LOCATION OF THE PILIATION FACTOR ON THE CHROMOSOME OF ESCHERICHIA COLI

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Pili are protein macromolecules synthesized by bacteria in the form of long, thin, rigid appendages radiating from the cell surface (Brinton et al. 1954). They can be visualized in the electron microscope and are easily concentrated and purified (Brinton and Stone, 1961). Recent observations have indicated that their genetic behavior may be amenable to analysis by the elegant tools of recombination (Lederberg and Tatum, 1946) and transduction (Zinder and Lederberg, 1952). Thus, Brinton and Baron (1960) have reported the transfer of piliation from Escherichia coli to Salmonella typhosa by sexual recombination. The S. typhosa recipient cells gained the ability to produce E. coli pili rather than Salmonella pili, and a possible chromosomal location for the piliation factor close to arabinose on the bacterial chromosome of E. coli was inferred. The transduction of the piliation factor has been demonstrated recently in E. coli by Brinton and Gemski (1961) using phage P 1.

This paper reports a more precise location of the piliation factor by means of interrupted mating experiments as well as certain findings related to the instability of the piliation factor in E. coli obtained by transductional analysis.

Genetic recombination studies: A non-interrupted mating was performed using E. coli K-12 Hfr Cavalli (W1895) $M^{-}P^{+}T^{+}ara^{+}L^{+}Sm^{s}$ as the donor and E.

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coli B/r $M^+P^-T^-ara^-L^-Sm^r$ as the recipient.** W1895 is stably piliated and B/r is non-piliated. The mating technique has been previously described (Brinton and Baron, 1960). Recombinants were selected for threonine synthesis, leucine synthesis, or arabinose fermentation and were scored for biochemical markers and piliation. Piliation was scored by a slide hemagglutination test (Duguid, 1955) (Brinton and Baron, 1960). All the recombinants were stable for both piliation and biochemical characteristics. The recombination frequencies for various pairs of markers as shown in Fig. 1 indicate that the piliation factor is located to the left of threonine.

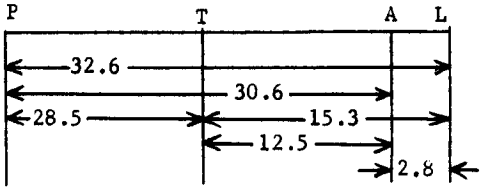

Figure 1

Genetic mapping by recombination is fairly accurate over a limited region of the chromosome, but over longer distances such mapping can be inaccurate due to the oriented transfer of markers from donor to recipient and the probability of random chromosomal breakage between the markers during transfer.

To investigate this possibility and to map the piliation factor by an independent method, the interrupted mating technique of Wollman and Jacob (1956) was used. The donor was E. coli K-12 W1895 which transfers its markers in the order T_6^r-lac-T_1^r-Az^r-L-ara-T-B_1-mtl-xyl-mal-gal. The recipient was a non-piliated strain of E. coli K-12, W945 $F^-lac^-L^-ara^-T^-B_1^-mtl^-xyl^-mal^-gal^-$. The donor and recipient were incubated together and at various times samples were removed and blended to interrupt the mating process. Recombinants were selected for lactose and scored for lactose, leucine, threonine, and piliation. The number of recombinants containing

** P = piliation, M = methionine, T = threonine, ara = arabinose, L = leucine, lac = lactose, B_1 = thiamine, mal = maltose, xyl = xylose, gal = galactose, mtl = mannitol, T_1^r = T_1 phage resistance, Az^r = azide resistance.

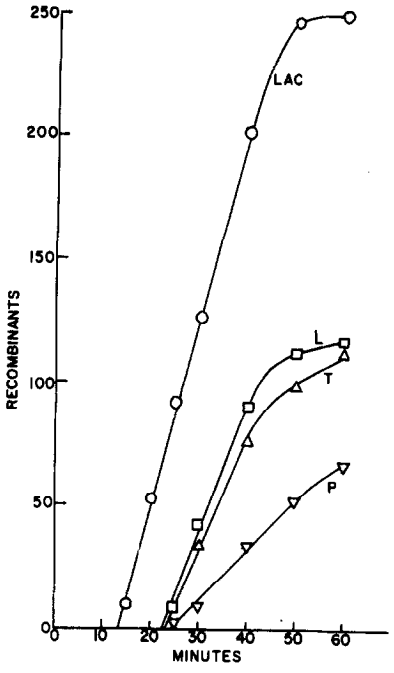

Figure 2

each marker is plotted as a function of time in Fig. 2. Extrapolation of the curves to zero recombinants shows piliation entering the recipient shortly after threonine. Another experiment using E. coli K-12 200U $P^-lac^-L^-T^-B_1^-$ as a recipient gave similar results. All recombinants were stably piliated.

