to test the hypothesis that this change may be an adaptation to increased DNA damage under stress. The organism used in my thesis is bacteriophage T4, which grows in its bacterial host, *Escherichia coli*. This system is widely employed as a model for studying the molecular events in genetic recombination. It is used here to determine whether an environmental stress, starvation, stimulates recombination. Experiments were also undertaken to understand the genetic basis of the enhancement that was found.

Evidence has been presented that starvation of *E. coli* leads to induction of the host *rec*A protein (McCreary, 1983). Thus, this work investigates the role of *rec*A protein in starvation-induced recombination of bacteriophage T4 upon infection of *E. coli*. The *rec*A protein is required in *E. coli* for genetic recombination (Radding, 1981) and for recombinational repair (Bernstein, 1981; Howard-Flanders, 1981). The *rec*A protein catalyzes exchange of strands between different DNA molecules, which is a key step in recombinational repair of damaged DNA. Experiments were undertaken to determine if a mutant *rec*A host allows starvation-induced bacteriophage recombination, and thus whether the *rec*A protein is needed for starvation-induced recombinational repair.

Starvation and Sexual Reproduction

There is evidence that some organisms which can alternate between parthenogenic and sexual reproduction favor sexual reproduction at higher population densities and under starvation conditions.

Birky and Gilbert (1971) found that the transition between asexual and sexual reproduction in some facultative species of rotifers is controlled by the environment. Starvation of amictic females induced the production of mictic daughters; the other consistent, clear-cut inducer of mictic females was population density.

Banta and Brown (1929) examined the factors responsible for male (and sexual egg) production in *Cladocera*. They found that crowded mothers produced a much higher percentage of males than did their uncrowded sisters, who rarely produced males. Bell (1982, p.175) noted

that laboratory work on *Hydra* has demonstrated the crucial role of crowding in eliciting sexuality, and work on other coelenterates strengthens this role. *Craspedacusta* are found to enter the sexual phase (they produce medusae) under crowded conditions (Bell, 1982, p.177) and when food supply is low. Crowding and starvation, then, are the most potent factors known to elicit sexuality in the freshwater coelenterates; that is, in cladocerans, *Hydra* and *Craspedacusta*. They also are a major factor in the switch to sexuality in plankton rotifers, aphids, and Cecidomyidae (Bell, 1982, p.370-1). In addition, several plant-parasitic nematodes show an increase in the frequency of males with crowding and starvation (Bell, 1982, p.371).

Among bacteria, *Bacillus subtilis* cells become competent for sexual interaction by DNA transformation under conditions of limited amino acid content in their growth media and at high density (Anagostopolous and Spizizen, 1961). Similarly, *Haemophilus influenzae* transforms best when grown to the end of log phase or when approaching the stationary phase of growth; i.e. under conditions of high density (Goodgal and Herriott, 1961).

These examples suggest that the change from asexual to sexual reproduction among microorganisms may often correlate with unfavorable environmental conditions such as crowding or starvation. This correlation is the basis for my expectation of enhanced recombination in phage T4 resulting from starvation.

Recombinational Repair

Recombinational repair is an important mechanism for overcoming DNA damages in a variety of organisms, including viruses, bacteria, and yeast (Bernstein, 1983). As this work assays for recombination between mutations in the rII locus of bacteriophage T4, I shall concentrate on the mechanism of recombinational repair in viruses.

Luria (1947) initiated investigation of recombinational repair with his study of bacteriophage T2. He found that when a phage suspension was treated with UV and allowed to infect host bacteria, the ability to produce progeny was greatly enhanced when the cells were multiply infected by the phage. This phenomenon was subsequently called multiplicity reactivation (MR). The basis for considering MR to be a recombinational repair process stems from the need for a least two genomes, and the fact that it depends on seven gene products which are also needed for spontaneous genetic recombination (Bernstein, 1983). In addition, the frequency of genetic recombination has been shown to increase under conditions where MR occurs, such as after treatment with UV, ethyl methane sulfonate (EMS), nitrous acid (HNO_2), and other DNA-damaging agents. The seven genes that appear to be necessary for both MR and spontaneous genetic recombination are gene 32 (helix-destablizing protein), genes 46 and 47 (which code for an exonuclease), gene 59 and *uvsW*, *uvsY*, and *uvsX* (which code for proteins with *recA* like functions) (Bernstein, 1983; see Bernstein, 1981 for review).

MR has been implicated in repairing DNA treated with a number of DNA-damaging agents including UV irradiation, mitomycin C (MMC),

psoralen-plus-near UV irradiation (PUVA), HNO_2, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), EMS, and others; starvation -induced damage due to a deficiency in resources for energy (ATP) or essential components of DNA might be similarly repaired.

A model for MR is shown in Figure 1, from Bernstein and Wallace (1983). Only a segment of the chromosome to be repaired is shown. This type of recombination -transfer of a single-stranded patch--appears to be the major type of recombination occurring during multiplicity reactivation repair (Rayssiguier and Vigier, 1972, 1977).

As shown in Figure 1, replication proceeds along one chromosome until damage is encountered, at which point a gap is formed in the new strand opposite the lesion. Exonucleases then begin the actual MR process, stripping away the ssDNA in the area around the gap. The second strand is now "cut-in-trans" to allow transfer of a strand of undamaged ssDNA complementary to the ssDNA in the gap in the first chromosome. Pairing of the homologous regions and exchange of the two strands is achieved, and the strand exchange is extended to elongate the heteroduplex region. Resolution of the chi-structure (Potter and Dressler, 1976), and subsequent repair synthesis and ligation are accomplished as in the current models of genetic recombination (Dressler and Potter, 1982; Meselson and Radding, 1975; Szostak *et al.*, 1983). The involvement of host *recA* gene product in MR processes was confirmed by McCreary (1983), as *recA*- mutants showed decreased multiplicity reactivation. Also, it was found that gene 49 endonuclease VII is not essential for MR (Hyman, 1983).

This model describes recombinational repair during mating in phage T4 as it is understood to date. The precise pathway of repair occurring in response to the DNA damage incurred upon starvation is not clear, nor is the involvement of *recA* protein clearly determined.

