Regulatory genes in the thermoregulation of *Escherichia coli* pili gene transcription

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Expression of several different pilus adhesins by *Escherichia coli* is subject to thermoregulation. The surface-located fimbrial structures are present during growth at 37°C but are not produced by cells grown at lower temperatures, such as 25°C. As a step toward understanding the molecular mechanism, we have studied the role of different cistrons of a cloned pilus adhesin gene cluster (*pap*) from a uropathogenic *E. coli* isolate. By promoter cloning, mRNA analysis, and expression of subcloned genes in *trans*, we have identified the *papI* gene as the mediator of thermoregulation at the level of pilus adhesin gene transcription. Expression of the major pilus subunit gene (*papA*) and several other pilus protein cistrons appeared to be dependent on stimulation by the *papB* and *papI* gene products. Constructs carrying different *pap* DNA regions indicated that none of the known Pap proteins acts directly as thermosensor. The chromosomal *rpoH* gene and RpoH σ factor did not appear to be required for *pap* transcription, and the thermoregulation of pilus gene transcription must be different from that of the heat shock regulon. By overexpressing the *papI* gene product from an expression plasmid in *trans*, we could circumvent the temperature regulation and turn on production of pilus adhesin at low temperature. Our results suggest that the level of mRNA encoding the PapI activator is limiting at low growth temperatures and that thermoregulation is due to a determinant in the *papI*–*papB* intercistronic region.

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Microorganisms presumably multiply as rapidly as environmental conditions permit. Studies of enteric bacteria such as *Escherichia coli* and *Salmonella typhimurium* show that they have evolved mechanisms that permit rapid growth under favorable conditions and aid in survival under conditions that are unfavorable for growth (Neidhardt 1987). Changes in the growth conditions may therefore cause more or less complex alterations in the biochemistry and properties of the cells. Regulatory networks such as the heat shock regulon, the SOS regulon, and the cAMP–CRP regulon are examples of how multigene systems may be affected coordinately by changes in environmental conditions. In addition to the networks and regulons that most bacteria seem to possess, one may find that individual isolates can express special properties lacking in the majority of a given species. The ability to adhere to surfaces in the surroundings is an example of such a property, and this kind of interaction between the microorganism and the environment is particularly important and evident in the case of infectious disease (Ofek and Beachey 1980). Adhesion by *E. coli* to human epithelial cells, as exemplified in the case of uropathogenic isolates, is commonly mediated by receptor-specific pilus adhesins (Korhonen et al. 1982; Svenson et al. 1983). The appearance of pili on the bacteria and ex-

pression of adhesive properties correlating to virulence are often temperature dependent, with optimum at 37°C and reduced at lower temperatures (De Graaf et al. 1980; Göransson and Uhlin 1984). Other conditional factors, e.g., growth substrate, may also influence the expression of pilus adhesins, and it appears that regulatory mechanisms have evolved to allow for successful colonization of a given niche under appropriate environmental conditions (Silverman et al. 1984).

Analysis of the molecular mechanism(s) involved in thermoregulation of adhesion should increase our knowledge about how external stimuli are transmitted to the level of gene expression. Molecular genetic studies of several determinants of pilus adhesins in *E. coli* have shown that there are multicistronic gene clusters encoding the different major and minor pilus proteins and the biogenesis of these surface organelles (for reviews, see Mooi and de Graaf 1985; Uhlin et al. 1985b; Normark et al. 1986). Using gene operon fusions to *lacZ*, we obtained evidence suggesting that the temperature regulation of digalactoside-binding pili (Pap pili) expression operates at the level of transcription of at least the major pilin gene, *papA* (Göransson and Uhlin 1984). Subsequent analysis established that there are two cistrons in the region upstream of *papA* (*papB* and *papI*), that *papA* is cotranscribed with *papB*, and that *papB* and *papI* seem to be involved in regulation of *papA* expression (Båda et al. 1985, 1988; see also Fig. 1). We

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have studied the role of different *pap* cistrons in the temperature regulation of pilus production and expression of the adhesion properties.

