THE ORIGIN AND DIRECTION OF CHROMOSOMAL REPLICATION IN *ESCHERICHIA COLI*

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Determination of the amount of specific region of chromosome may be possible using a DNA-DNA hybridization technique, if isolated F'DNA can be considered as a specific segment of chromosomal DNA. A preliminary study with this method showed that the chromosomal replication of *Escherichia coli* appears to start from an origin located near *ilv* (Yahara 1971). However, the experiment did not disclose the direction of replication because of two uncertainties. One of them is the possible occurrence of the redundant base sequences (Cutler and Evans 1967) which could obscure the hybridization results. The other is the possibility of incomplete synchronization of bacterial growth by means of amino acid starvation technique, as was pointed out by Caro and Berg (1969). Re-examining the first possibility and circumventing the second, the present report describes experiments the results of which cannot be explained by a *fixed origin-unidirectional* model of chromosomal replication in *E. coli*.

MATERIALS AND METHODS

(a) *Bacterial strains*. The strains of bacteria used in this investigation are described in Table 1.

(b) *Media*. M3 medium was Difco prepared Bacto-penassay broth medium 3. TY medium was described in Yamagata and Uchida (1969). TG medium contained 0.1 M Tris-HCl (pH 7.2), 0.092 M NaCl, 0.04 M KCl, 0.02 M NH₄Cl, 0.1 mM CaCl₂, 0.64 mM KH₂PO₄, 0.16 mM Na₂SO₄, 1.0 mM MgCl₂, 0.01 mM FeCl₃, 0.2% glucose and 1 µg/ml thiamine. Tris-succinate medium was the same as TG medium except that glucose was substituted by 0.5% Na-succinate. M9 medium was composed of 0.042 M Na₂HPO₄, 0.022 M KH₂PO₄, 0.8 mM MgCl₂, 8.5 mM NaCl, 0.019 M NH₄Cl, 0.2% glucose and 1 µg/ml thiamine. M9 agar was M9 medium containing 1.35% agar. M9 *lac* agar was M9 agar containing 0.5% lactose in place of glucose. Dihydrostreptomycin, when required, was added to give a concentration of 100 µg/ml.

(c) *Isolation of P. mirabilis harboring F'-factors*. *E. coli* F'-factors were transferred to *P. mirabilis* according to the procedure described by Falkow, Wohlhieter, Citarella and Baron (1964): Donor and recipient bacteria were separately grown in M3 medium to a concentration of 1×10^9 cells/ml, and equal volumes of each were mixed and incubated at 37°C for 3 hr. Appropriate dilutions of the mating mixture were then spread on suitable selective plates. Mating efficiency was usually about 1×10^{-6} per input donor

Table 1. Bacterial strains used

strain	characters	origin
<i>E. coli</i> strains		
W 3747	F'13+/met ⁻ thi ⁻ T 6 ^r λ ⁺	Hirota & Sneath (1961)
AB 1206	F'14+/met ⁻ ilv ⁻ arg ⁻ thi ⁻ str ^r his ⁻ gal ⁻ T4 ^r lac ⁻ pro ⁻	Pittard et al. (1963)
W 4580	F'15+/met ⁻ thi ⁻	Ishibashi et al. (1964)
JC 1552/KLF 3	F'KLF 3/his ⁻ leu ⁻ argG ⁻ metB ⁻ str ^r lac ⁻ malA ⁻ xyl ⁻ mtl ⁻	B. Low
AY 5	F ⁻ met ⁻ arg ⁻ thy ⁻ thi ⁻	derived from JC 66 F ⁺
AB 1315	F ⁻ pro ⁻ lac ⁻ gal ⁻ str ^r xyl ⁻ thi ⁻ λ ⁻ χ 357 ⁺	K. Horiuchi
AY 11	F'13+/AB 1315	
<i>P. mirabilis</i> strains		
PY 2	lac ⁻ nic ⁻ str ^r	derived from AC 2505 (Adelberg)
AC 2626	lac ⁻ nic ⁻ ilv ⁻	K. Horiuchi
Pm 17	lac ⁻ nic ⁻ gal ⁻ ura ⁻ trp ⁻ thy ⁻	R. Nakaya
PY 6	lac ⁻ nic ⁻ his ⁻ str ^r	derived from PY 2
PFY 3	F'13/PY 2	
PFY 13	F'14/AC 2626	
PFY 26	F'15/Pm 17	
PFY 33	F'KLF 3/PY 6	