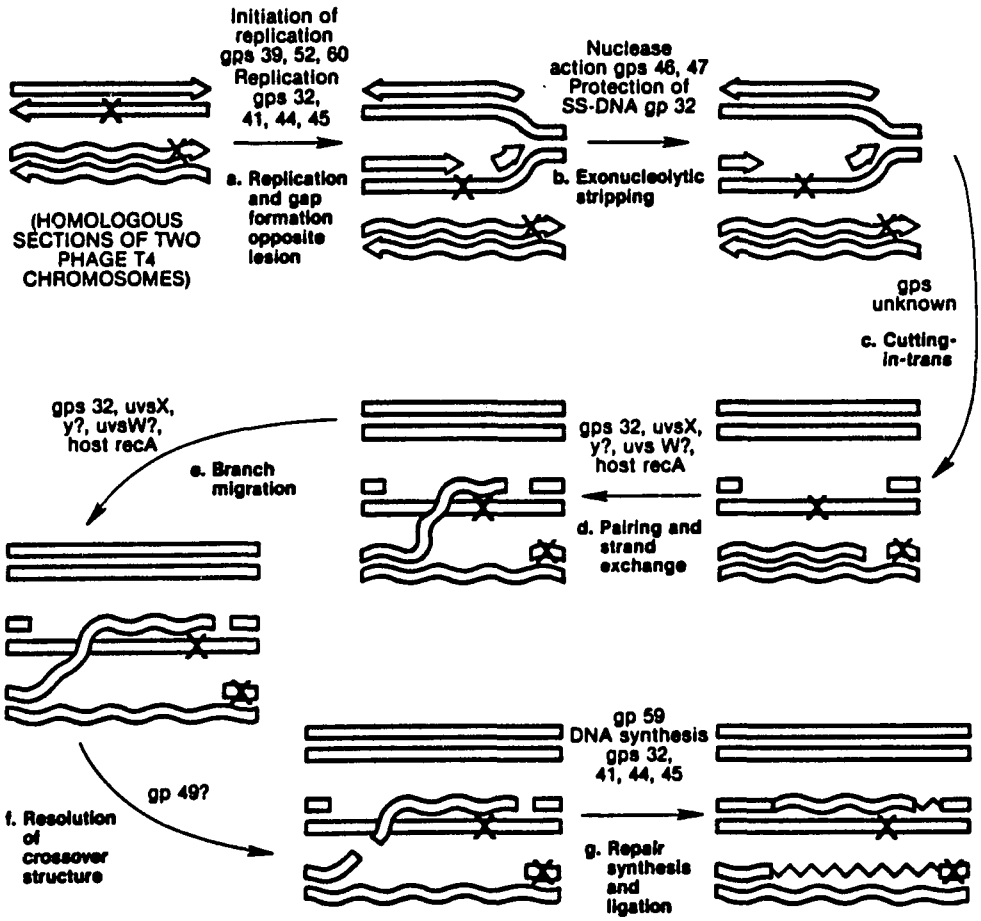

Figure 1. A model of MR in T4-infected cells. (From Bernstein and Wallace, 1983; used with permission.)

recA

The *E. coli* *recA* gene product appears to be essential for multiplicty reactivation. It seems to be involved in controlled repair functions in two ways: as a protease and as a recombinase (McCreary, 1983).

The functionally integrated, highly versatile *recA* is a 37.8 kdal (MW = 37.842) protein and has been isolated in crystalline form. The purified protein has been sequenced and its amino acid composition determined (Horii, Ogawa and Ogawa, 1980; Sancar *et al.*, 1980). Both the recombinase and protease functions of the isolated protein are reduced by a single mutation in the structural gene (Hickson *et al.*, 1981). The *recA* product was discovered by Clark and Margulies (1965) to take part in a major DNA repair process distinct from excision repair . They found that in addition to being extremely sensitive to damage by ultraviolet radiation and x-rays, *recA*$^-$ cells were deficient in recombination. To test the relationship between the new repair pathway defined by *recA* and the previously-known excision repair pathway, double mutants were tested. A *uvrA*$^-$ mutant which had already beeen implicated in the excision repair pathway was combined with a *recA*$^-$ mutant. The *uvrA*$^-$*recA*$^-$ double mutants were found to be approximately 50 times more sensitive than either single mutant. This determined the involvement of *recA* in post-replication recombinational repair (PRRR), this new repair process. This was later confirmed by more studies with excision repair-deficient *uvrA*$^-$ mutants (Howard-Flanders, 1981; Rupp and Howard-Flanders, 1968).

The role of the *recA* gene product in PRRR is three-fold,

analogous to its role in the current models of genetic recombination (Dressler and Potter, 1982; Messelson and Radding, 1975; Potter and Dressler, 1976). It functions as a recombination enzyme which manipulates strands of DNA to a) promote homologous pairing between two DNA molecules, b) to act as an ATPase (ATP hydrolysis effects the release of *rec*A protein from the DNA molecule, and c) to promote strand exchange, driving branch migration.

*rec*A protein promotes homologous pairing by bringing regions of DNA having a complementary sequence of bases into precise alignment in preparation for strand exchange. The *rec*A protein was shown to promote homologous pairing between a single DNA strand (ssDNA) and a duplex. In addition, it was shown to promote homologous pairing between two circular duplex molecules if one of the duplexes has an ss region equivalent to a post-replication gap (Radding, 1981; West *et al.*, 1980). *rec*A protein also promotes base pairing between ssDNA and its complement in a supercoiled duplex circle. The single strand invades the circle to displace the original partner to its complement, forming a D loop. This reaction is called single-strand uptake or assimilation, and is potentially related to the heteroduplex formation step common to recombination models (Holliday, 1964; Meselson and Radding, 1975). *rec*A then, in its roles in recombination, requires both ssDNA as a cofactor and ATP (Dressler and Potter, 1982; McEntee, Weinstock and Lehman, 1979; McEntee, Weinstock, and Lehman, 1981; Shibata *et al.*, 1979a). ATP stimulates the binding of *rec*A protein with ssDNA and promotes the binding of the protein with dsDNA. Assimilation of the

single strand into dsDNA is then achieved following ATP-hydrolysis. The reaction is driven by the binding of *rec*A to ssDNA as its cofactor at an optimum rate of one monomer of ATP for every 5-10 nucleotides of ssDNA (McEntee *et al.*, 1981). Basically, ssDNA governs all *rec*A protein activities: it is a cofactor for the ATPAse activity, the protease activity, the binding of the duplex DNA, and the unwinding of duplex DNA (Radding, 1981). The amount of single-stranded DNA determines the stoichiometric requirement for purified *rec*A protein in the pairing reaction (Shibata *et al.*, 1979b) and the binding to ssDNA appears to be cooperative (McEntee *et al.*, 1981). In addition, *E. coli* single-stranded binding protein (SSB) has a profound effect in sparing *rec*A protein, and may also interact with it (Glassberg, Meyer and Koraby, 1979; McEntee, Weinstock and Lehman, 1980; Shibata *et al.*, 1980).

Following the formation of the D-loop, produced as *rec*A effects the reciprocal exchange between the two ds regions (West, Cassuto and Howard-Flanders, 1981), two heteroduplexes are left in which the four strands have exchanged partners. This is, in other words, a ss crossing-over, the intermediate in the Holliday model of recombination (Holliday, 1964). In the presence of ATP, *rec*A protein then carries out its last recombinational role, as it drives branch migration, during which the crossover site advances along the molecules, resulting in an actual exchange of strands. This heteroduplex region is extended at the rate of approximately four base pairs/second (Cox and Lehman, 1981a), and requires the continued presence of active *rec*A protein and homology of the pairing strands; a pyrimidine dimer slows the rate 50-fold (Cox and Lehman, 1981b; DasGupta and Radding, 1982; Livneh and

Lehman, 1982). At this point *recA* has completed its task; enzymes act to resolve intermediates, fill gaps, ligate the sugar-phosphate backbone, and fulfill remaining repair functions.

