Vol. 132, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1985 Pages 42-48

HYBRID BETWEEN TEMPERATE PHAGE P22 AND VIRULENT PHAGE MB78

M. Verma and M. Chakravorty*

Molecular Biology Unit
Institute of Medical Sciences
Banaras Hindu University
Varanasi-221005, INDIA

Received August 5, 1985

Bacteriophage MB78, a virulent phage of Salmonella typhimurium can not grow in rifampicin resistant mutant of the host. However, the temperate phage P22 which grows normally in this host helps MB78 to grow in its non-permissive host. P22 can not itself multiply under the condition of mixed infection and the burst size of MB78 is very much reduced. The burst sizes of both are reduced even when the permissive host LT2 is mixedly infected with P22 and MB78. When rifampicin resistant mutants are mixedly infected, only P22-specific mRNAs are produced in the early stage. This is followed by transcriptions from both P22 and MB78 genomes. Subsequently, only MB78-specific messengers are detected in the infected host. Hybrids between the two phages have been isolated from the mixedly infected cells. These hybrids which contain only 15-20% of P22 genome can grow in the rifampicin resistant mutants of the host. The analysis of hetero-duplex between MB78 and hybrid phages revealed the presence of at least three mismatch regions. Experiments are in progress to identify the parts of P22 genome present in the hybrid phage.

Rifampicin resistant mutants of the hosts which have altered (rifampicin resistant) RNA polymerase are sometimes defective in transcribing phage genomes (1-3). Rifampicin resistant mutants of E. coli poorly support the growth of phages like T4D and λ (1,2.). In case of phage T4 the synthesis and the assembly of the tail fibers are blocked in such host mutants (2). In a class of E. coli mutants called groN, the growth of λ is arrested because the N gene product (regulatory gene for phage development) fails to function. The groN mutation produces a structural change in the DNA polymerase in such a way that it can no longer interact properly with the phage gene N product (1). The E. coli mutants affecting phage T7 DNA replication produce RNA polymerase

*To whom all correspondence may be made.

0006-291X/85 $1.50

resistant to the inhibition by the T7 gene 2 protein (3). Further, rifampicin resistant mutant (rif39) of Salmonella typhimurium is lysogenised at a reduced frequency upon infection with temperate phage P22 (4).

In course of our studies with MB78, a newly isolated virulent phage of S. typhimurium (5), it was observed that the rifampicin resistant mutant of S. typhimurium (rif39) can not support the development of the phage MB78 whereas other Salmonella phages like the temperate phage P22 and the virulent one 9NA (6) can grow in rif39. However, MB78 can grow to some extent in rif39, if mixedly infected with P22. The present communication deals with the development of phage MB78 in rif39 in presence of phage P22 and the formation of the hybrid between the two.

Materials and Methods

[^{3}H]-thymidine (6,700 Ci/mol) and α-[^{32}P]-labelled nucleoside triphosphates were purchased from New England Nuclear Corporation, Chicago, Ill, USA. [^{14}C]-uridine (49.3 Ci/mol) was purchased from Bhabha Atomic Research Centre, Bombay, India. 2,5-diphenyloxazole (PPO) and dimethyl 1,4-bis-(5-phenyloxazolyl)benzene(POPOP) were products of Amersham/Searle Corporation, Arlington Heights, Ill, USA. Cesium chloride was obtained from Schwarz/Man. Orangeburg, N.Y. Nitrocellulose membrane filters were obtained from Schleicher and Schull Company, West Germany or Millipore Company, USA.

Salmonella typhimurium (LT2, strain 18) and the C_1 mutant (clear plaque forming mutant of phage P22 (7) were originally obtained from Myron Levine of the Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA. Rifampicin resistant mutant (4) of S. typhimurium (rif39), was obtained from Bruce Ames, Berkeley, California, USA. Phage MB78 was isolated in our laboratory (5).

Pulse labelled RNA as well as S. typhimurium DNA were isolated as described by Taneja et al. (8).

Phage DNA was isolated according to the method described by Smith (9).

DNA-RNA hybridisation and DNA-DNA hybridisation were carried out as per methods of Gillespie and Spiegelman (10) and Denhardt (11) respectively.

Results and Discussion

Burst sizes of MB78 and P22 in LT2 and rif39 in single and mixed infections

The results presented in Table 1 show that phage MB78 can not multiply in rifampicin resistant host rif39. However, phage P22 helps MB78

Table 1. Burst sizes of P22 and MB78 in LT2 and rif39 under conditions of single and mixed infections

Host Cell	Infecting Phages	Burst Sizes	
		P22	MB78
LT2,	P22	150	----
	MB78	---	148
	P22 + MB78	12	18
rif39	P22	180	----
	MB78	----	0.5
	P22 + MB78	zero	11.0

In case of single infection the m.o.i. was 10 whereas in case of mixed infection, m.o.i. of each type of phage was 5.

to grow in such host although the burst size is rather small. A strong interference between the two phages P22 and MB78 is observed even when a permissive host (LT2) is mixedly infected with these two phages. In order to find out whether the inability of phage MB78 to grow in rif39 is due to the alteration in the RNA polymerase or any other second mutation in rif39, a number of spontaneous rifampicin resistant mutants of S. typhimurium were isolated and a few of them chosen at random were tested. Out of 6 mutants tested, only one supported the growth of both P22 and MB78. The reason for this is not known. The remaining five mutants behaved like rif39 and did not support the growth of MB78 (data not presented). Most probably the mutation in RNA polymerase makes the host nonpermissive for phage MB78.