Table 2. Mating couples for isolation of *P. mirabilis* harboring F' factor

F'	donor	recipient	selected marker	mating efficiency (per input donor)
F'13	W 3474	PY 2	lac ⁺ (a)	1.2×10 ⁻⁶
F'14	AB 1206	AC 2626	ilv ⁺ (b)	0.9×10 ⁻⁶
F'15	W 4580	Pm 17	thy ⁺ (c)	2.3×10 ⁻⁶
F'KLF 3	JC 1552/KLF 3	PY 6	his ⁺ (d)	2.0×10 ⁻⁶

P. mirabilis F'-merogenotes were selected on (a) M9-lac plate containing nicotinic acid (10 µg/ml) and streptomycin; (b) M9-plate containing nicotinic acid; (c) M9-plate containing nicotinic acid, uracil (10 µg/ml) and tryptophan (50 µg/ml); (d) M9-plate containing nicotinic acid and streptomycin.

bacteria. *P. mirabilis* F' merogenotes thus obtained were generally unstable and segregated F⁻ cells with high frequency, but after five successive single colony isolations, stable clones could be selected (Table 2).

(d) *Preparation of F' DNA.* *Proteus mirabilis* strains PFY 3 and PFY 13 were grown in TY medium at 37°C until bacterial concentrations reached to 1.5×10⁹/ml. F⁻ segregants were less than 2% of the populations. PFY 26 and PFY 33 were grown in M9 medium containing required supplements. Cells were harvested by centrifugation, and DNA was extracted by the method described by Marmur (1961). Concentration of episomal DNA was accomplished according to the procedure described by Wohlhieter, Falkow

Table 3. Preparations of partially purified F'DNA used

DNA	Preparation No.	Heating Temperature (°C)	F'DNA Content (%)	Recovery of F'DNA (%)
PFY 3			9	
PFY 13			7	
PFY 26			16	
PFY 33			4	
F'13	304	78.5	35	91
	307	78.5	36	—
	309	79.0	53	64
F'14	401	78.5	37	100
	402	79.0	50	94
	404	79.0	63	58
F'15	505	79.0	59	56
	506	79.0	50	70
KLF 3	601	79.0	62	55

and Citarella (1966). The differential denaturation of chromosomal *P. mirabilis* DNA and episomal *E. coli* DNA was carried out by heating at 78°–80°C for 5 min in 0.1×SSC (SSC contains 0.15 M NaCl and 0.015 M Na-citrate), followed by filtration of the heated DNA solution through Millipore filters HA to remove denatured fractions. The filtrates were dialyzed against 1×SSC. The purity of the F'DNA preparations thus obtained was examined in a Spinco Model E ultracentrifuge, and ultraviolet photographs were traced by a Joyce-Loebl microdensitometer. Characteristics of some of the F'DNA preparations used in this study were listed in Table 3.

(e) *Preparation of labeled DNA.* *E. coli* K12 F[−] AY 5 was labeled with ³²P (6 μC/μmole P) in TY medium, TG medium and Tris-succinate medium. The procedure for extraction of DNA from labeled cells was described previously (Yahara 1971). The recovery of DNA after purification was usually about 40%. W 4580 and AY 11 were labeled with ³²P (6 μC/μmole P) in TG medium. AY 5 DNA's used in the experiments described in Tables 4 and 5 were labeled with ³H-thymine in supplemented M 9 medium at specific activities of 50 μC/μmole and 15 μC/μmole, respectively.