The role of *recA* in genetic recombination and related processes in recombinational repair is clear; its catalysis of the annealing of ssDNA and of the transfer of strands from one molecule to another is well-defined. The other major function of *recA* protein, however, is equally important-- its role as a protease in regulating the expression of other genes. The regulatory activity was first detected when Clark, Luria and others found that *recA*$^-$ lysogenic cells are not induced, even when the cells are exposed to uv irradiation (Brooks and Clark, 1967; Hertman and Luria, 1967). Shortly thereafter, it was discovered that when DNA synthesis was inhibited by thymidine starvation or nalidixic acid, a specific protein was induced (Inouye and Pardee, 1970). This protein was identified through further studies as the protein X from a "tif" (thermal induction and filamentation) mutant (Castellazi, George and Butlin, 1972), actually a *recA* mutant (Castellazi, *et al.*,1977). Thus protein X was identified as the product of gene *recA* (Emmerson and West, 1977; Gudas and Pardee, 1976; McEntee, 1977). By now several responses to distress had been elucidated and collectively termed the "SOS response". This response to distress includes prophage induction, increased mutagenesis (error-prone repair), the interruption of DNA replication and cell division, and the synthesis of a protein now known to be the *recA* gene product (Howard-Flanders, 1981).

The protease activity of *recA* was studied with phage lambda

repressor protein as substrate (Craig and Roberts, 1980, 1981; Phizicky and Roberts, 1980, 1981; Roberts, Roberts and Craig, 1978). The other substrate of *rec*A is the *lex*A gene product (Horii *et al.*, 1981; Little *et al.*, 1980). Cleavage of both lambda repressor and *lex*A protein greatly reduces the repressor activity of both proteins, and indicates the regulatory role of *rec*A protein in controlling prophage induction by λ repressor, and in controlling the SOS response by the *lex* repressor (Little and Mount, 1982).

The model for the SOS repair system is straightforward. *lex*A protein represses during exponential growth at least 17 unlinked genes, including *rec*A. To put this in perspective, this means that approximately 0.5% of the genes in *E. coli* are part of this complex regulatory system (Walker, 1984). DNA damage and/or inhibition of replication produces some inducing signal which reversibly activates a protease activity of the *rec* protein, allowing for cleavage and inactivation of the *lex*A repressor. Consequently the products of the SOS target genes are expressed at much higher levels, and the secondary SOS functions are expressed. (Actually, without induction there is low level expression of most of the target gene products; e.g. uninduced *E. coli* cells contain approximately 800 to 1200 *rec*A molecules/cell.) Following stress such as uv treatment, *rec*A protein increases rapidly, peaking after 60-90 minutes at an amplification as high as 20-55 times the basal protein level (Karu and Belk, 1982; Salles and Paoletti, 1983). The SOS response remains turned on until the repair is completed, at which point the inducing signal fades, the *rec*A protease activity is halted, and the *lex*A repressor accumulates and represses

the target genes: the SOS system is now turned off.

The significance of the protease activity in this repair system is clear. The feedback process allows *rec*A protein to accumulate during *lex*A derepression, maintaining the needed SOS processes as well as freeing *rec*A protein for recombinational processes. In distressed lysogenic cells, the lambda repressor is cleaved by proteolytic *rec*A protein and the bacteriophage are able to leave the cell for a healthier host and enhanced survival (Horii *et al.*, 1981; Roberts and Roberts, 1975). In non-lysogenic cells, activated *rec*A protein turns on the SOS system; as lesions are healed the *rec*A proteolytic activity diminishes, *lex*A accumulates, and the system shuts down (Little and Mount, 1982).

The roles of *rec*A protein in recombination and proteolysis are incredibly diverse; its significance in the repair processes which utilize these functions is fundamental. A greater understanding of *rec*A and all the other enzymes involved in these cellular responses to damaging agents should enhance our understanding of how DNA integrity is maintained.

Recombinational Repair and Sexual Reproduction

Recombinational repair, discussed here in bacteriophage T4, occurs in a range of organisms, including prokaryotes and eukaryotes (for brief review, see Bernstein, 1983). Recombinational repair circumvents a wide variety of induced lesions in phage DNA, and is very efficient in overcoming potentially lethal damage (for review, see Bernstein, 1981). Due to this ability, it has been proposed (Bernstein,

1977; Bernstein, 1979) that spontaneous recombination may actually be largely a reflection of recombinational repair of naturally occurring lesions, and that this repair may provide for an important selective advantage for the recombinational process.

This advantage is independent of variation and genetic diversity, the selective advantage of recombination put forth by Fisher (1930) and Muller (1932) in the early 30's. This view of recombination has classically been linked to the maintenance of sexual reproduction by natural selection, since the variation effected by recombination allows for a more diverse response to selective pressures. Maynard Smith (1978) and Williams (1975), however, have recently detailed serious weaknesses in the variation hypothesis of sexual reproduction.

An alternative hypothesis is suggested by Bernstein *et al.*, (1981), (also Bernstein, 1977; Bernstein, 1979; Martin, 1977). They propose that sexual reproduction may have evolved, in large part, as a mechanism for recombinational repair of genetic material. Sex is then maintained by natural selection because it promotes repair and insures the integrity of germ-line DNA. Medvedev (1981) concluded in a review of the phenomena contributing to the immortality of the germ line that meiotic recombination and repair are the most important means of rejuvenating germ cells and restoring the integrity of their genetic material. Gensler and Bernstein (1981) have cited efficient recombinational repair during meiosis as the mechanism for protecting germ-line DNA of multicellular organisms from damage and, consequently, from aging.

Meiosis is a sexual process seemingly designed to promote recombination. The main steps of the sexual cycle, though variable among different organisms, are probably of general occurrence.

"These are: (i) two genomes or parts of genomes come together within a shared cytoplasm; (ii) the genomes pair, so that homologous sequences are adjacent; (iii) accurate exchange of genetic material occurs between the two genomes; and (iv) the exchange is followed by separation of the products of the interaction."

These steps are the same as those presumed to be required for recombinational repair in phage T4 (Bernstein and Wallace, 1983).

It seems evident that efficient recombinational repair is possible -- and probable -- during sexual processes. One can therefore infer that recombinational repair in the germ-line of a range of organisms is effective against a variety of lesions which may be produced by the natural environment. The occurrence of such lesions would have provided the selective basis for the evolution of recombinational repair and hence sexual reproduction. By this reasoning recombinational repair could be an important (perhaps the most important) function of meiosis and sexual reproduction (Bernstein, 1983; Bernstein and Wallace, 1983).