Analysis of mRNAs synthesized in rif39 following mixed infection with P22 and MB78.

As P22 and MB78 DNAs have no detectable homology (5), phage-specific transcriptions under the condition of mixed infection could be studied by molecular hybridization technique (10). Pulse-labelled RNAs isolated at different times following mixed infection were separately hybridized to host, P22 and MB78 DNAs in identical reactions mixtures. As shown in Fig. 1 there was mostly host-specific transcriptions up to 3 min

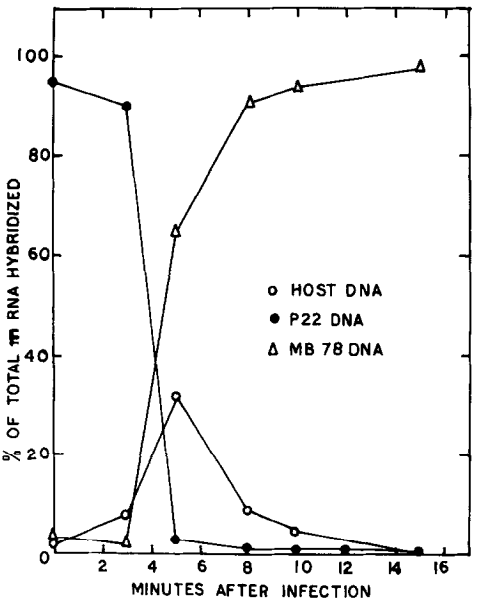

Fig. 1 Transcription of host and phage genomes following mixed infection of rif39 cells with P22 and MB78

[^{14}C]-uridine labelled RNAs pulsed for 1 min only were isolated from rif39 cells at different times following mixed infection with P22 and MB78. Three hybridization experiments were carried out with each sample of pulse labelled mRNAs where the DNAs were either from MB78 or P22 or rif39. Each incubation was in duplicate. Results from duplicate samples were in close agreement. The average of the two results has been plotted. Input radioactivity was in the order of 10^5 cpm. Total counts hybridized was in the order of 10^4. Values are corrected for blank (filter with No DNA) which varied from 40-50 cpm.

following infection. The transcription of phage P22 DNA was detectable at this stage (3 min or so) but MB78-specific message was not detectable. Host specific transcription stopped after 5 min following infection while the transcriptions of both P22 and MB78 genomes continued. At that stage about one third of the total mRNAs were P22-specific and the rest were from phage MB78. With time P22-specific mRNA synthesis slowed down. From 8 min onwards mostly MB78-specific mRNAs were synthesized. Thus phage P22 genome is transcribed at the early stage and most probably helps phage MB78 to overcome the transcription inhibition in rif39. Once MB78 transcription, starts it inhibits the synthesis of P22 mRNAs and interferes with the development of P22. It should be mentioned here that no MB78

specific messages are detected in rif39 infected with MB78 alone (data not presented).

Isolation of hybrid phages between P22 and MB78

Since MB78 can grow in rif39 (to some extent) in presence of P22 attempt was made to isolate hybrids between the two phages. Cells (rif39) were mixedly infected with P22 and MB78 and the progeny particles were plated on rif39. The plaques obtained under such conditions were big in size like those of MB78. Phages from a number of such plaques were purified by repeated growth in rif39. These purified phages resembled MB78 serologically but unlike MB78 grew in rif39 and were named PMB expecting those to be the hybrids between P22 and MB78. The efficiencies of plating of these phages were found to be variable (data not presented). As the phages PMB1 and PMB3 showed good efficiency of plating on rif39 those were used for further investigations. These two phages resembled phage MB78 so far as serological properties, type of lysozyme production, sensitivity to EDTA etc. are concerned (data not presented). To find out whether these phages are hybrids between phages P22 and MB78, DNA-DNA hybridization experiments were carried out. The results presented in Table 2 indicate that these so called hybrid phages indeed contain 15-20% of P22 genome. Literature survey indicates that such recombinants between temperate and virulent phages having no detectable homology has not so far been reported. It may, however, be emphasized that a small stretch of homologous region, if any, is enough for a crossover to take place and under the present set of experiments a strong selection pressure has been applied which made selection of such hybrids possible. It was necessary to confirm that the hybrid phages do contain hybrid DNAs. Heteroduplexes between MB78 and hybrid phages were formed and visualized through electron microscope (Fig. 2). From the survey it appeared that there are at least three mismatch regions. No such mismatch regions were observed when homoduplex formation was carried out. Complete heteroduplex map could not be constructed as phage MB78 DNA seems to have nicks or

