REPLICATION OF EXTRACHROMOSOMAL ELEMENTS IN A DNA SYNTHESIS INITIATION MUTANT OF SALMONELLA TYPHIMURIUM.

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SUMMARY

The replication of two large extrachromosomal elements has been followed in a mutant of Salmonella typhimurium which is temperature-sensitive for DNA synthesis initiation. The replication of both the pi factor (M.W. 62 million daltons) and an F'lac (M.W. 80 million daltons) is reduced approximately in proportion to the overall reduction in DNA synthesis at the restrictive temperature. It is considered that the gradual cessation of plasmid replication is a reflection of a coupling between this process and the chromosome replication cycle.

INTRODUCTION

Studies on extrachromosomal elements (plasmids) in bacteria have suggested that distinct elements replicate independently[1]. That this is to some extent true is shown by the isolation of several mutant F factors that are unable to replicate at high temperatures. The majority of these mutants map within the F factor itself and have no discernible effect on chromosomal DNA replication[1]. On the other hand many extrachromosomal elements are stably maintained and strictly segregated into daughter cells although only present at one or two copies per chromosome group. This strict control could be achieved by a coupling of extrachromosomal DNA replication to chromosome replication or to some other aspect of cell growth.

In order to obtain more information about the regulation of the replication and segregation of bacterial replicons several workers have studied the replication of extrachromosomal elements under conditions that block chromosomal DNA synthesis[2-6].

In this work I have followed the replication of two large extrachromosomal elements in a mutant of Salmonella typhimurium which is temperature-sensitive for DNA synthesis initiation[7].

METHODS

Organisms. 11GT is a mutant of Salmonella typhimurium which is temperature-sensitive in the initiation of DNA synthesis and is probably the equivalent to dnaC in Escherichia coli[7]. 11GT is a low thymine requirer and also requires methionine, tryptophan, isoleucine and valine.

11GT/F'lac is an F'lac derivative of 11GT. The F'lac has a molecular weight of approximately 80 x 10^6 daltons (Spratt, unpublished results).

Media. 11GT was grown in glucose/casamino acid minimal medium containing 4 µg/ml thymine[7]. 11GT/F'lac was grown in the same minimal medium containing 0.2% lactose in place of glucose.

Extrachromosomal DNA synthesis. Both the plasmids exist as closed circular DNA and the radioactivity incorporated during a pulse of ^{3}(H)-thymine was analysed for closed circular DNA by sedimentation in alkaline sucrose. Samples were cooled, washed in cold 0.05M tris/HCl buffer pH 8.0 and resuspended in TES buffer (0.05M tris/HCl; 0.02M EDTA; 0.05M NaCl pH 8.0) and lysed with 1% sodium lauroyl sarcosinate in 0.8M NaOH and sheared as described by Freifelder, Folkmanis & Kirschner[8]. 0.2 ml of the sheared lysate was layered on to an 8 ml linear 5-20% alkaline sucrose gradient[8] and centrifuged at 20° for 40-45 min in the 3 x 10 swing-out rotor of an M.S.E. superspeed 50 centrifuge at 30,000 r.p.m. 41, 0.2 ml fractions were collected directly on to drop reaction paper using an ISCO density gradient fractionator. The papers were processed and counted as described previously[7].

RESULTS

Salmonella typhimurium LT2 and its derivatives possess a closed

circular plasmid DNA[9]. This DNA (pi) is present at about 1 copy/ chromosome and has a molecular weight of approximately 60 x 10^6 daltons (Meynell, Spratt & Rowbury, in preparation). The closed circular pi DNA can be seen as a fast sedimenting minor peak in alkaline sucrose gradients, Fig. 1a. On shifting strain 11GT from 25° to 38° DNA synthesis continues at a gradually decreasing rate for about 60 min and then stops[7].

An exponentially growing culture of 11GT was divided into three 25 ml portions and one was maintained at 25° and the other two shifted to 38°. 500μCi of ^{3}(H)-thymine (29Ci/mMol) was added to two of the cultures for a period of 80 min at 25° and 38° respectively. 500μCi of ^{3}(H)-thymine was also added to the third culture for 80 min at 38° after a prior 80 min at 38°. The initial absorbance at the time of addition of label was 0.1 in each case. The total incorporation during the three pulses was measured and the incorporation into pi and chromosomal DNA measured by alkaline sucrose centrifugation. Fig. 2; Table I, experiment 1 shows that 1.6% of the DNA synthesised is closed circular pi DNA at 25°. At 38° the total incorporation is decreased and the incorporation into closed circular pi DNA is also greatly decreased. The percentage of the total counts incorporated into pi DNA at 38° is about half that at 25° during both the first and second 80 min pulses. Similar results were obtained when the DNA synthesised at 38° was analysed by isopycnic centrifugation in CsCl in the presence of ethidium bromide (data not shown).

Table 1, experiment 2 shows the result of an identical experiment with 11GT/F'lac (Fig. 1b shows a typical profile from an alkaline sucrose centrifugation of a lysate of 11GT/F'lac - F'lac DNA appears as a second faster sedimenting peak well separated from closed circular pi DNA). As before synthesis of both pi and F'lac decreases at 38° and the percentage incorporation into pi and F'lac is about half that at 25°.