Statement of the Problem

Recombinational repair of germ-line DNA may be the major function of meiosis and sexual reproduction (Bernstein, 1983) and the selective basis for their evolution (Bernstein and Wallace, 1983). In somatic cell lines repair is less efficient than in the germ line and hence DNA

damage would be expected to accumulate. This accumulation of DNA damage may be the primary cause of aging (Bernstein, 1979; Gensler and Bernstein, 1981).

Starvation conditions stimulate the sexual cycle in the organisms mentioned above. Thus, starvation may cause DNA damage, and this damage may induce recombination as a repair mechanism. The first goal of this research was to see whether starvation induces recombination in phage T4, an organism commonly used as a standard to investigate the mechanism of recombination. Having found that starvation did indeed increase recombination, my second goal was to see if the *E. coli recA* gene were required. Implication of the *recA* gene product would be extremely helpful in determining whether starvation causes DNA damage and in elucidating the recombination pathway induced by starvation.

MATERIALS AND METHODS

The bacterial and viral strains used in my thesis are listed in Table 1. The *E. coli* strains AT713, GY3428, and GY3448 were obtained from Dr. Barbara Bachman, *E. coli* Genetic Stock Center, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 96510. *E. coli* strains S/6/5 and K594 λ, and phage strains T4D, *r71*, and *rED144* were obtained from the stock collection maintained by Dr. Harris Bernstein, Department of Microbiology and Immunology, University of Arizona, College of Medicine, Tucson, Arizona, 85724.

Media

The following growth media were used:

1. Hershey broth (Steinberg and Edgar, 1962)

Component	Amount (g/l)
Bacto Nutrient Broth (Difco)	8.0
Bacto Peptone (Difco)	5.0
NaCl	5.0
Glucose	1.0

pH adjusted to 7.2-7.4 with 5N NaOH

2. M9 complete medium (Adams, 1959)

Component	Amount(g/l)
$NaHPO_4$	6.0
KH_2PO_4	3.0
NH_4Cl	1.0
$MgSO_4.7H_2O$	0.25
Glucose	40.0
$MgSO_4$	0.27
$FeCl_3$	2.7
Casamino Acids - Vitamin Free (Difco)	2.5

pH adjusted to 6.8 with 0.2N HCl

Both media were supplemented with 40.0 mg/l of thymine when used to grow strains AT713, GY3428, and GY3448.

Plating agar was made as described by Steinberg and Edgar (1962):

Component	Amount (g/l)
Bactor agar (Difco)	10.0
Bacto Tryptone (Difco)	13.0
NaCl	8.0
Sodium Citrate	1.0
Glucose	3.0

Soft agar (top agar) was made with the same ingredients as plating agar except the agar concentration was reduced to 6.5 g/l and the glucose reduced to 1.3 g/l (Steinberg and Edgar, 1962).

TABLE 1. Bacterial and Viral Strains

Strain	Relevant Genotype	Repair Phenotype	Reference
E. coli			
AT713	wild-type	wild-type	Taylor & Trotter, 1967
GY3428	*rec*A431	pro$^+$/rec$^-$ #	Morand, Blanco, and Devoret, 1977
GY3448	*rec*A430	pro$^-$/rec$^+$ *	Morand *et al.*, 1977
S/6/5	wild-type	wild-type	Bernstein's lab
K594λ	wild-type	wild-type	Bernstein's lab
Bacteriophage T4			
T4D	wild-type	wild-type	Bernstein's lab
r*71*	r*IIA*$^-$	wild-type	Edgar *et al.*, 1962
r*ED144*	r*IIA*$^-$	wild-type	Edgar *et al.*, 1962

recombination deficient only

* protease deficient only

Adsorption salts (M9 salts) contained $NaHPO_4$, KH_2PO_4, NH_4Cl and $MgSO_4.7H_2O$ at the same concentrations used in M9 complete medium (Adams, 1959).

Phage Stock Preparation

High titer phage stocks were prepared using *E. coli* S/6/5 as the host. The cells were grown to about 5 x 10^7 cells/ml in 250 ml of Hershey's broth at 37°C with aeration. Approximately 10^5-10^6 phage were then added and allowed to grow until cell lysis was visible (usually a minimum of 5 hours) or overnight. A few drops of $CHCl_3$ were added to lyse any remaining cells and the cell debris was removed by centrifugation in a Sorval GSA rotor (3,000 rpm for 15-20 minutes). The 250 ml of supernatant was divided into six equal portions and the phage pelleted using a Sorval SS34 rotor (16,000 rpm for two hours). The supernatant was discarded and the pellets gently resuspended in 2 ml of an M9 salt solution (i.e., M9 medium without glucose or casamino acids). The portions were combined and the phage concentration assayed.

All phage and bacterial assays were conducted using the soft agar overlay technique described by Adams (1959). A dilution of bacteria or phage plus three to five drops of concentrated indicator bacteria (for phage only) were added to approximately 3 ml of melted top agar and the mixture was then poured onto an agar plate in an even layer. Indicator bacteria were prepared by growing 250 ml of strain *E. coli* S/6/5 or K594 λ to 1-2 x 10^8 cells/ml in Hershey broth, pelleting them at 3,000 rpm for 15-20 minutes, and resuspending them in 20-25 ml of fresh broth. In all tests, the plates were incubated at 37°C for 18-24 hours

and the phage plaques were counted using a quebec colony counter.

Phage stocks were stored at 4°C with 2-3 drops $CHCl_3$ added. A fresh stock was prepared when the titer dropped below $2x10^{10}$ phage/ml.

Induction of Recombination In Phage T4 by Starvation of Phage-Infected Cells

Crosses of phage T4 mutants *r71* and *rED144* were performed using a protocol for phage mating described by Steinberg and Edgar (1962) with modifications suggested by Carol Bernstein. Fifteen-hundredths of a ml from a fresh culture of cells grown overnight to approximately $2x10^9$ cells/ml were transferred to 15 ml of complete M9 medium. This culture was grown with aeration to about $1.5x10^8$ cells/ml. The cells were then centrifuged and resuspended in 15 ml M9 salts solution. This resuspension in solution lacking a carbon source imposed starvation conditions upon the cells. Nine ml of resuspended cells were immediately transferred to a bubbler tube at 37°C, and 1 ml of a mixed phage suspension was added. This was time zero (0). The phage mixture consisted of equal parts of two *rII* cistron mutants, *r71* and *rED144*, each at $2x10^{10}$ phage/ml. The multiplicity of infection (moi) was 10. The mixture of starved cells and phage was aerated slowly by bubbling at 37°C for ten minutes to allow adsorption of the phage to the bacteria. Then, at predetermined time intervals of 15 minutes, 30 minutes, 60 minutes, 120 minutes, 140 minutes, 160 minutes, 180 minutes, 210 minutes and 240 minutes, samples were transferred to complete M9 medium at 37°C and assayed on the permissive host *E. coli* S/6/5 for infectious centers. Samples were also assayed for unadsorbed

phage by adding several drops of $CHCl_3$, which kills all infected bacteria, but allows free phage (unadsorbed input) to survive.