Results

Thermoregulation of Pap pilus adhesin is mediated by the papI–papB region

In addition to the major pilin subunit gene, *papA*, the formation of pilus adhesin involves several accessory genes and minor pilus protein genes (i.e., *papC–papH*, Fig. 1). Most distal from *papA* is the *papG* gene, and it has been shown to encode the pilus protein, which is responsible for the binding specificity of the Pap adhesin (Lund et al. 1987). The *papA* gene is part of an operon starting with *papB*, and although most transcripts seem to terminate between *papA* and *papH*, there is evidence that some transcription continues through the *papH–*

papC region and perhaps even farther (Båga et al. 1985, 1987, 1988; Uhlin et al. 1985a). Therefore we asked whether or not expression of all genes required for biogenesis of pili and adhesin was dependent on the regulatory genes and sequences located upstream of *papA*. One way to test this was to determine whether pilus production and expression of adhesin could be made constitutive at both high and low temperatures if the region upstream of *papA* were replaced by DNA sequences encoding a temperature-independent promoter region. Our studies with the *E. coli alaS* gene promoter suggested that it would be suitable, considering the relative strength and apparent temperature independence of that promoter upon cloning into operon fusion vectors (Sjöberg et al. 1986, and unpubl.; see also Table 2). The plasmid pHMG93 contains all of the *pap* genes required for production of pili and adhesin, whereas the region including *papI* and part of the *papB* gene has been replaced by DNA encoding the 5' end and the promoter of

Figure 1. Genetic and physical maps of plasmids: (A) Constructs expressing Pap pilus–adhesin genes or *lacZ* operon fusions; (B) PapB and PapI overproducing plasmids are shown below a map of the *papI–papB–papA* region. The parts representing cloning vector DNA are indicated by interrupted lines and are not drawn to scale. The filled parts of the bars show the coding regions of the indicated *pap* genes. Hatched portions show that only a part of a gene is remaining. Vertical arrowheads indicate cleavage sites for different restriction endonucleases: (E) *EcoRI*; (H) *HindIII*; (B) *BamHI*; (S) *SphI*; (T) *TaqI*; (A) *ApaI*; (V) *EcoRV*. Horizontal arrows indicate direction of transcription from promoters, as discussed in the text. (Δ) The insertion mutation in *papB*, which constitutes the difference between pHMG15 and the wild-type derivative pHMG1.

Figure 2. Electron microscopic analysis of pilus expression: (A) HB101/pPAP5 at 37°C; (B) HB101/pPAP5 at 26°C; (C) HB101/pHMG93 at 37°C; (D) HB101/pHMG93 at 26°C; (E) HB101/pBR322 at 37°C; (F) HB101/pBR322 at 26°C.

the *alaS* gene. *E. coli* strain HB101 harboring the different plasmids was grown at 37°C and at 26°C. The bacteria were then analyzed for production of pili by electron microscopy (Fig. 2) and for expression of hemagglutinating ability. As summarized in Table 1, the cloned wild-type *pap* genes (pPAP5) were temperature dependent in their expression, but the *alaS* promoter substitution (pHMG93) resulted in constitutive expression. The strain HB101/pHMG93 showed very similar numbers of pili per cell at the two temperatures, whereas no pili were seen on the surface of HB101/pPAP5 cells (only occasional flagella were observed) after growth at 26°C (Fig. 2). The fact that expression of hemagglutination by HB101/pHMG93 was also independent of growth temperature indicated that all the thermoregulatory effects on the *pap* genes were mediated by genes and/or sequences in the *papI*–*papB* region.

To determine whether or not the gene products of *papB* or *papI* were the direct cause of thermoregulation (e.g., as a thermolabile protein), we analyzed the expression of β -galactosidase from *papA*–*lacZ* constructs (Fig. 1) with an intact or mutated *papB* gene (pHMG1, pHMG15) and from *papB*–*lacZ* operon fusions with intact or deleted *papI* genes (pPAP226, pPAP218). When tested at different temperatures (25–37°C), the level of β -galactosidase expression was reduced, in all cases, at the lower temperature. Absence of either of the gene products from *papB* or *papI* did not seem to abolish thermoregulation of *papB*–*papA* transcription, although the steady-state level at 37°C was reduced by 10-fold or

Table 1. Temperature-dependent and constitutive expression of *Pap* pilus adhesin

Strain	Pilus phenotype ^a		Adhesin phenotype ^b	
	37°C	26°C	37°C	26°C
HB101/pPAP5	+	–	+	–
HB101/pHMG93	+	+	+	+
HB101/pBR322	–	–	–	–
J96	+	–	+	–
J96/pHMG94	+	+	+	+

^a Examined by electron microscopy.