(f) *Hybridization.* DNA-DNA hybridization was performed by the membrane-filter technique as described by Denhardt (1966). Heat denatured F'DNA was attached to membrane filter (Sartorius MF 30, 18 mm in diameter). Each F'DNA-filter was incubated in the preincubation mixture (Denhardt 1966) for 6 hrs at 65°C just prior to hybridization. Test DNA's to be annealed with F'DNA immobilized on filter were denatured and fragmented by heating in 0.5 N NaOH-0.5×SSC at 100°C for 10 min followed by neutralization with HCl (Tomizawa and Ogawa 1968; Yahara 1971). Hybridization was carried out by an incubation at 65°C for 18 hrs. Each side of the filter was washed with 100 ml of 2×SSC, and the filters were dried and counted in toluene-liquifluor in a Beckman scintillation spectrometer. Each experiment was carried out in duplicate or in triplicate, and standard deviations were calculated from all the data obtained.

RESULTS

(a) *Conditions of hybridization*

Fig. 1 shows the amounts of labeled *E. coli* DNA annealed to various amounts of F'DNA immobilized on filters. The plateau value of each curve is proportional to (size of DNA homologous with F'DNA) $\times$ (frequency of DNA homologous with F'DNA among total DNA). When 1 μ g F'DNA is immobilized on each membrane filter, the amount of radioactivity annealed to the F'DNA filter is proportional to the amount of heat-alkali denatured labeled *E. coli* DNA added to hybridization mixture, as far as the labeled DNA in the hybridization mixture does not exceed 2 μ g (Fig. 2).

(b) *Effect of P. mirabilis DNA contaminating partially purified F'DNA on hybridization results*

Preparations of partially purified F'DNA were more or less contaminated with *P. mirabilis* DNA (Table 3). If labeled *E. coli* DNA interacts with the *P. mirabilis* DNA,

Fig. 1. Various amounts of F'DNA's were immobilized on filters, and were hybridized with 1 μ g each of heat-alkali denatured AY 5 DNA (9,500 cpm) labeled with 32 P (4 μ C/ μ mole P). F'DNA preparations used were the same as those used in Table 7.

Fig. 2. Membrane filters each carrying 1 μg of heat denatured F'13 DNA (#309) were incubated with various amounts of ^{32}P labeled, alkali-heat denatured AY 5 DNA in hybridization mixture. The ordinate indicates the amount of AY 5 DNA hybridized with F'13 DNA.

addition of heat-alkali denatured *P. mirabilis* DNA to a hybridization mixture should diminish the amount of labeled *E. coli* DNA hybridized to the F'DNA-filter. As can be seen from the data presented in Table 4, decrease in hybridization of ^3H -*E. coli* DNA to F'DNA-filter due to an addition of cold *P. mirabilis* DNA to the hybridization mixture was less than 10%. The observed inhibition seems to be at least in part due to nonspecific reaction, since an addition of heat-alkali denatured heterologous calf thymus DNA to the mixture resulted in a considerable decrease in hybridization between partially purified F'DNA and labeled *E. coli* DNA (Table 4). It was noted, furthermore, that the ratio of the amounts of labeled *E. coli* DNA hybridized with F'14 DNA to that with F'13 DNA was unchanged by the addition of cold *P. mirabilis* DNA to the hybridization mixture (data not shown).

(c) Homology between F factor and *E. coli* chromosome

DNA-DNA hybridization study by Falkow and Citarella (1965) has suggested that a substantial portion of F-factor should possess nucleotide sequences homologous with the

Table 4. Effect of adding *P. mirabilis* or calf thymus DNA into hybridization mixture

F'DNA (F'14)	^3H - <i>E. coli</i> DNA	<i>P. mirabilis</i> DNA	Calf thymus DNA	Radioactivity bound	
				cpm	%
μg	μg	μg	μg		
1	0.2	0	0	286	100
1	0.2	1	0	269	94
1	0.2	5	0	253	89
1	0.2	10	0	251	88
1	0.2	0	1	292	102
1	0.2	0	5	252	88
1	0.2	0	10	260	91

Each hybridization mixture contained in 2.0 ml of $6\times\text{SSC}$ 1 μg of F'14 DNA (#401) immobilized on filter and 0.2 μg (16,000 cpm) of heat-alkali denatured AY 5 DNA which was homogeneously labeled with ^3H -thymine (50 $\mu\text{C}/\mu\text{mole}$). Competitor DNA's when present were also heat-alkali denatured. Each experiment was carried out in duplicate.