After one-and-a-half hours of growth in complete M9 media at 37°C, $CHCl_3$ was added to the growth tubes and mixed well to lyse all infected bacteria, and the samples were measured for total phage progeny (TP) on permissive *E. coli* S/6/5, and for wild-type phage recombinant progeny (REC) on *E. coli* $K594_\lambda$, which only allows growth of wild-type phage. Recombination frequency is calculated as 2(REC)/TP. The factor of two is included to take into account r*71*, r*ED144* double mutant recombinants which are assumed to form at equal frequency to wild-type recombinants. The cells and phage were maintained at a constant 37°C throughout the procedure to minimize induction of repair mechanism due to heat shock (Mitchel and Morrison, 1983).

The protocol for carrying out the infections in *recA*$^-$ *E. coli* mutants was similar to that just described, except that thymine was added to the complete M9 medium to aid growth.

RESULTS

Table 2 lists the results obtained in three replicate experiments in which the phage mutants r*ED144* and r*71* were crossed in a wild-type host *E. coli* S/6/5. The recombination frequencies and burst sizes (phage progeny per infected bacterium) for each time of starvation of the infected cells are shown. The data for the three experiments are plotted in Figures 2, 3, and 4 respectively. The curves show the percent recombination frequencies from the three phage crosses plotted versus the starvation time. In each case there is an increase in percent recombination as starvation time increases, peaking at about two hours. The first two experiments have five time points; the final run had nine time points, and probably gives the most accurate indication of the shape of the curve. Figure 5 depicts normalized curves for all three experiments to illustrate the rough similarity between the three sets of results. In Figure 6, it can be seen that burst size generally declines with increase in starvation, suggesting accumulation of unrepaired damages.

The results obtained using two different *recA*$^-$ mutants as host as well as an isogenic wild-type host are shown in Table 3. More experiments were run than are indicated (three runs for AT713, two each for GY3428 and GY3448), but because the phage growth was poor (burst sizes were less than 10 phage/bacterium) the results were not included. When the wild-type host (AT713) was used, a strain isogenic with the

recA- strains, an increase is seen in percent recombination as starvation time increased. This is most evident in experiment 2 (Table 3), but is also weakly indicated in experiment 1. The *recA*- mutant data were incomplete, and more work is necessary before a clear conclusion can be drawn as to the role of *recA* protein in starvation-induced recombination. If further experiments give curves for GY3428 and GY3448 similar to those seen for AT713, it would imply that the host *recA* gene product is not necessary for starvation-stimulated recombination. If recombination is not induced by starvation in these mutant hosts, it would imply that *recA* function is essential.

TABLE 2. The Effect of Starvation on Recombination and Burst Size in Crosses of *rII* Mutants in a Wild-type Host

Duration of Starvation (min)	EXP 1		EXP 2		EXP 3	
	* % rec	burst size	% rec	burst size	% rec	burst size
7.5	0.90	46	-	-	-	-
15	0.94	52	1.34	76	1.31	48
30	-	-	1.45	61	1.69	45
60	1.69	27	1.61	47	2.03	41
105	-	-	-	-	2.07	39
120	1.72	31	2.31	31	2.17	28
140	-	-	-	-	1.87	41
160	-	-	-	-	1.76	43
180	-	-	-	-	1.72	28
210	-	-	-	-	1.33	30
240	1.35	18	2.05	19	-	-

* percent recombination = recombination frequency x 100 =

100 x 2(REC)/TP

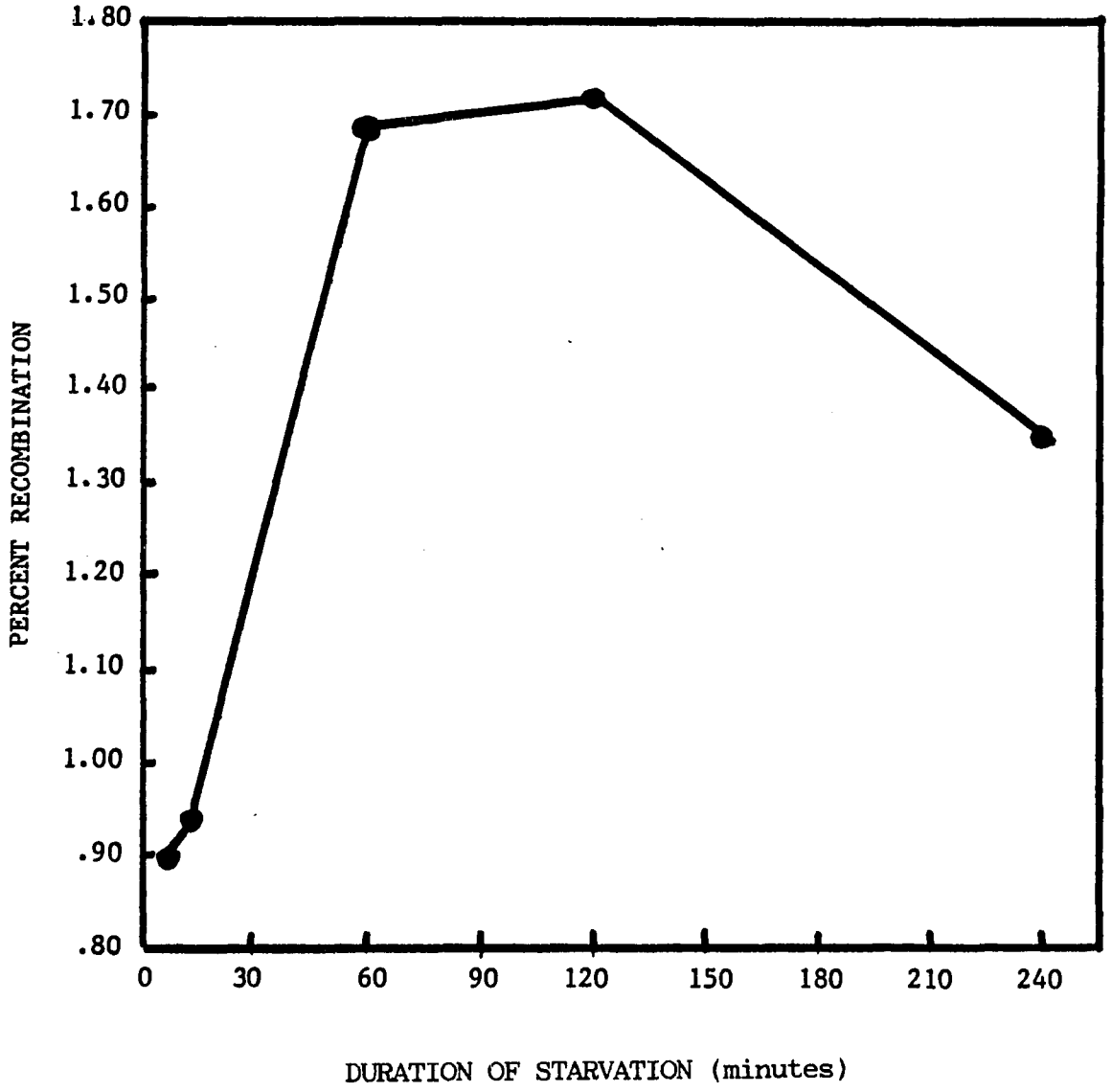

Figure 2. Recombination frequencies of T4 in wild-type *E. coli* with starvation from data of Experiment 1.