^b Mannose-resistant hemagglutination assay (MRHA).

more (M. Göransson, K. Forsman, B.E. Uhlin, unpubl.). Temperature-shift experiments (from 25–37°C) with bacteria carrying the different fusion plasmids also showed that the kinetics of increase in β -galactosidase expression was similar (Fig. 3). These results suggested that the gene products from *papB* and *papI* by themselves were not the cause of the temperature effect.

The *rpoH* (*htpR*) gene product of *E. coli* has been shown to activate transcription of a set of genes (the heat shock regulon) upon shifts from low to high growth temperatures while acting as an alternative σ -factor in the

Figure 3. Expression of β -galactosidase by *papA*–*lacZ* and *papB*–*lacZ* operon fusions during a temperature upshift. *E. coli* K-12 strain MC1029 carrying the different plasmids was grown in L broth and shifted from 25°C to 37°C, as indicated. Samples for measurements of β -galactosidase specific activity were taken at the indicated times.

RNA polymerase complex (Grossman et al. 1984; Landick et al. 1984; Yura et al. 1984). To test directly for the possible involvement of the *rpoH* gene product in the thermoregulation of *pap* gene transcription, we introduced *papA-lacZ* and *papB-lacZ* operon fusion plasmids into the *rpoH* mutant *E. coli* strain K165 and its *rpoH*⁺ parental strain SC122 (Cooper and Ruettinger 1975; Neidhardt and VanBogelen 1981). Although the strain K165 loses viability at 37°C, we were able to monitor the expression of β -galactosidase from the fusion constructs during the initial cell doubling after a shift from 30–37°C. The results from such an experiment with the *papB-lacZ* fusion plasmid pPAP218 are shown in Figure 4. Similar results were obtained with *papA-lacZ* fusions (M. Göransson, K. Forsman, B.E. Uhlin, unpubl.). As observed with the *rpoH*⁺ strain MC1029 (Fig. 3), there was a gradual increase in β -galactosidase expression after shifts of cultures of the *rpoH* mutant and wild-type derivatives of strain SC122. The results, therefore, did not provide any evidence that the heat shock σ -factor would be directly involved in thermoregulation of *pap* transcription.

Figure 4. Effect of an *rpoH* mutation on the *papB-lacZ* expression by plasmid pPAP218 during a temperature shift from 30°C to 37°C. (A) Growth was monitored by optical density measurements. (B) β -Galactosidase expression. Open symbols represent strain SC122/pPAP218; closed symbols show strain K165/pPAP218.

Temperature-dependent transcription of the *papI* and *papB* operons

Our earlier studies indicated that the gene products of *papB* and *papI* act at the transcriptional level of expression of the pilin genes (Baåga et al. 1985, 1988; Göransson et al. 1988). Therefore, it seemed possible that the cellular level of one of these proteins might be temperature dependent and mediated the effect on *papB-papA* transcription. To confirm the *papB-papA-lacZ* operon fusion data and to determine whether or not the *papI* gene would be thermoregulated at the transcriptional level, we extracted RNA and performed Northern blot analysis after growth of strains HB101/pPAP5 and J96 at 37°C and 26°C. The RNA blots were probed with a *papI*-specific probe and with a *papA* probe. The *papA* probe revealed both the 1300-nucleotide-long *papB-papA*-specific transcript and the 800-nucleotide-long *papA*-specific processed mRNA product (Baåga et al. 1988). The RNA was also analyzed with a *bla* gene probe to utilize the β -lactamase gene of the vector plasmid as an internal control. The alteration in growth temperature appeared to have little effect on plasmid copy number, as revealed by the relative amounts of *bla*-specific mRNA produced (Fig. 5B). The *bla* gene in the vector pBR322 is transcribed from two different promoters, and there are three major terminators (von Gabain et al. 1983). One of the promoters (in the *tet* gene promoter region) was removed in the construction of pPAP5, consequently, there is less transcription of the *bla* gene in this plasmid. The steady-state levels of RNA-BA and RNA-A transcripts were very much reduced at 26°C in both the original *E. coli* J96 strain (Fig. 5A, lanes 2 and 3) and in strain HB101 carrying the cloned *pap* determinant (Fig. 5A, lanes 6 and 7). In addition to the temperature regulation of the *papB-papA* operon, the results also established that *papI* transcription was thermoregulated. As shown in Figure 5C, the amount of *papI* mRNA was reduced at 26°C in a manner similar to that of the *papB-papA* transcripts. These results made it likely that the PapB and/or PapI proteins might be a limiting factor in transcription of the pilus adhesin operon at lower temperatures.

