Function and Expression of an N-Acetylneuraminic Acid-Inducible Outer Membrane Channel in Escherichia coli

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The Escherichia coli yjhA (renamed nanC) gene encodes a protein of the KdgM family of outer membrane-specific channels. It is transcribed divergently from fimB, a gene involved in the site-specific inversion of the region controlling transcription of the fimbrial structural genes but is separated from it by one of the largest intergenic regions in E. coli. We show that nanC expression is induced by N-acetylneuraminic acid and modulated by N-acetylglucosamine. This regulation occurs via the NanR and NagC regulators, which also control fimB expression. nanC expression is also activated by the regulators cyclic AMP-catabolite activator protein, OmpR, and CpxR. When the NanC protein was reconstituted into liposomes, it formed channels with a conductance of 450 pS at positive potential and 300 to 400 pS at negative potential in 800 mM KCl. The channels had a weak anionic selectivity. In an ompR background, where the general porins OmpF and OmpC are absent, NanC is required for growth of E. coli on N-acetylneuraminic acid as the sole carbon source. All these results suggest that NanC is an N-acetylneuraminic acid outer membrane channel protein.

Porins are proteins that form water-filled channels across the outer membrane of gram-negative bacteria. These channels allow the diffusion of hydrophilic molecules (≤600 Da for the general porins OmpF and OmpC) into the periplasm with no particular substrate specificity. In Escherichia coli this family of proteins is represented by the trimeric porins OmpC, OmpF, and PhoE. Elucidation of the structure of these proteins showed that each monomeric barrel is formed by 16β-strands (5). Other outer membrane channels catalyze the specific diffusion of a particular class of nutrient and thus allow the uptake of compounds that would diffuse too slowly through the general porins (15).

The E. coli maltose-specific channel protein LamB and the sucrose channel protein ScrY are the best studied of this class of channel proteins. These proteins also form trimers, and each monomer consists of 18 transmembrane β-strands (24). A few other specific channels are known in E. coli: the aryl-β-D-glucoside channel protein BglH, the nucleoside transporter Tsx, and the long-chain fatty acid channel protein FadL (30, 13, 2, 28).

Recently a new class of specific channel proteins has been described in Erwinia chrysantheni, the oligogalacturonate-specific channel protein KdgM (3). This small protein (216 amino acids) seems to be monomeric and forms a channel of 14 transmembrane β-strands (18). Proteins of the KdgM family are found in other gram-negative bacteria, including Erwinia carotovora, Yersinia pestis, Salmonella enterica serovar Typhimurium, Escherichia coli, Klebsiella pneumoniae, Vibrio harlottii, and Pseudomonas species. A comprehensive genomics analysis has shown that most of the KdgM orthologue-encoding genes are clustered with genes involved in pectin degradation and that a binding site for KdgR, the regulator of pectin degradation pathways, can be detected in their upstream regulatory regions (22). Thus, most of these KdgM homologues could be involved in the transport of oligogalacturonates. The V. harlottii gene is clustered with alginate lyase genes, and the channel could transport alginate degradation products. Two KdgM homologues exist in E. coli, YjhA and YshA. The genes yjhA and yshA are not controlled by KdgR, and the chromosomal location of these genes does not allow prediction of their substrates. The channel function of YshA (OmpL) has been proven, but its substrate was not characterized (6).

yjhA is the first gene of the presumed yjhATS operon. The function of YjhT and YjhS is not known, and they have no homology with proteins of known function. This operon is transcribed divergently from fimB, a gene whose product catalyzes the site-specific inversion of the region controlling transcription of the fimbrial structure genes (fimA to fimH) (7). The intergenic yjhA-fimB region is exceptionally large for E. coli (1.4 kb). Moreover, elements that regulate fimB and which could potentially regulate yjhA have been identified near the center of this region and include a NanR binding site. NanR is a regulator of N-acetylneuraminic acid (Neu5Ac or sialic acid) metabolism in E. coli (10). Sialic acids are a family of more than 40 nine-carbon monosaccharides present mostly in eukaryotes, where they play roles in cell-cell and cell-molecule interactions (29).