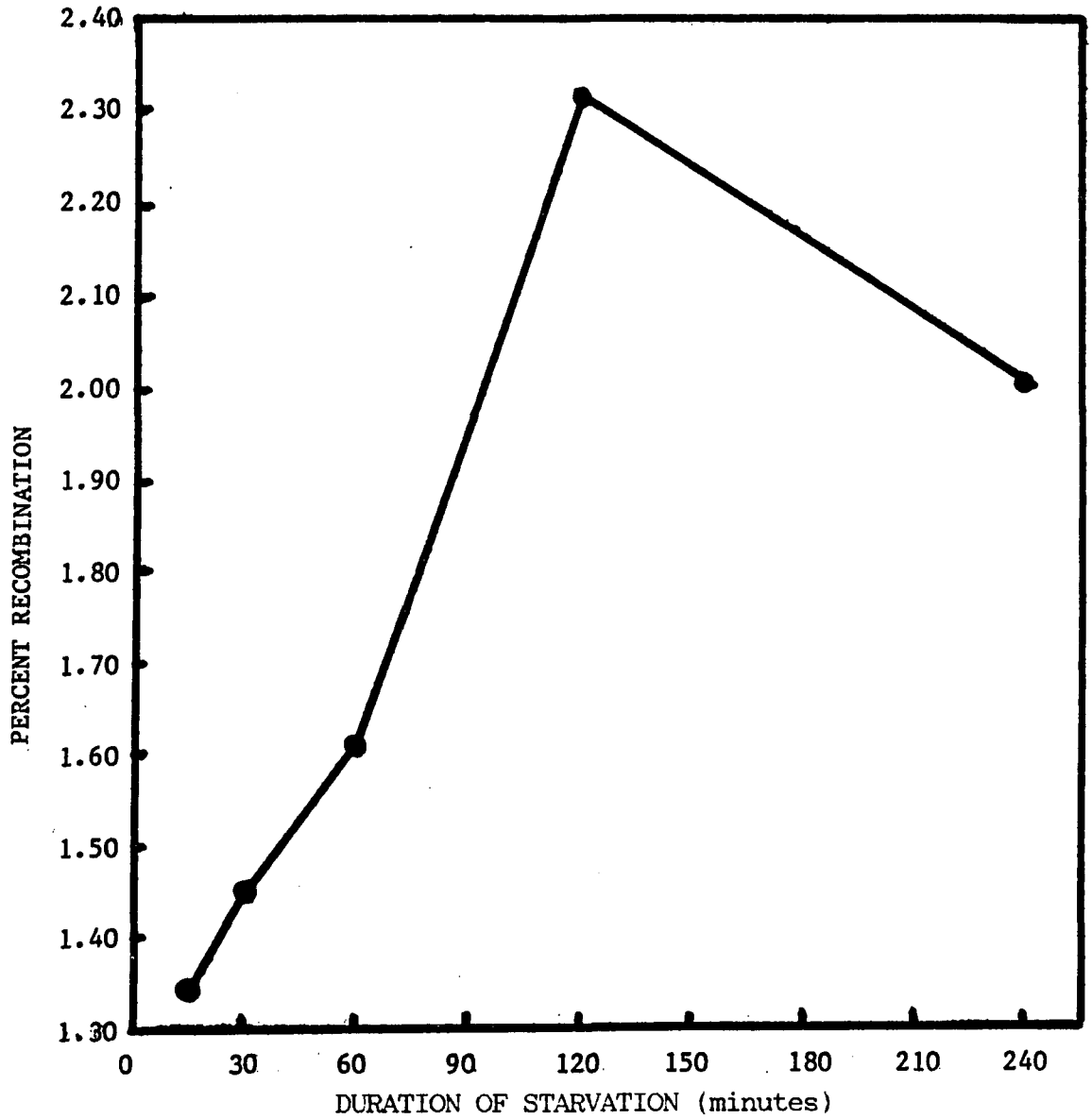

Figure 3. Recombination frequencies of T4 in wild-type *E. coli* with starvation from data of Experiment 2.