E. coli chromosomal DNA. Such molecular homology would certainly affect the hybridization results, and it may obscure the present estimations. Therefore, in order to detect the possible binding of the F-factor moiety of episomal F'DNA with labeled *E. coli* chromosomal DNA, heat-alkali denatured F'DNA different from the F'DNA immobilized on the filter was added to the hybridization mixture. Table 5 shows that no remarkable interference by the added F'DNA was observed. An argument that the amount of the competitor F'DNA added was insufficient to suppress the hybridization between F-factor moiety of F'DNA and *E. coli* chromosomal DNA is unlikely, since 1 μ g of the competitor F'DNA presently added should be equivalent in *mole* to the corresponding DNA segment contained in 20–40 μ g of *E. coli* DNA. Therefore, even if *E. coli* chromosomal DNA is partially homologous with F-factor DNA, the present method does not detect, and should not be affected by these possible molecular homology.

Table 5. Competition by different species of F'DNA

Immobilized F'DNA	Competitor F'DNA		Radioactivity bound (cpm)
	F'	(μ g)	
F'13	—	—	419
F'13	F'14	1	403
F'14	—	—	340
F'14	F'13	1	350

Each hybridization mixture contained in 2.0 ml of 6 $\times$ SSC 1 μ g of F'13 DNA (#309) immobilized on a filter and 1 μ g (24,000 cpm) of heat-alkali denatured AY5 DNA labeled with 3 H-thymine (15 μ C/ μ mole). Competition experiments were carried out with heat-alkali denatured F'13 or F'14 DNA.

(d) *Absence of redundancy in base sequences of E. coli chromosomal DNA*

It is necessary to know whether or not the hybridization is proportional to an amount of the chromosomal region corresponding to F'DNA. To test the point, partial diploid F'cells were compared with haploid F⁻ cells: Exponentially growing separate cultures of haploid (AY5) and partial diploid strains (AY11 and W4580) were labeled with 32 P. The labeled DNA's were extracted from the cells and hybridized with various F'DNA's. As shown in Table 6, increased hybridization to a factor of about 2 was observed for partial diploid strains. The hybridization observed with a partial diploid strain should depend on the time of replication of F'-factor and that of chromosomal region corresponding to the F'DNA. If a F'-factor replicates at the earliest stage of replication cycle of chromosome, and the corresponding region to the F'DNA replicates at the latest stage, the ratio should come up to 3. Although it has been suggested that the replication of F-factor may be completed during an early stage of chromosomal replication (Jacob, Brenner and Cuzin 1963; Nishi and Horiuchi 1966; Donachie and Masters 1966), we do not know the exact timing of the replication of F-factor. Moreover, the number of F-factor per chromosome has not been determined beyond doubt (Stouthamer, de Haan and Bulen 1963; Jacob, Brenner and Cuzin 1963; Yamagata and

Table 6. Comparison of partially diploid strains with haploid

Immobilized F'DNA	³² P hybridized (cpm)			Ratio	
	AY 5 (F ⁻)	W 4580 (F'15)	AY 11 (F'13)	AY 11/AY 5	W 4580/AY 5
F'13	233	254	472	1.1	1.9
F'14	173	170	151	0.98	0.88
F'15	123	240	117	2.0	0.90

E. coli K 12 strains AY 5, W 4580 and AY 11 were labeled separately with ³²P (4 μ C/ μ mole P) in TG medium supplemented with required nutrients. DNA's were extracted from the labeled cells as described in the text. F'DNA preparations # 307, # 404 and # 505 were used for hybridizations (Table 3). Each hybridization mixture contained in 2.0 ml of 6 $\times$ SSC 1 μ g of F'DNA immobilized on a filter and 1 μ g (9,500 cpm) of heat alkali denatured labeled *E. coli* DNA to be tested.