Transduction studies:

Since only closely linked chromosomal markers can be jointly transduced at an appreciable frequency (Lennox, 1955), transduction of piliation by phage could offer a method of investigating in finer detail the linkage relationships involved. The donor E. coli strains were B-L(E)F^+ (Brinton, 1959), Bam F^+ (Brinton, unpublished), K12 Hfr Cavalli W1895 $M^-P^+\lambda^+$ and K12 Hfr Cavalli W1485 $M^-P^+\lambda^-$. B-L(E)F^+ and Bam F^+ are unstably piliated strains while W1895 and W1485 are stably piliated. The phage strains were P1kc which transduces both E. coli K12 and E. coli B, and P1bt which transduces E. coli B. The recipient non-piliated strain was E. coli B/r $P^-T^-ara^-L^-$. The transduction techniques were essentially as described by Lennox. Phage infected recipients were plated on media selective for threonine, arabinose, and leucine. The transductants were scored for biochemical markers and for piliation and the frequencies of joint transduction with each selective marker are shown in Table I.

Piliation was linked to all three markers with a maximum joint frequency of transduction of about 30%. The joint transduction frequencies of piliation with the biochemical markers in any given experiment were not very different but were nearly always greatest for threonine, inter-

Table I

Frequencies of joint transduction among piliation, threonine, arabinose and leucine expressed as per cent total transductants. ± indicates the standard error. The recipient strain was *E. coli* B/r $P^-T^-ara^-L^-$.

Donor Strain	Scored Marker	Selective Marker		
		threonine	arabinose	leucine
W1485	P	30.0 ± 6.4	13.1 ± 1.4	28.3 ± 1.9
	T	100	6.7	6.7
	A	1.7	100	40.3 ± 6.5
	L	1.1	32.9 ± 4.8	100
W1485	P	25.0 ± 11.0	21.1 ± 2.7	31.1 ± 4.2
	T	100	-	-
	A	2.1	100	50.0 ± 7.2
	L	2.6	39.4 ± 8.1	100
W1895	P	30.0 ± 15.0	12.7 ± 3.3	18.1 ± 4.2
	T	100	-	-
	A	8.3	100	58.3 ± 14.2
	L	7.7	54.0 ± 13.0	100
Bam P^+	P	18.5 ± 2.7	10.9 ± 1.4	14.7 ± 1.7
	T	100	11.7 ± 5.6	2.9
	A	2.7 ± 2.7	100	58.3 ± 7.7
	L	3.9 ± 2.7	48.0 ± 7.0	100
B-L(E) P^+	P	10.0 ± 4.8		
	T	100		

mediate for leucine, and lowest for arabinose. The joint transduction frequencies among pairs of biochemical markers were essentially the same as those found by Lennox: arabinose-leucine about 50%, arabinose-threonine 0-12%, leucine-threonine 0-4%.

All piliated transductants were unstable and spontaneously segregated P^- cells which formed larger, less dense colonies. On the other hand, most of the transductants were stable for the biochemical markers, although a few were unstable for a non-selected biochemical marker (Lennox, 1955). Instability of a biochemical marker, however, was not correlated with loss of piliation.

Essentially the same results were obtained whether the donor strain was stably piliated or not.

Discussion: Although sexual crosses of a piliated Hfr donor with non-piliated recipients show the piliation character to be capable of chromosomal integration at a specific locus, the frequencies of joint transduction of piliation with other markers and the instability of piliated transductants are not consistent with a stable, exclusively chromosomal determinant at this location. In attempting to explain these results, we have noticed some striking similarities between the genetic determinant of piliation and the class of genetic elements called "episomes" (Jacob, Schaeffer, and Wollman, 1961).

1) Pili are not essential cellular components.
2) The piliation factor can be irreversibly lost since, in most instances, non-piliated cells arising from unstably piliated strains do not revert.
3) The piliation factor can have a specific chromosomal location.
4) The piliation factor can be transduced not linked in the usual way to known chromosomal markers.
5) The piliation factor can exist in the same strain in different states, depending on the method of genetic transfer. (Non-piliated B/r can be made stably piliated by conjugation with W1895 or unstably piliated by transduction with phage grown on the same donor).

The possibility that the piliation factor might be episomal is under investigation.

The chromosomal location for the piliation factor found here is in disagreement with the location between T_1 and leucine reported by Maccacaro et al (1959). No evidence for a plasmagenic mode of inheritance as proposed by these authors was found.

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