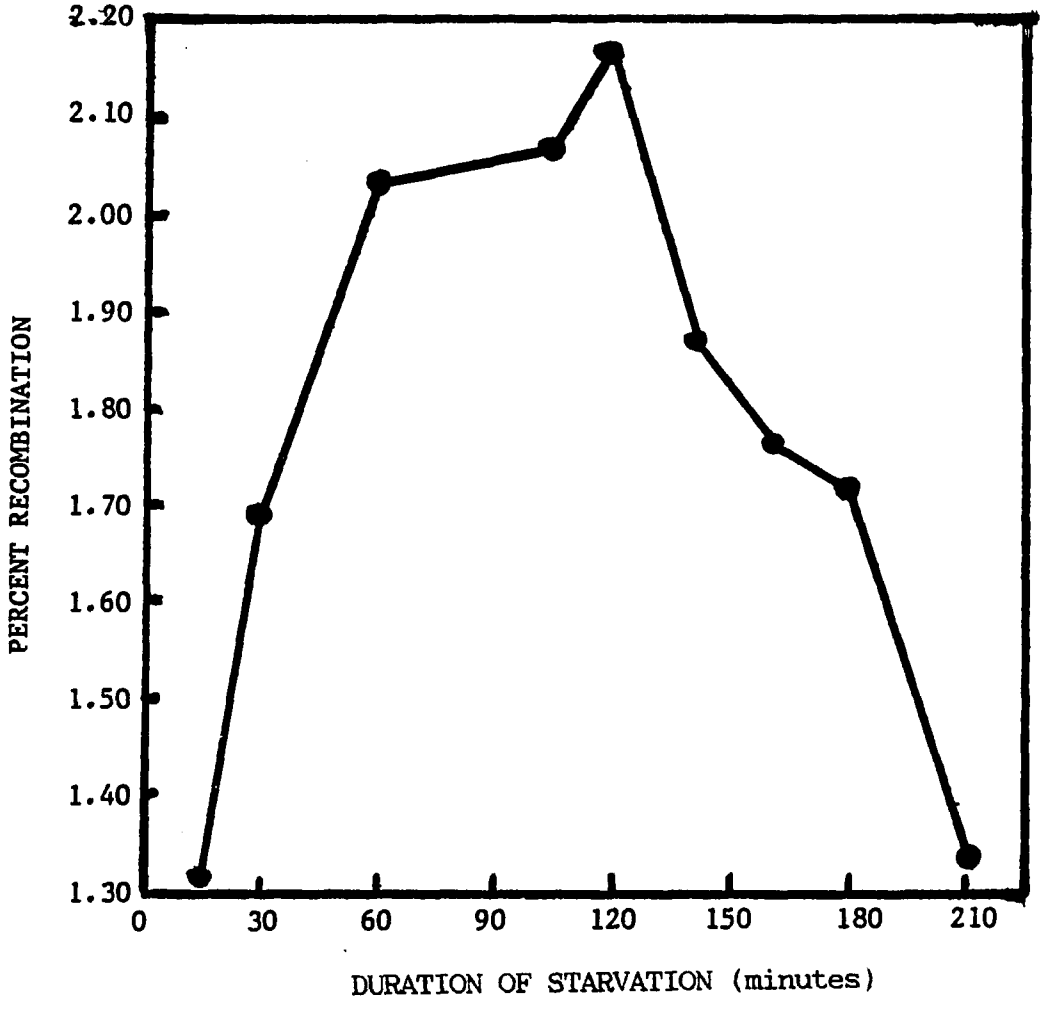

Figure 4. Recombination frequencies of T4 in wild-type *E. coli* with starvation from data of Experiment 3.

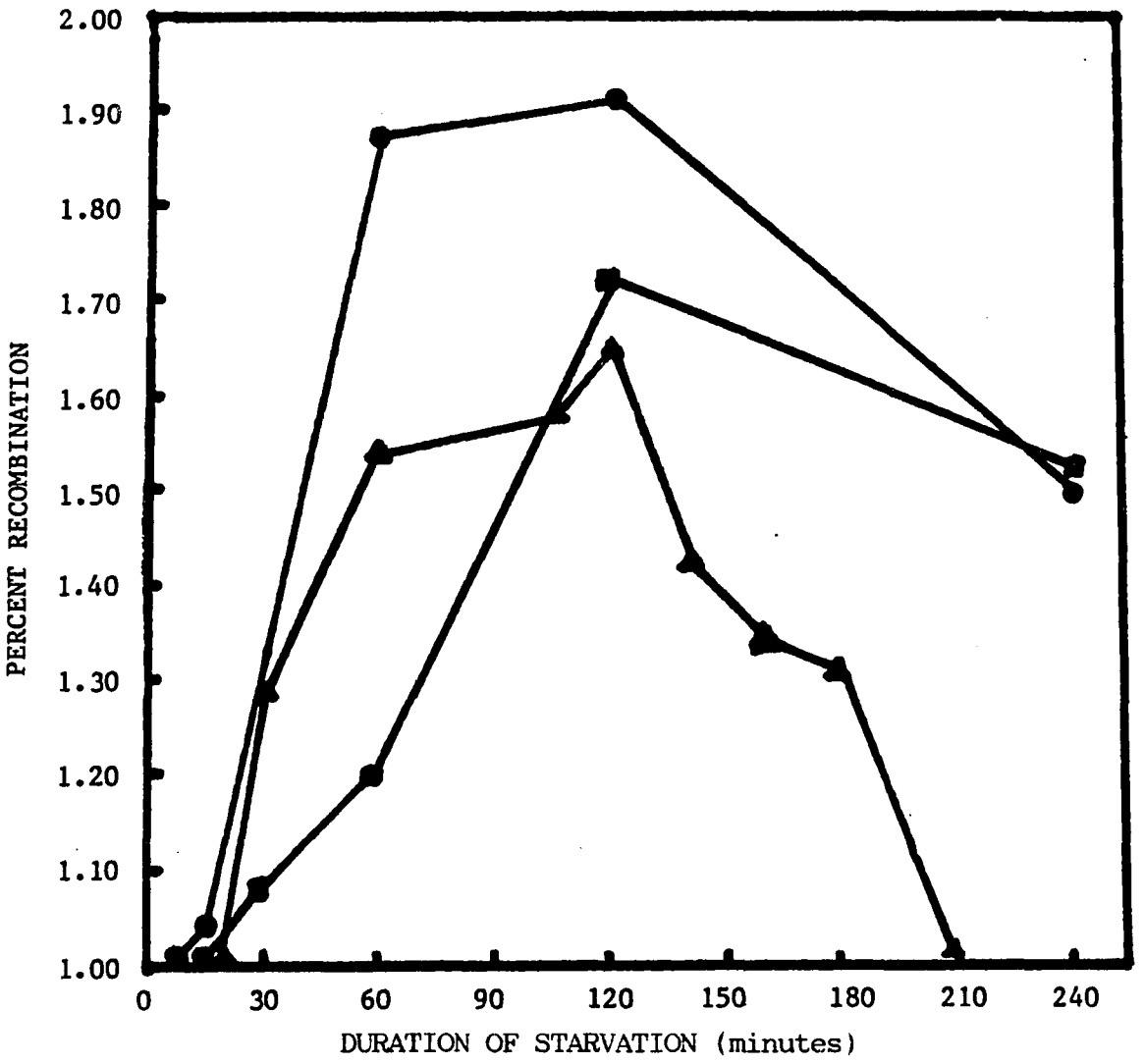

Figure 5. Recombination frequencies of T4 in wild-type *E. coli* with starvation. All values in each experiment are normalized against initial value in that experiment.