Uchida 1969). Therefore, it cannot be concluded whether the increased hybridizations of 2.0-2.2 fold observed in the present experiment are reasonable or not. However, Pl phage lysate prepared from a *E. coli* partially diploid strain for *pro* and *ade* markers gave about twice more transductants for these markers than those for other markers (Berg and Caro 1967). The value of 2.0-2.2 obtained in the present study is in good agreement with those observed on the relative frequency of transductants directed by Pl phage. Since recombination necessitated in the process of Pl transduction is thought to occur only between homologous DNA regions, the coincidence of the values appears to suggest that F'DNA can hybridize only with the region of chromosomal DNA corresponding to the F'DNA. In another words, these results suggest that the redundancy in base sequences extensive enough to influence the hybridization results does not exist in the chromosomal DNA of *E. coli*.

(e) *Marker frequency analyses on exponentially growing cultures of various growth rates*

The rationale of the following experiment is based on an equation: $P(x) = 2^{n(1-x)}$ (Sueoka and Yoshikawa 1965), where $P(x)$ represents a frequency for a given marker to be located at a position x ($0 \leq x \leq 1$; $x=0$: origin of replication, $x=1$: terminus of replication). An average number of replication points per chromosome (N) can be expressed by an equation: $N = 2^n - 1$ ($n \geq 1$) where n is reciprocally proportional to generation time of the cell. When n_1 is greater than n_2 , if a ratio of $P_{\text{obs}}(x, n_1)$ to $P_{\text{obs}}(x, n_2)$ for a certain marker a is greater than that for another marker b , we may infer that the marker a replicates earlier than the marker b in the cycle of chromosomal replication. In this way, therefore, we are able to determine the order of replication of various markers on the chromosome.

Amounts of DNA which hybridize with various F'DNA's (F'13, F'14, F'15 and F'KLF 3) were measured with three ³²P-DNA's prepared from K 12 F⁻ AY 5 cells grown at three different growth rates. The division times of cells were 25 ± 2 , 48 ± 3 and 89 ± 6 min, in TY medium, TG medium and Tris-succinate medium, respectively. Since n for

Fig. 3. The circular genetic map of *Escherichia coli* K12 (Taylor 1970) showing locations of relevant markers and extents of partial diploidies of episomes used.

E. coli grown in glucose minimal medium with generation time of about 40 min is thought to be 1.0 (Cooper and Helmstetter 1968), n -values for *E. coli* cells grown in TY medium and Tris-succinate medium were estimated to be about 2 and 0.5, respectively. Results presented in Table 7 shows that the replication order of four markers tested should be *ilv*—(*thy*, *lac*)—*his*. The result is consistent with that obtained by synchronous labeling and DNA-DNA hybridization (Yahara 1971). Neither the clockwise replication model (Calc. 1) nor the counter-clockwise model (Calc. 2) appears to be consistent with the results presented in Table 7.

Table 7. Marker frequency analysis on K12F⁻ AY5

F'DNA	succinate medium DNA	TG medium DNA	TY medium DNA	TY medium DNA	
				succinate medium DNA	TG medium DNA
F'13 (<i>lac</i>)	216±8 cts/min	258±11	213±6	0.99±0.07	0.83±0.66
F'14 (<i>ilv</i>)	108±3	159±8	210±5	1.94±0.11	1.32±0.08
F'15 (<i>thy</i>)	83±5	116±5	102±3	1.23±0.14	0.88±0.07
F'KLF 3 (<i>his</i>)	77±5	93±4	64±4	0.83±0.11	0.69±0.06

"TY DNA/TG DNA" normalized by F'14			"TY DNA/succinate DNA" normalized by F'14		
Observed	Calc. 1	Calc. 2	Observed	Calc. 1	Calc. 2
0.63±0.08	0.82	0.61	0.51±0.06	0.74	0.48
1.00	1.00	1.00	1.00	1.00	1.00
0.67±0.09	0.58	0.86	0.63±0.11	0.44	0.80
0.52±0.06	0.65	0.76	0.43±0.08	0.53	0.66

AY5 was labeled with ³²P (4 μC/μmole P) in TY-medium, in TG-medium and in Tris-succinate medium. DNA's were extracted and 1 μg (8,500 cpm) each was alkali-heat denatured, and hybridized with various DNA's. F'DNA's immobilized were #307, #402, #506 and #601 for F'13, F'14, F'15 and F'KLF 3, respectively (Table 3). Expected values were calculated by assuming either clockwise (Calc. 1) or counter-clockwise replication (Calc. 2).