To test whether an increased cellular level of the gene products could possibly activate *pap* gene expression at lower temperatures, we constructed *papB*⁺ and *papI*⁺ expression plasmids, in which the genes were put under the transcription of another promoter (Fig. 1). The plasmids pHMG79 and pHMG94 express the PapB and PapI proteins at high levels under the control of the *lac* promoter [*lacP(UV5)*], and we introduced these plasmids into the strain carrying the *papA-lacZ* operon fusion pHMG1 and measured the effect on *papA* expression at 37°C and 26°C (Table 2). In the case of the *papI* expression plasmid, pHMG94, there was a clear stimulation of *papA* expression at both temperatures. The β -galactosidase level was even somewhat higher at 26°C than at 37°C. These results suggested that high-level expression of *papI* could lead to temperature-independent expression. In contrast, the presence of pHMG79 led to only a

Figure 5. Northern blot hybridization analysis of *papB* and *papI* transcripts after growth at 26°C and 37°C. (A–C) Results after probing the RNA blots with *papA*-, *bla*-, and *papI*-specific probes, respectively. (Lane 1) Labeled DNA fragments of *Hind*III-digested phage λ DNA as molecular size markers. The RNA samples were J96 at 26°C (lane 2); J96 at 37°C (lane 3); HB101/pBR322 at 26°C (lane 4); HB101/pBR322 at 37°C (lane 5); HB101/pPAP5 at 26°C (lane 6); HB101/pPAP5 at 37°C (lane 7). Strain J96 was grown in L broth, and the HB101 derivatives were grown in the casamino acids medium. (A,B) Results were obtained by probing the same blotted filter (Hybond-N) sequentially with a washing step in between, according to the procedure described by the manufacturer (Amersham International). The total amount of RNA on the gel used for the blot in A and B was 25 μ g in lanes 2 and 3 and 10 μ g in lanes 4–7. (C) Results were obtained with a separate blot, and all lanes of the gel contained 50 μ g RNA samples.

slightly increased expression of β -galactosidase from pHMG1 at 26°C; there was an apparent decrease at 37°C, and the level of β -galactosidase activity at both temperatures was only ~20–25% of the *papA-lacZ* expression from the pHMG1 control strain at 37°C (Table 2).

The *papI* expression plasmid pHMG94 was also introduced into the *E. coli* isolate (strain J96) from which the *pap* DNA was cloned, and analysis of the phenotype showed that PapI overproduction resulted in temperature-independent expression of the native, chromosomal

Table 2. Effect of overproduction of *PapB* and *PapI* on *papA-lacZ* expression

Fusion plasmid	Coresiding plasmid	β -Galactosidase sp. act. (units)	
		37°C	26°C
pHMG1 (<i>papI</i> ⁺ , <i>papB</i> ⁺ , <i>papA-lacZ</i>)	pACYC184(vector)	293	12
pHMG1	pHMG79[<i>lacP(UV5)-papB</i> ⁺]	54	74
pHMG1	pHMG94[<i>lacP(UV5)-papI</i> ⁺]	390	490
pHMG61 (<i>alaS-lacZ</i>)	—	259	253

ally located pilus adhesin genes (Table 1). Both pilus production and adhesion were fully expressed at 26°C. Evidently, the biogenesis of functional pilus adhesins can occur at the lower growth temperature, and there did not appear to be any limitation at the level of translation or surface localization and interaction of the subunits. Taken together, the results suggested that the transcription of the gene for the activator PapI would be limiting in the thermoregulated wild-type situation.

Discussion

The expression of adhesive properties by bacteria appears to be regulated to fit the environmental circumstances. We have shown that the temperature-dependent expression of bacterial adhesion by digalactoside-binding *E. coli* is due to regulation at the transcriptional level. The polycistronic *papB* operon and the monocistronic *papI* operon are divergently oriented, and transcription of both was essentially turned off at 26°C, in comparison with 37°C (Fig. 5). The intact *papB* and *papI* genes were needed for high-level transcription of pilus adhesin genes (i.e., from the *papB* promoter) at 37°C. The PapB protein was suggested recently to act as autoregulator of its own transcriptional unit, because overproduction in *trans* appeared to reduce expression of the *papB* operon, and in vitro studies showed that the protein may bind to DNA sequences overlapping the *papB* promoter (K. Forsman, M. Göransson, and B.E. Uhlin, in prep.; see also Table 2). Depending on the cellular concentration, PapB would thereby have a dual role in activating and repressing transcription. The role of the *papI* gene has been less clear, but the present results showed that the PapI protein functioned as an activator of the *papB* operon. Furthermore, PapI appeared to be the limiting factor that became critical for pilus adhesin expression at low growth temperatures.