Table 2. DNA-DNA hybridization between P22, MB78 and hybrid phage DNAs

DNA on filter	^{32}P-DNA in solution	Per cent hybridized
P22	P22	100
MB78	P22	0.40-0.50
PMB1	P22	15-20
PMB3	P22	14-25
None	P22	0.1-0.3
P22	MB78	0.5-0.6
MB78	MB78	100
PMB1	MB78	79-83
PMB3	MB78	80-83
None	MB78	0.2-0.3
P22	PMB1	15-22
MB78	PMB1	80-85
PMB1	PMB1	100
PMB3	PMB1	88-90
None	PMB1	0.7-0.8
P22	PMB3	19-20
MB78	PMB3	82-85
PMB1	PMB3	86-90
PMB3	PMB3	100
None	PMB3	0.6-0.7

The values are averages of four different sets of experiments carried out at different times while in each experiment the samples were in duplicate. In some experiments DNA was labelled in vivo (12) and in others in vitro through nick translation (13). Input radioactivity varied between $5x10^4$-10^5 counts/min depending on the preparation. The extent of hybridization between homologous DNAs has been taken as 100 per cent and calculations are made with respect to that. Filter with no DNA was used to determine unspecific adsorption of labelled DNA (blank value).

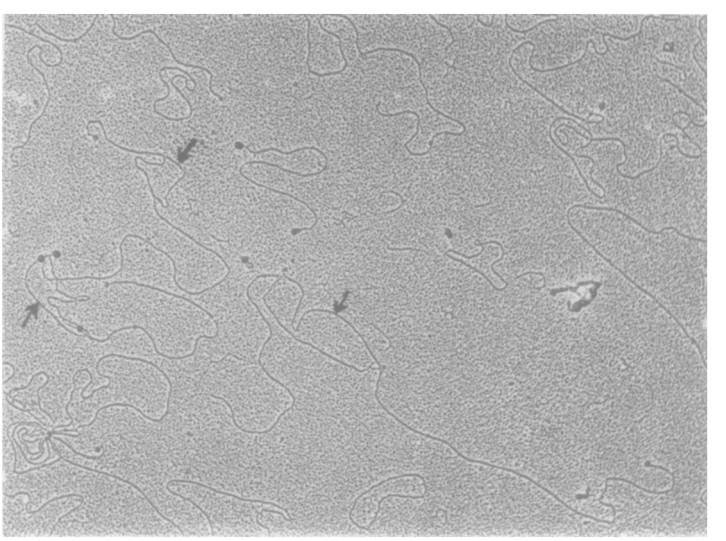

Fig. 2 Heteroduplex between the DNAs from hybrid phage PMB1 and MB78

Two heteroduplex molecules with three mismatch regions (marked with arrows) are visible. Circular DNAs were those of pBR322 which was used as internal marker for length measurement.

ribonucleotides in the DNA as evident from alkaline sucrose gradient analysis (unpublished results from this laboratory). Experiments are in progress to identify the part(s) of P22 genome present in the hybrid phage, map their physical location and study the interaction between P22 and MB78 DNAs with the host RNA polymerase.

Acknowledgements

Heteroduplex analysis was carried out in the laboratory of Prof. A.A. Alexandrov, Institute of Molecular Genetics, Moscow, USSR during the visit of one of us (MC) under exchange of programme between Indian National Science Academy and Academy of Sciences of USSR. The authors are extremely thankful to the scientists of the Institute, specially to Maya Krupipenka for providing all the necessary help for heteroduplex analysis. The financial assistance from the Department of Science and Technology, Government of India, Council of Scientific and Industrial Research, University Grants Commission and Indian National Science Academy, is being gratefully acknowledged.

References

1. Georgopoulos, C.P. (1971) Proc. Natl. Acad. Sci. USA, 68, 2967.
2. Snyder, L.R. (1972) Virology, 50, 396.
3. DeWynagert, M.A., and Hinkle, D.C. (1980) J. Virol., 33, 780.
4. Hong, J.S.,Smith, G.R. and Ames, B.N. (1971) Proc. Natl. Acad. Sci. USA, 68, 2258.
5. Joshi, A., Siddiqui, J.Z., Rao, G.R.K. and Chakravorty, M. (1982) J. Virol., 41, 1038.
6. Wilkinson, R.G., Gemski, P. and Stoker, B.A.D. (1972) J. Gen. Microbiol., 70, 527.
7. Levine, M. (1957) Virology, 3, 22.
8. Taneja, S., Khandekar, P.S. and Chakravorty, M. (1975) Molec. Gen. Genet., 137, 315.
9. Smith, H.O. (1968) Virology, 34, 203.
10. Gillespie, D. and Spiegelman, S. (1965) J. Mol. Biol., 12, 829.
11. Denhardt, D.T. (1966) Biochem. Biophys. Res. Commun., 22, 641.
12. Botstein, D. (1968) J. Mol. Biol. 34, 621.
13. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol., 113, 237.