Extrusion of Sex Pili by Rapidly Washed R⁺ *Escherichia coli*

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Although plasmid-determined sex pili play an essential part in conjugation by enterobacteria, their exact role is still uncertain (Meynell, 1972). It was originally suggested that the sex pilus provides a tube through which the donor DNA passes to the recipient, the donor and recipient remaining otherwise apart (see Brinton, 1971). Other hypotheses endow it with a more dynamic role, e.g. that the donor DNA is drawn across by pili polymerized in the donor and de-polymerized in the recipient (see Brinton, 1971), or that, after attachment of the free end of the pilus to the recipient, the pilus retracts so that the surfaces of donor and recipient are brought into contact (see Curtiss, 1969). The demonstration of extrusion or retraction in response to an external stimulus would favour a dynamic role; stimulated retraction has been proposed as an explanation for pilus disappearance after treatment with arsenate or cyanide (O'Callaghan, Bundy, Bradley & Paranchych, 1973; Novotny & Fives-Taylor, 1974) and for their shortening after attachment of donor-specific phage (Jacobsen, 1972). The possibility that certain stimuli cause extrusion was suggested by our finding that adsorption of specific antibody to sex pili determined by an I-like sex factor increased the numbers of pili per bacterium from the normal value of between 1 and 2 to about 30 (Lawn & Meynell, 1972). That effect could be attributed to prevention of retraction, or to stabilization of continuously growing pili by prevention of breakage or de-polymerization. However, we show here that large numbers of sex pili also appear suddenly in response to a treatment not involving antibody, namely, rapid washing.

w945(R538I_{drd}2) is a strain of *Escherichia coli* K12 having no common pili but forming I-like sex pili because it carries the de-repressed I-like R factor, R538I_{drd}2 (Lawn & Meynell, 1970). Bacteria from a nutrient broth culture in late logarithmic phase at 37 °C were collected on a membrane filter of pore size 0.22 µm and washed for 1 min by passing broth at 37 °C rapidly through the filter by applying a suction of 140 mm Hg to its lower surface. The bacteria were then suspended in broth at 37 °C and, after a period of 2 to 5 min, a sample was prepared for electron microscopy by collection on a second filter, using gentle suction as described by Lawn & Meynell (1970).

We expected that pili would be removed by the rapid washing but found, instead, that the bacteria carried exceptionally numerous, uniformly short, sex pili (Fig. 1). In one experiment, where the pili on 75 bacteria were counted, the average number per bacterium was 25.3 and the maximum number 68. In another experiment, the average number was 18.8 and the maximum 41. Control samples washed for twice the time (i.e. for 2 min) with gentle suction, or prepared by a single gentle filtration without preliminary washing, showed an average of less than 2 pili per bacterium with only a very occasional bacterium carrying more than 5. Samples of rapidly washed bacteria transferred to specimen grids directly

Fig. 1. *Escherichia coli* K12 carrying the de-repressed I-like R factor, R538Idrd2, after rapid washing on a membrane filter with warm broth. About 60 short I-like sex pili are seen closely applied to the surface of the bacterium.

from the first filter without the intermediate stage of resuspension in broth also showed increased numbers of pili. Rapid washing with phosphate buffered saline (phosphate, 0.01 M; NaCl, 0.14 M; KCl, 0.03 M; pH 7.3) or with aqueous sucrose solution (10 %, w/v) at 37 °C instead of broth also produced an increase. As with antiserum (Lawn & Meynell, 1972), rapid washing with ice-cold broth had no effect, even when the bacteria were suspended in broth at 37 °C immediately afterwards. After an increase in pilus numbers had been produced by antiserum, there was no further increase on subsequent washing with antiserum-free broth: most pili were strongly antibody-labelled (produced before washing) and only a few were unlabelled (produced during washing). In contrast, prior exposure to non-specific serum did not prevent a subsequent increase upon rapid washing with broth.

The increased numbers of pili after rapid washing or antibody treatment is most simply explained by stimulated extrusion, and this is the only possible explanation if the 1 or 2 pili per bacterium present in ordinary cultures of donor bacteria are stable structures. If, on the other hand, the normal complement of pili is the net result of a dynamic equilibrium between extrusion and loss, then an increase could be due to prevention of loss. Loss in ordinary cultures is unlikely to result from breakage or de-polymerization because rapid washing would have been expected to exaggerate such loss rather than to diminish it. There remains the possibility that retraction normally regulates the number of pili and that specific antiserum or rapid washing both inhibit retraction: if so, the rapid effects of washing imply an extremely high rate of pilus turnover in ordinary cultures. A temporary imbalance between extrusion and retraction cannot explain the results of rapid washing, for the increased numbers of pili produced as a result of the washing persisted for at least 5 min after the bacteria had been resuspended in broth.

In addition to explaining the increased numbers of pili, any satisfactory hypothesis must also explain the uniformly short length of the pili that appeared. If pilus growth is arrested by exhaustion of the supply of pilus protein, the appearance of large numbers of pili of nearly uniform length is more consistent with simultaneous extrusion than with inhibition of retraction, where the pili, having been extruded asynchronously, would consequently be expected to show more variation in length.

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REFERENCES

- BRINTON, C. C. (1971). The properties of sex pili, the viral nature of 'conjugal' genetic transfer systems, and some possible approaches to the control of bacterial drug resistance. *Critical Reviews of Microbiology* **1**, 105-160.
- CURTISS, R., III (1969). Bacterial conjugation. *Annual Review of Microbiology* **23**, 69-136.
- JACOBSEN, A. (1972). Role of F pili in the penetration of bacteriophage ϕ 1. *Journal of Virology* **10**, 835-843.
- LAWN, A. M. & MEYNELL, E. (1970). Serotypes of sex pili. *Journal of Hygiene* **68**, 683-694.
- LAWN, A. M. & MEYNELL, E. (1972). Antibody-stimulated increase in sex pili in R⁺ enterobacteria. *Nature, London* **235**, 441-442.
- MEYNELL, G. G. (1972). *Bacterial Plasmids*. London: Macmillan.
- NOVOTNY, C. P. & FIVES-TAYLOR, P. (1974). Retraction of F pili. *Journal of Bacteriology* **117**, 1306-1311.
- O'CALLAGHAN, R. J., BUNDY, L., BRADLEY, R. & PARANCHYCH, W. (1973). Unusual arsenate poisoning of the F pili of *Escherichia coli*. *Journal of Bacteriology* **115**, 76-81.