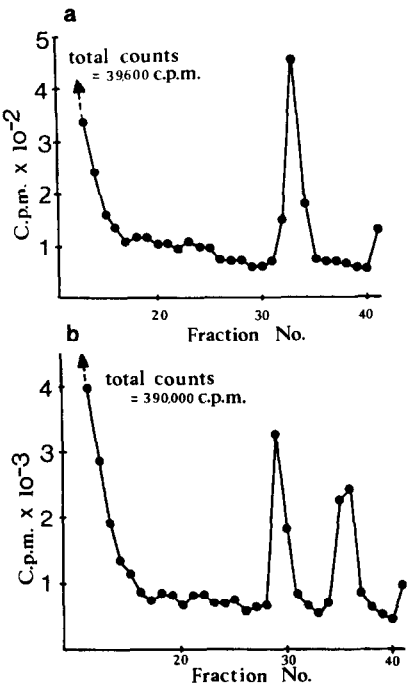

Fig. 1.

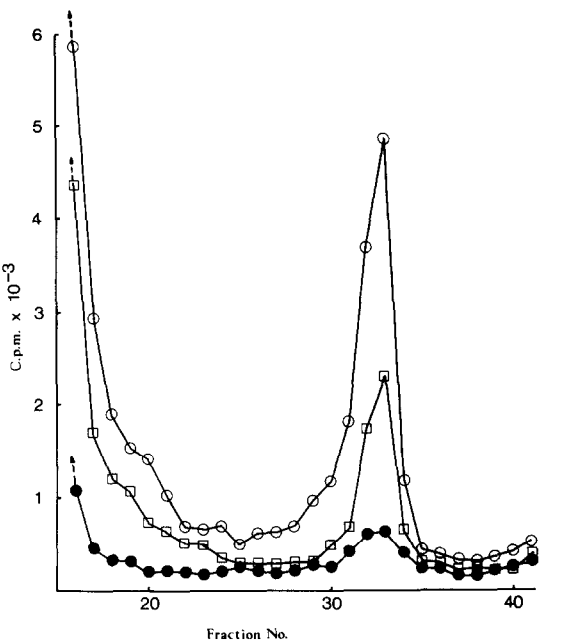

Fig. 2.

Legend to Figure 1.

Separation of closed circular DNA of pi and F'lac by sedimentation in alkaline sucrose.
a) A sheared lysate of ^{3}(H) labelled 11GT was centrifuged for 45 min at 30,000 r.p.m. and fractionated as described in methods. Sedimentation is from left to right. The fast sedimenting peak at fraction 33 is closed circular pi DNA.
b) A similar lysate of 11GT/F'lac centrifuged for 40 min at 30,000 r.p.m. The pi bands at fraction 29 and F'lac sediments rather faster at fraction 36 .

Legend to Figure 2.

Replication of closed circular pi DNA in 11GT at the restrictive temperature.
A culture of 11GT was pulse labelled for 80 min with 3-(H) thymine at 25° (○) or in the first (□) or second (●) 80 min after a shift to 38°. Sheared lysates were centrifuged for 45 min at 30,000 r.p.m. on alkaline sucrose gradients to separate pi DNA. Total counts in the three lysates were 734400; 567800 and 149260 c.p.m. respectively.

TABLE I Replication of Pi and F'lac in 11GT

	EXPERIMENT 1		EXPERIMENT 2		
	Counts in 0.1 ml culture at end of 80 pulse (c.p.m.)	% in Pi	Counts in 0.1 ml culture at end of 80 pulse (c.p.m.)	% in Pi	% in F'-lac
0'-80' (25°)	30733	1.6	15260	1.13	0.65
0-80' (38°)	23061	0.83	8723	0.47	0.23
80'-160' (38°)	7456	0.85	1777	0.54	0.25

DISCUSSION

Both the replication of pi and F'lac (in the presence of pi as a pi$^-$ strain of 11GT has not yet been obtained) continues to a certain extent at the restrictive temperature. Pi and F'lac synthesis during the second 80 min at 38° is reduced to 5% and 4% respectively of the synthesis in an 80 min period at 25°. The synthesis of closed circular DNA of both elements decreases rather faster than the reduction in total incorporation such that at 38° both pi and F'lac are synthesised at about half their differential rates at 25°. The decrease in synthesis of these elements is not due to loss of closed circular DNA at 38° as prelabelled plasmid is stable in this strain when the culture is shifted to the restrictive temperature (Spratt, unpublished results). Several explanations of these results are possible but it would seem that the lesion in DNA synthesis initiation in strain 11GT is affecting the replication of two plasmids. It is possible that the temperature sensitive component of the chromosomal initiation apparatus in strain 11GT is also involved in the regulation or replication of pi and F'lac or that the effect is due to a coupling between the replication of these elements and an event in the DNA replication cycle e.g. the completion of a round of DNA replication or DNA segregation.

Bazaral & Helinski[3] have shown that when protein synthesis is inhibited the replication of the F factor decreases in a rather similar manner to the synthesis in 11GT. As the F factor seems to replicate late in the DNA replication cycle[6] it seems possible that the residual synthesis of plasmid is due to the replication of plasmid triggered by the completion of rounds of chromosomal DNA replication in 11GT at the restrictive temperature. As no new rounds of chromosomal DNA synthesis are initiated under these conditions plasmid replication gradually ceases.

Zeuthen & Pato[6] have obtained similar results for F'lac replication in DNA synthesis initiation mutants of Escherichia coli using an enzyme induction technique.

The replication of ColE1 DNA in a temperature-sensitive initiation mutant of Escherichia coli stops immediately at the restrictive temperature[4] but the replication of this small plasmid probably differs considerably from that of larger plasmids[3,5]. The replication of phage DNA is also clearly controlled by a quite separate means and phage develops extensively in DNA synthesis initiation mutants[2,10].

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