E. coli is able to use Neu5Ac as sole carbon source for growth. Catabolism of Neu5Ac requires the products of the nanATEK operon, yielding pyruvate and N-acetylgalactosamine-6-phosphate (GlcNAc-6-P). GlcNAc-6-P is further metabolized to fructose-6-phosphate by NagA and NagB, two enzymes of the N-acetylgalactosamine (GlcNAc) degradation pathway, whose synthesis is controlled by the nagC-encoded repressor (10, 20). Neu5Ac regulates transcription of fimB, and it has been suggested that it could also control the expression of the
differently expressed yjhA TS operon (7, 29). We show in this work that yjhA is indeed regulated by NanR and several other regulators and that it encodes a Neu5Ac-specific channel.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and growth conditions.** Bacterial strains were grown in Luria-Bertani (LB) or M63 medium (14). M63 medium deprived of an ammonia source was used to test the effect of ammonia concentration, and M63 medium diluted twofold with various sucrose concentrations was used to test the effect of osmolality. P1 transduction were carried out with P1vir (14). Strain NM522 was used for cloning experiments. Other *E. coli* strains used are 11814 (MG1655 Δacr) (A. Vianney), IBPC356 (thiA1 leuB6 histG4 argR3 Δ[ter-lac]Y2 thi-1 galK2 ara-14 ysl-5 m1-1 tss-33 kdxK1 psl-1 supF44 recB21 recC22 sibB15 sibC201 nagC::tcr) (19), TK281 (MC4100 ompr::Tn10) (8), MV679 (MG1655 ΔacrΔZYA ΔwamR) (I. Blomfield), PHP1242 (MG1655 ΔacrR) (G. Jubelin, pop4129 (chl::Tn10 Δrpl) (J.-C. Lazzaroni), and IBPC1016 (JM101 mle::tc nanR) (20).

**Molecular biology techniques.** Plasmid DNA preparation, DNA restriction and electrophoresis, and bacterial transformation and electrophoration were all performed according to Sambrook et al. (23).

**S1 nuclease protection and DNase footprint analysis.** Total RNA was purified by the hot phenol method, and S1 nuclease transcript mapping was carried out as previously described (10). The probes used were the PCR fragments Fim3-Fim2 and Fim1-Fim4, labeled at the Fim3 and Fim1 oligonucleotides prior to the PCR. *Fim3* (5'-ACGTACGTCCAGTGTCGCAG) corresponds to amino acids 29 to 28 of the yjhA open reading frame. The relative positions of Fim1 (5'-CTACCGATGAGATCATCGG), Fim2 (5'-AACATCAACAGGCCCTCC), and Fim3 (5'-AGACCCGCAAGTGTTGCG) are indicated on Fig. 2A. DNase I footprint analysis was carried out as previously described with the Fim1-Fim2 PCR fragment as the probe labeled at the Fim1 site. NagC with a C-terminal His6 tag was the gift of Charles Bell and Mitchell Lewis, and chloroamphenicol (CAP) was the gift of Annie Koh.

**YjhA expression and purification.** Production of porins in inclusion bodies followed by a renaturation step is now a well-established method to obtain active proteins (25, 3). To produce YjhA without its signal peptide, a DNA fragment followed by a renaturation step is now a well-established method to obtain active proteins (25, 3). To produce YjhA without its signal peptide, a DNA fragment followed by a renaturation step is now a well-established method to obtain active proteins (25, 3). To produce YjhA without its signal peptide, a DNA fragment followed by a renaturation step is now a well-established method to obtain active proteins (25, 3). To produce YjhA without its signal peptide, a DNA fragment followed by a renaturation step is now a well-established method to obtain active proteins (25, 3).

**Construction of a yjhA-lacZ fusion strain.** Primers yjh-3 (5'-GGTTCCTAGC GATTATCTTCG) and yjh-4 (5'-GCCATTTGCGATAATCCCGG) were used to amplify a DNA fragment extending 1 kb upstream and downstream of the yjhA coding sequence. The amplified fragment was cloned into plasmid pGEM-T (Promega, Madison, Wis.), and a lacZ-kanamycin resistance cassette was inserted into the unique BclI site of yjhA located at nucleotide 125. This construct was introduced into plasmid pKO3 and recombined into the *E. coli* chromosome according to the method described by Link et al. (12).