DISCUSSION

The results summarized in Table 7 suggest that among four markers tested (*lac*, *ilv*, *thy* and *his* which correspond to F'13, F'14, F'15 and F'KLF 3 DNA's respectively), *ilv* replicates earlier than others, and *his* replicates latest during the cycle of chromosomal replication in K 12 F⁻ AY 5 cells. Since *ilv* is located just opposite to *his* on the circular genetic map of *E. coli* (Fig. 3), the results obtained cannot be explained by the theory of a *fixed-origin and unidirectional replication* (Cairns 1963). The observed values of the ratios (F'TY/F'TG)/(F'14 TY/F'14 TG) and (F'TY/F'Suc)/(F'14 TY/F'14 Suc) shown in Table 7 are obviously not consistent with those calculated by assuming either a clockwise or a counterclockwise replication.

The present result is in good agreement with an experiment described previously (Yahara 1971), the result of which suggested one of the following possibilities:

- (1) the synchronization of cellular growth achieved by amino acid starvation was incomplete (Caro and Berg 1969).
- (2) Two or more regions with homologous base sequences exist separately on the *E. coli* chromosome (Cutler and Evans 1967).
- (3) The result obtained reflects an intrinsic nature of the replication.

Since the marker frequency analysis presented in Table 7 is independent of synchrony of cells, the first one of the above possibilities needs not be considered. As for the second possibility, the result shown in Table 6 suggests that hybridization results reflect the amounts of DNA segments corresponding to F'DNA used. In various species of eucaryotic DNA's redundancy in DNA base sequences has been found (Britten and Kohne 1968; Thomas *et al.* 1970). On the other hand, it is generally understood that redundancy in DNA base sequences is rare in bacteria (McCarthy and Church 1970). Cutler and Evans (1967), however, suggested the existence of redundancy in *E. coli* DNA, based on an observation that 26% of DNA which replicated at the middle stage of replication cycle interacted with DNA which replicated at the last stage. However, bromouracil used in their experiment is known to induce premature initiation of replication in *E. coli* (Abe and Tomizawa 1967), the cross reaction observed by them may be explained without assuming an existence of redundancy in DNA base sequences.

It has been suggested that 5S ribosomal RNA cistrons might be redundant and located separately at different sites on the *E. coli* chromosome (Jarry and Rosset 1970). Such a redundancy in base sequences may possibly be found also in other regions. The conclusion of the present study, however, seems not to be affected by those possible redundancies, since the hybridization was sufficiently specific as far as presently examined (Table 6), and gave results (Table 7) which were compatible with observations by others (reviews by Lark 1969; Bonhoeffer and Messer 1969). Therefore, the possibility (2) mentioned above also cannot account for the present observations. Although Caro and Berg (1969) have reported a clear result which could be interpreted by a fixed origin-unidirectional replication model, their model cannot explain the present results shown in Table 7.

The present results indicate that a semi-circle portion of the circular chromosome covering the *lac* region (see Fig. 3) replicates in the order *ilv*—*lac*—*his*. This is compatible with conclusions of others (Lark 1969; Bonhoeffer and Messer 1969). However, the replication sequence of markers located between 45–74 minutes on the standard genetic map of *E. coli* (Taylor 1970) appears to contradict to a fixed origin and unidirectional replication model. Masters (1970) also observed an anomalous sequence of replication of markers around this area; the present result is similar to observation of Masters.