● ---- Experiment 1
■ ---- Experiment 2
▲ ---- Experiment 3

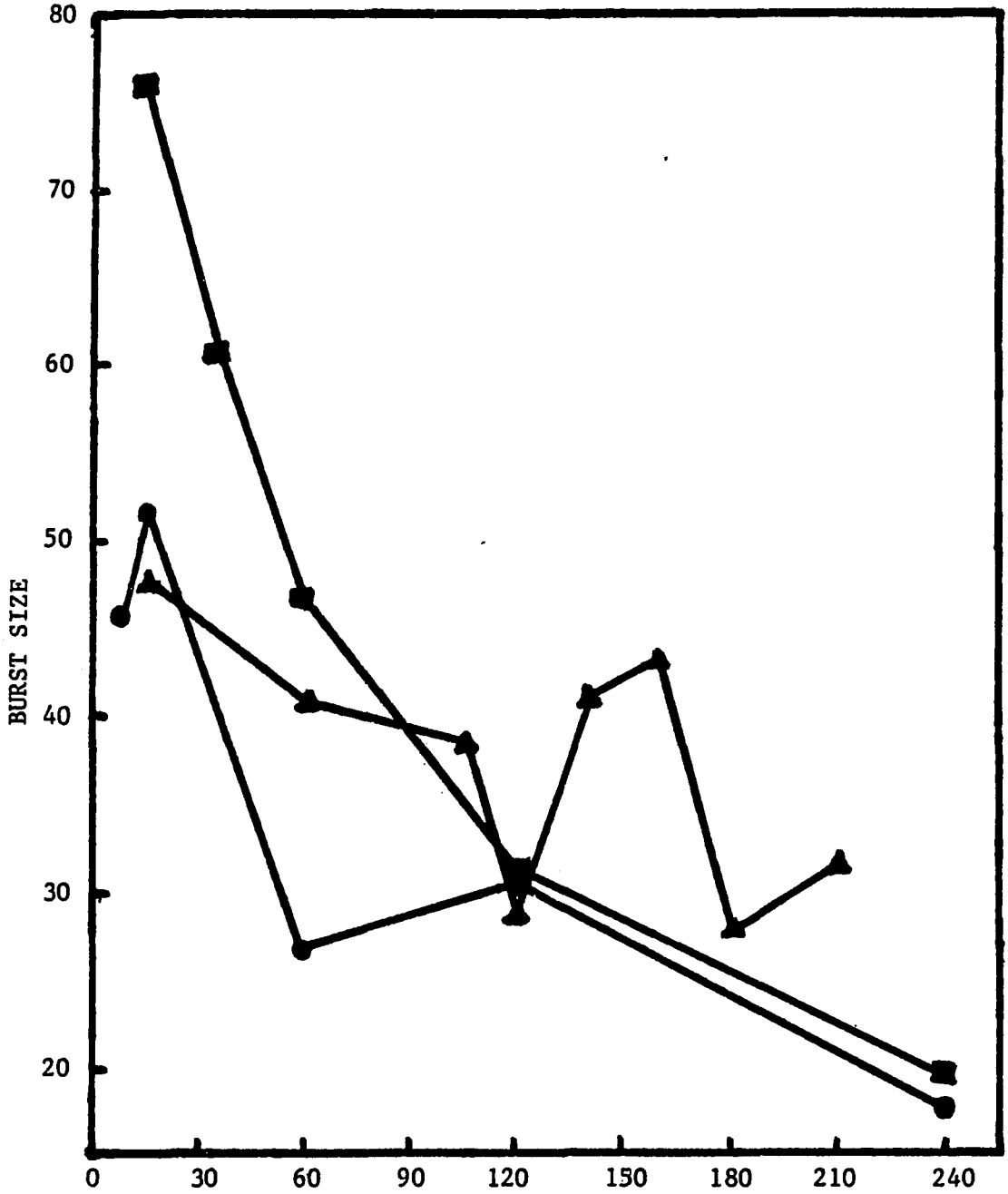

Figure 6. Average number of phage produced per cell with increased starvation.

● ---- Experiment 1
■ ---- Experiment 2
▲ ---- Experiment 3

TABLE 3. *rec*A Mutants

Starvation (min)	AT713+ (wild-type) EXP 1 %rec#	burst size*	AT713 EXP 2 %rec	burst size	GY3428 (pro⁺rec⁻) %rec	burst size	GY3448 (pro⁻rec⁺) %rec	burst size
15	.85	90	.98	33	.51	39	1.10	31
30	.82	117	1.00	20	.72	90	2.27	17
60	1.05	131	1.47	32	-	-	2.24	31
105	.96	141	1.81	33	.64	56	1.91	58
120	.93	173	1.76	79	.44	19	2.16	53
140	.78	148	1.69	27	.51	17	-	-
160	2.67	125	1.81	79	.66	35	2.58	42
180	.89	158	.69	33	.63	32	3.57	32
210	1.13	129	1.89	14	.79	23	2.43	49

+ Two experiments using AT713 are included in the table.

* In determinations where burst size was less than ten phage per bacterium, the recombination data are not included.

percent recombination = 100 x 2(REC)/TP.

DISCUSSION

The induction of genetic recombination in bacteriophage T4 infected cells by starvation is illustrated by the increase in percent recombination seen in Figures 2-5. When host *E. coli* (S/6/5) were coninfected with two *rIIA*$^-$ mutants of T4 and starved for increasing amounts of time, the recombination frequency increased about two times within two to two-and-a-half hours. This increase is consistent with the increase seen in multiplicity reactivation, a recombination process, when host cells are starved prior to treatment with the DNA-damaging agent mitomycin C (McCreary, 1983).

To determine if the induction of recombination by starvation depends on the host *rec*A function, *rec*A$^-$ mutant strains of *E. coli* were tested. Two *rec*A$^-$ strains, described by Morand *et al* (1977), each carrying a different *rec*A allele, were used in an attempt to determine if either of the two main functions of the *rec*A gene product are involved in the starvation-induced recombination pathway. *rec*A$^-$ strain GY3428, derived from *rec*A$^+$ strain AT713, carried the *rec*A431 allele. This allele is characterized as being protease proficient, as measured by its ability to induce lambda prophage, but recombination deficient. The *rec*A$^-$ strain GY3448, also derived from AT713 but carrying the *rec*A430 allele, is protease deficient, but retains near-normal recombinational capability.

Two sets of crosses were carried out with the wild-type strain AT713. Experiment 2 (Table 3) showed a similar increase in

recombination to that obtained in wild-type *E. coli* (S/6/5), the strain used in the first set of experiments (Figures 2-5). Although the first experiment also showed an increase with starvation, there was considerable fluctuation in the percent recombination in sequential points, suggesting a defect in the experimental procedure. The results of the experiments involving the two *rec*A- strains are also listed in Table 3. They appear to implicate the recombination function as essential for the increase in recombination and the protease function as unnecessary. However, the results are not adequate to draw a reliable conclusion. More data are necessary to determine if *rec*A is utilized in either or both of its capacities.

A possible interpretation of the dramatic increase in recombination of phage T4 with starvation is that depleting the energy reserves of the infected cell results in damage to the phage DNA. This damage might induce recombination as a repair mechanism. It would be helpful to quantitate the damage incurred by measuring the amount of DNA fragmentation. Wielckens *et al.*, (1983) found that "starvation" conditions induce a reversible metabolic adaptation in Ehrlich ascites tumor cells which was associated with a fragmentation of DNA. It would also be interesting to substitute ultraviolet irradiation for starvation to introduce DNA damage and induce *rec*A protein and then to measure stimulation of recombination.

My main observation that recombination in phage T4 was induced by starvation can be interpreted in terms of repairing DNA damaged in an energy-depleted system. In a range of microorganisms (see Introduction

for examples) it is observed under natural conditions that crowding and starvation stimulate sexual reproduction and hence also presumably recombination. The phage T4 system offers an opportunity for studying this phenomenon at the molecular level. My results are consistent with the hypothesis that the primary evolutionary advantage of meiosis, and hence of sexual reproduction, is the opportunity it provides for recombinational repair of germ-line DNA.

Summary

The influence of an environmental stress, starvation, on the enhancement of recombinational repair of DNA in phage T4 was studied. It was found that recombination increased dramatically with starvation. After two to two-and-a-half hours of co-infection and suspension under starvation conditions in a salt solution, there is an approximately two-fold increase in recombination.

Attempts to elucidate the molecular pathway of this stimulation and the involvement of the host *recA* protein in the recombination induced by starvation were inconclusive.

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