**Reconstitution of yjhA in liposomes.** The purified protein YjhA (500 ng) was added to 2 ml of buffer (500 mM KCl, 10 mM HEPES-KOH, pH 7.4, 33 mM octylglucoside) containing 1 mg of sonicated lipids (asolectin from soybean, type IV-S). Detergent was removed with SM-2 Bio Beads (Bio-Rad, Hercules, Calif.) as previously described (3), and the proteoliposomes were recovered by ultra-centrifugation. For planar bilayer experiments, the proteoliposomes were resuspended in 0.1 ml of 500 mM KCl–10 mM HEPES-KOH, pH 7.4. To obtain giant proteoliposomes amenable to patch-clamp recording, the proteoliposomes were subjected to a dehydration-rehydration cycle as previously described (1). Rehydration was performed in 100 mM KCl–10 mM HEPES-KOH, pH 7.4.

**Electrophysiological recording.** Bilayers were formed from a solution of asolectin lipids dissolved in n-decane (30 mg/ml) across a 250-μm-diameter hole. The purified protein YjhA (500 ng) was added to 2 ml of buffer (500 mM KCl, 10 mM HEPES-KOH, pH 7.4, 33 mM octylglucoside) containing 1 mg of sonicated lipids (asolectin from soybean, type IV-S). Detergent was removed with SM-2 Bio Beads (Bio-Rad, Hercules, Calif.) as previously described (3), and the proteoliposomes were recovered by ultra-centrifugation. For planar bilayer experiments, the proteoliposomes were resuspended in 0.1 ml of 500 mM KCl–10 mM HEPES-KOH, pH 7.4. To obtain giant proteoliposomes amenable to patch-clamp recording, the proteoliposomes were subjected to a dehydration-rehydration cycle as previously described (1). Rehydration was performed in 100 mM KCl–10 mM HEPES-KOH, pH 7.4.

**Results**

**Regulation of yjhA expression.** *fimB* expression is regulated by Neu5Ac through the binding of the NanR regulator at a specific site in the *fimB*-yjhA intergenic region (Fig. 1A) (7). To verify whether NanR could also control yjhA expression, we measured expression of a yjhA-lacZ fusion in the absence and presence of Neu5Ac. In the absence of inducer, the expression of the fusion was very low and it was induced 20-fold by Neu5Ac (Table 1). When the fusion was introduced into a nanR background, its expression was identical to that in the Neu5Ac-induced strain and it was not further inducible by Neu5Ac, confirming that induction of yjhA by Neu5Ac occurs through NanR.

Since *yjhA* is regulated by nanR, we renamed it nanC for *nanR*-regulated channel. Neu5Ac metabolism converges with that of GlcNAc. We tested the effect of GlcNAc and of a mutation in the GlcNAc metabolic pathway regulator, *nanC*, on nanC expression. In the wild-type background glucose and GlcNAc produced a 10-fold decrease in nanC expression which could be due to catabolite repression. However, in the nanR background only glucose or introduction of a *crp* mutation produced a strong decrease in expression, while the introduction of a *nanC* mutation produced a 50% increase in expression (Table 1), suggesting that GlcNAc and NagC have additional functions in nanC expression (see below).

Expression of porins and channels is often regulated in response to variations in environmental conditions (15). Neu5Ac and GlcNAc can be used by the bacteria as sole ammonia sources. However, ammonia deprivation did not induce expression of *nanC* (data not shown). Similarly, *nanC* expression was not modified by oxygen limitation. Expression of the general porins OmpC and OmpF is regulated by osmolarity via the two-component regulatory system EnvZ-OmpR (8). Expression of *nanC* was reduced 2.5-fold in an *ompR* background (Table 1). *nanC* expression was predicted to be regulated by the two-component regulatory system CpxR-CpxA by transcriptome analysis (16). Indeed, *nanC* expression was reduced twofold in a *cpxR* background. The effect of the cpxR and *ompR* mutations was cumulative (Table 1).

**NagC and CAP binding to the nanC promoter.** The transcriptional start for *nanC* was mapped on mRNA prepared from a
wild-type and nanR mutant (JM101 and IBPC 1016) strain during growth on glycerol, glucose, or sialic acid. A single start site was located 428 bp upstream of the nanC open reading frame using a probe starting within the nanC open reading frame. This transcript was mapped precisely using a shorter probe (Fim 1-Fim 4, Fig. 1B). The transcript was detected in the wild-type strain grown on sialic acid or in the nanR mutant in all three media, although it was low in RNA from the strain grown on glucose, confirming the strong catabolite repression. The start site corresponds to a TATAAC sequence and lies within the predicted NanR binding site containing two repeats of the GGTATA hexamer (10). NanR binds to this sequence (27). It can be noted that the nanC promoter is located in exactly the same position relative to the NanR binding site as the nanA promoter is within the NanR operator. There is no obvious −35 promoter sequence, but a potential CAP site is detectable on the sequence (Fig. 1A), centered at position −61.5, which is consistent with the strong catabolite repression observed with the nanC fusion. A NagC binding site is centered 65 nucleotides upstream of the transcription start site, so that it overlaps the CAP site.