Taking into accounts the present findings that *thy* appears to replicate later than *ilv*, and that the origin of replication is located near *ilv*—*thy* (Yahara 1971, 1972), the replication of *E. coli* chromosome seems to proceed according to one of the following three models:

Model A: The replication starts from a fixed origin located between *ilv* and *thy*, and proceeds bidirectionally, similarly to a model proposed to explain the replication of λ phage (Schnös and Inman 1970). However, a part of counterclockwise replication ceases within a short time before the *his* has rarely been replicated.

Model B: The replication starts from a fixed origin and proceeds unidirectionally, but two populations are there, one of which is clockwise and the other is counterclockwise. The latter population is rarer than the former.

Model C: The replication origin is not fixed, but is chosen by chance from a number of sites which are located between *ilv* and *his* (mainly between *ilv* and *thy*) and the replication proceeds clockwise.

Model A requires two replication points on a chromosome, which sounds to be incompatible with observations of Cairns (1963) and Bonhoeffer and Gierer (1965) demonstrating that every chromosome possesses only one replicating point when cells grow exponentially in minimal glucose medium at 37°C. However, the paradox may not be serious, if one of the two replication points postulated presently will be lost within a short time. Model B and Model C also seem to contradict with the findings that every site of a chromosome tends to replicate periodically with regular time interval which is equal to the generation time of the cell (Meselson and Stahl 1958; Lark, Repko and Hoffman 1963; Nagata and Meselson 1968). However, taking into account the precision of the experiment, their evidences seem not to be strong enough to exclude the above two models, as was already discussed by Nagata and Meselson (1968) themselves.

SUMMARY

A DNA-DNA hybridization technique using isolated F'DNA was examined for its qualification as a method of determining amounts of several specific regions of bacterial chromosome, and applied to a study on the origin and direction of chromosomal replication in *Escherichia coli* K12. Marker frequency analysis gave an evidence against the fixed origin-unidirectional replication model. Three possible models are proposed for the origin and direction of chromosomal replication of *E. coli*.

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LITERATURE CITED

- Abe, M., and J. Tomizawa, 1967 Replication of the *Escherichia coli* K 12 chromosome. Proc. Natl. Acad. Sci. **58**: 1911-1918.
- Berg, C. M., and L. G. Caro, 1967 Chromosome replication of *Escherichia coli*. I. Lack of influence of the integrated F factor. J. Mol. Biol. **29**: 419-431.
- Bonhoeffer, F., and W. Messer, 1969 Replication of the bacterial chromosome. Ann. Rev. Genetics **3**: 233-246.
- Bonhoeffer, F., and A. Gierer, 1963 On the growth mechanism of the bacterial chromosome. J. Mol. Biol. **7**: 534-540.
- Britten, R. J., and D. E. Kohne, 1968 Repeated sequences in DNA. Science **161**: 529-540.
- Cairns, J., 1963 The chromosome of *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **28**: 43-45.
- Caro, L. G., and C. M. Berg, 1969 Chromosome replication in *Escherichia coli*. II. Origin of replication in F⁻ and F⁺ strains. J. Mol. Biol. **45**: 325-336.
- Cooper, S., and C. E. Helmstetter, 1968 Chromosome replication and the division cycle of *Escherichia coli* B/r. J. Mol. Biol. **31**: 519-540.
- Cutler, R. G., and J. E. Evans, 1967 Isolation of selected segment from the genome of Hfr *Escherichia coli*. J. Mol. Biol. **26**: 81-90.
- Denhardt, D. T. 1966 A membrane-filter technique for the determination of complementary DNA. Biochem. Biophys. Res. Comm. **23**: 641-646.
- Donachie, W. D., and M. Masters, 1966 Evidence for polarity of chromosome replication in F⁻ strains of *Escherichia coli*. Genet. Res. **8**: 119-124.
- Falkow, S., J. A. Wohlhieter, R. V. Citarella, and L. S. Baron, 1964 Transfer of episomic elements to *Proteus*. I. Transfer of F-linked chromosomal determinants. J. Bacteriol. **87**: 209-219.
- Falkow, S., and R. V. Citarella, 1965 Molecular homology of F-merogenote DNA. J. Mol. Biol. **12**: 138-151.
- Hirota, Y., and P. H. A. Sneath, 1961 F' and F mediated transduction in *Escherichia coli* K 12. Japan. J. Genetics **36**: 308-318.
- Ishibashi, M., Y. Sugino, and Y. Hirota, 1964 Chromosomal location of thymine and arginine genes in *Escherichia coli* and F' incorporating them. J. Bacteriol. **84**: 554-561.
- Jacob, F., S. Brenner, and F. Cuzin, 1963 On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **27**: 329-348.
- Jarry, B., and R. Rosset, 1970 An attempt to localize 5 S RNA cistrons in *Escherichia coli*. Biochem. Biophys. Res. Comm., **41**: 789-797.
- Lark, K. G., 1969 Initiation and control of DNA synthesis. Ann. Rev. Biochem. **33**: 569-604.
- Lark, K. G., T. Repko, and E. H. Hoffman, 1963 The effect of amino acid deprivation on subsequent DNA replication. Biochim. Biophys. Acta **76**: 9-24.
- Marmur, J., 1961 A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**: 208-218.
- Masters, M., 1970 Origin and direction of replication of the chromosome of *E. coli* B/r. Proc. Natl. Acad. Sci. **65**: 601-608.