The best understood case of temperature regulation of bacterial gene expression is probably the heat shock regulon (Neidhardt 1987). The results of our experiments with an *rpoH* mutant strain strongly suggested that the temperature regulation of *pap* transcription is a novel mechanism different from the heat shock regulon. The RpoH σ -factor was not required, and in contrast to the

transient induction of heat shock genes, the temperature-induced transcription of *pap* genes, upon a temperature upshift, resulted in a high steady-state level expression of pilus adhesins. It is interesting to note, however, that the PapI protein may play similar role as the *rpoH* gene product in the sense that it functions as a temperature-regulated activator of some specific promoter(s). The transcription of the *rpoH* gene itself is temperature dependent, and the increased expression of the RpoH protein at higher temperatures accounts for the induction of mRNA synthesis from different genes of the heat shock regulon (Tilly et al. 1986; Erickson et al. 1987; Straus et al. 1987). The RpoH σ -factor thereby functions as an acceptor of the sensory information that is forwarded, by some unknown mechanism, in the cell upon a sudden change in temperature (Straus et al. 1987).

Whether or not PapI may interact with RNA polymerase, with the DNA (or elsewhere), is not known at present, and we can only hypothesize about how *papI* gene transcription is regulated. In the case of *rpoH*, it was proposed that the induction occurs by a post-transcriptional derepression, i.e., by regulation of translation (Erickson et al. 1987). Furthermore, there appeared to be a transient increase in the stability of the protein upon heat shock (Straus et al. 1987). It has not been ruled out that the PapI activation could be affected by similar mechanisms. However, we find it more likely that the *papI* gene is regulated at the transcriptional level because we could increase its expression efficiently at low temperature by simply substituting its promoter. There was a low-level *papB-lacZ* expression that showed temperature-dependent characteristics (e.g., kinetics during upshift similar to the wild-type case) even in a *papI*-deficient construct, pPAP218 (Fig. 3). This observation suggested that the intercistronic region between *papI* and *papB* in itself may have temperature-dependent properties. Furthermore, the Northern blot analysis established that the two transcriptional units from this region were temperature regulated to a similar extent (Fig. 5).

Pili-adhesin gene systems such as *pap*, which are associated with virulence of *E. coli*, represent additional genetic information not present in the genome of most (normal) intestinal *E. coli* populations. Often, a given clinical isolate of, e.g., uropathogenic *E. coli*, may carry several pilus adhesin determinants, and recent studies suggest that the DNA sequences corresponding to the *papI-papB* region could be more conserved than the other regions (e.g., pilus subunit genes) among different adhesin determinants (Göransson et al. 1988).

Complementation tests with *papB* and *papI* mutants showed that the corresponding regulatory gene products of other gene systems can act in *trans* on Pap pili-adhesin expression. The PapI and PapB proteins may allow for regulatory interaction and coordination of expression of separate adhesin determinants according to the environmental circumstances. The role of PapI, as elucidated in this paper, would therefore include temperature regulation of several transcriptional units in such strains, and one may consider the multiple pilus adhesins to constitute a regulator network or regulon.

Materials and methods

Bacterial strains and plasmids, media, and growth conditions

The *E. coli* strains HB101 (Boyer and Roulland-Dussoix 1969) and MC1029 (Casadaban and Cohen 1980) were used as hosts of plasmids in most experiments. The uropathogenic *E. coli* isolate J96 was the strain from which the Pap pili-adhesin determinant was cloned initially (Hull et al. 1981). The strains K165 and SC122 (Cooper and Ruettinger 1975; Neidhardt and VanBogelen 1981) were utilized in tests of the possible influence by the *rpoH* gene.