- McCarthy, B. J., and J. B. Church, 1970 The specificity of molecular hybridization reactions. *Ann. Rev. Biochem.* **39**: 131-150.
- Meselson, M., and F. W. Stahl, 1958 The replication of DNA in *Escherichia coli*. *Proc. Natl. Acad. Sci.* **44**: 671-682.
- Nagata, T., and M. Meselson, 1968 Periodic replication of DNA in steadily growing *Escherichia coli*: The localized origin of replication. *Cold Spring Harbor Symp. Quant. Biol.* **33**: 553-557.
- Nishi, A., and T., Horiuchi, 1966 β -Galactosidase formation controlled by an episomal gene during the cell cycle of *Escherichia coli*. *J. Biochem.* **69**: 338-340.
- Pittard, J., J. S. Loutit, and E. A. Adelberg, 1963 Gene transfer by F' strains of *Escherichia coli* K 12. *J. Bacteriol.* **85**: 1394-1401.
- Schnös, M., and R. B. Inman, 1970 Position of branch points in replicating DNA. *J. Mol. Biol.* **51**: 61-73.
- Stouthamer, A. H., P. G. deHaan, and E. J. Bulten, 1963 Kinetics of F-curing by acridine orange in relation to the number of F-particles in *Escherichia coli*. *Genet. Res.* **4**: 305-317.
- Sueoka, N., and H. Yosukikawa, 1965 The chromosome of *Bacillus subtilis*. I. Theory of marker frequency analysis. *Genetics* **52**: 747-757.
- Taylor, A. L., 1970 Current linkage map of *Escherichia coli*. *Bacteriol. Rev.* **34**: 155-175.
- Thomas, Jr., C. A., B. A. Hamkalo, D. N. Misra, and C. S. Lee, 1970 Cyclization of eucaryotic deoxyribonucleic acid fragments. *J. Mol. Biol.* **51**: 621-632.
- Tomizawa, J., and T. Ogawa, 1968 Replication of phage lambda DNA. *Cold Spring Harbor Symp. Quant. Biol.* **33**: 533-551.
- Wohlhieter, J. A., S. Falkow, and R. V. Citarella, 1966 Purification of episomal DNA with cellulose nitrate membrane filters. *Biochim. Biophys. Acta* **129**: 475-481.
- Yahara, I., 1971 On the origin of replication of *Escherichia coli* chromosome. *J. Mol. Biol.* **57**: 373-376.
- Yahara, I., 1972 On the attachment of replication origin to membrane in *Escherichia coli*. *Japan. J. Genetics* **47**: 45-51.
- Yamagata, H., and H. Uchida, 1969 Effect of acridine orange on sex factor multiplication in *Escherichia coli*. *J. Mol. Biol.* **46**: 73-84.