To obtain compatible combinations of plasmids in some experiments, the plasmid constructions were based on derivatives of either of the two cloning vectors, pBR322 (Bolivar et al. 1977) and pACYC184 (Chang and Cohen 1978). The *pap-lacZ* operon fusion plasmids pHMG1, pHMG15, pPAP218, and pPAP226 are all based on the vector pRZ5202 (Reznikoff and McClure 1986), and the constructions were reported previously (Göransson and Uhlin 1984; Båga et al. 1985). Cloning of a 1.4-kb *EcoRI-BamHI* fragment carrying the *alaS* gene promoter (Putney et al. 1981) into *EcoRI-BamHI*-digested pRZ5202 gave plasmid pHMG61. The plasmid pPAP5 contains all of the *pap* genes within the *EcoRI-BamHI* fragment originating from *E. coli* strain J96. In pHMG93 the *papI-papB* control region of pPAP5 has been replaced by cloning of the *alaS EcoRI-SalI* promoter fragment from pHMG61 into *EcoRI-XhoI*-digested pHMG76 (Båga et al. 1988). The PapI expression plasmid pHMG94 was constructed in three steps. First a 275-bp *papI*-encoding *SphI-TaqI* fragment was cloned into *AccI-SphI*-digested pUC19 (Yanisch-Perron et al. 1985) to give plasmid pHMG96. Next, the *BamHI-SphI papI* fragment from pHMG96 was inserted behind the *lacP(UV5)* promoter in the *BamHI-SphI*-restricted expression vector pJJS300 (Sninsky et al. 1981). The resulting plasmid, pHMG95, was then digested with *EcoRI-HincII*, and the *lacP(UV5)-papI+* fragment was cloned into *EcoRI-PvuII*-restricted pACYC184 to give plasmid pHMG94. The PapB expression plasmid pHMG79 was constructed from pHMG72 (Båga et al. 1985). pHMG72 was made blunt-ended at its *Apal* site with T4 DNA polymerase and was then digested with *EcoRI*. The *papB* promoter fragment was then replaced with an *EcoRI-BamHI lacP(UV5)* promoter fragment (blunt-ended at its *BamHI* end) derived from plasmid pJJS300. That plasmids pHMG79 and pHMG94 mediated high-level expression of the PapB and PapI proteins, respectively, was confirmed by gel electrophoretic analysis of protein extracts of labeled minicell derivatives (data not shown).

Bacteria were routinely grown in L-broth or medium E with 1.5% casamino acids, and solid media contained 1.5% agar, as described earlier (Göransson and Uhlin 1984; Båga et al. 1985; Göransson et al. 1988).

Recombinant DNA methods

Plasmid constructions, transformation, DNA preparation, and analysis by agarose gel electrophoresis were carried out according to standard procedures (Maniatis et al. 1982). Restriction endonuclease digestions and DNA ligation reactions were carried out under the conditions recommended by the enzyme manufacturers (Boehringer-Mannheim GmbH and New England Biolabs).

Determination of β -galactosidase activity

The specific activity of β -galactosidase was assayed according to Miller (1972). Units were defined according to the following formula:

$$\text{Units} = 1000 \times \frac{\text{OD}_{420} - 1.75 \times \text{OD}_{550}}{t \times v \times \text{OD}_{600}}$$

Where OD₄₂₀ and OD₅₅₀ are read from the reaction mixture, OD₆₀₀ reflects the cell density just before assay, *t* is the time of the reaction in minutes, *v* is the volume of culture used in the assay (in milliliters).

Electron microscopy

Bacterial suspensions in buffer [10 mM Tris-HCl (pH 7.5); 10 mM magnesium chloride] were allowed to sediment on copper grids coated with thin films of 2% Formvar. After negative staining with 1% sodium silicotungstate (pH 6.0), the grids were examined in a JEOL 1003 microscope.

Hemagglutination assay

Tests of MRHA with human erythrocytes on glass slides were performed as described previously [Norgren et al. 1984].

Northern blot analysis of mRNA

Procedures for extraction of bacterial RNA and analysis by Northern blot hybridization were essentially as described previously [Båga et al. 1985, 1988]. The buffer used was 20 mM sodium HEPES, 1 mM EDTA, and 5 mM sodium acetate (pH 7.0). The RNA was blotted onto a nylon filter (Hybond-N, Amersham International) and was cross-linked to the filter by irradiating with a UV-light illuminator. After hybridization, the filter was washed four times for 5 min at room temperature in 2 × SSC (1 × SSC contained 150 mM NaCl, 15 mM sodium citrate), 0.5% SDS and, subsequently, during three 20-min washes at 50°C in 0.1 × SSC, 0.5% SDS. The M13 phage clone used as *papA*-specific probe was labeled using a probe primer, as described previously [Båga et al. 1985]. Purified DNA fragments employed as *papI*- and *bla*-specific probes were labeled with a multiprimer DNA labeling system (Amersham International). The *papI* probe was a 275-bp *SphI*-*TaqI* fragment covering the gene. A 692-bp *DraI* fragment from pBR322 was used as a *bla*-specific probe.

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