DNase I footprinting confirmed that both proteins bind to the same region (Fig. 2). Although the CAP site does not show very high affinity, when both proteins were added simultaneously, CAP (50 nM) seems to bind preferentially, as shown by the formation of the classic pattern of three hypersensitive
The intracellular formation of GlcNAc releases NagC from its site and in consequence allows the binding of CAP to its overlapping site. Thus, in the absence of NagC binding, even in the presence of the lowered cyclic AMP (cAMP)/CAP concentration produced by growth on GlcNAc or glucose, cAMP/CAP can bind and activate nanC expression. In support of this interpretation, in the nanR strain, expression was low on glucose but high on GlcNAc, whereas in the nanR nagC strain it was high on both sugars.

Sialic acid is degraded via GlcNAc-6-P and so it should produce inducing signals for both regulators. This dual regulation by Neu5Ac and GlcNAc means that it is probably only when the flux through the combined nan-nag pathways is high enough to generate both inducing signals (a condition seen when both compounds are present in the growth medium) that the operon is fully induced. However, when both Neu5Ac and GlcNAc were added simultaneously to the growth medium, the level of nanC expression was lower than when Neu5Ac alone was added (Table 1), probably because of the catabolite repression provoked by addition of a high concentration of GlcNAc.

**Physiological role of NanC.** The induction of nanC expression by Neu5Ac and GlcNAc let us suppose that it could be a channel allowing entry of these compounds into the bacteria. To test this hypothesis, growth of a nanR strain, a nanR ompR strain, and its nanC derivative was tested with these compounds as the sole carbon source. The presence of the nanR mutation prevents induction problems that could arise if the substrates cannot efficiently induce their transport systems, and the ompR mutation prevents expression of the general porins OmpC and OmpF through which these compounds could enter. While no difference was observed for growth with glucosamine, glucose (used as a control, data not shown), or GlcNAc as the sole carbon source for the three strains (Fig. 3A), the nanC mutant was unable to grow with Neu5Ac (Fig. 3B). In contrast, a nanR nanC mutant (which is OmpF+ OmpC+) could grow with this substrate (data not shown). This indicates that, in the absence of the porins OmpF and OmpC, the channel formed by nanC is the only way for Neu5Ac to enter the bacteria. Glucose and GlcNAc probably enter by other weakly expressed general porins.

**Porin activity of NanC.** Purified NanC proteins were reconstituted into liposomes. Addition of these liposomes to the cis compartment of a bilayer chamber, at low protein concentration, resulted in insertion in the planar lipid bilayer of channels that were open at 0 mV and at low, positive, or negative voltage (Fig. 4). At high voltages, positive or negative, the channels closed in a manner characterized by large, slow transitions. Sporadic fast closures were often superimposed on the slow kinetic closure. The tendency to close was higher at positive than at negative potential. Similar characteristics were observed in patch-clamp experiments performed on giant proteoliposomes reconstituted with NanC. The main conductance of the channel in symmetrical 800 mM KCl medium was 450 pS at positive potential and 300 to 400 pS at negative potential. Lower subconductances were also observed. The channels were weakly selective for anions with a reversal potential of around 20 mV under asymmetrical conditions (800 mM KCl versus 10 mM KCl).

The effect of different compounds on channel conduction through the NanC channel was examined in patch-clamp experiments performed on giant proteoliposomes reconstituted with NanC. The main conductance of the channel in symmetrical 800 mM KCl medium was 450 pS at positive potential and 300 to 400 pS at negative potential. Lower subconductances were also observed. The channels were weakly selective for anions with a reversal potential of around 20 mV under asymmetrical conditions (800 mM KCl versus 10 mM KCl).

![Image](https://via.placeholder.com/150)

**FIG. 2.** NagC and CAP binding to the nanC regulatory region. The Fim1-Fim2 PCR fragment labeled at the Fim1 site was mixed with NagC and/or CAP at the concentration indicated. Cyclic AMP was included with CAP at 0.2 mM. The region protected by NagC and the overlapping site. The marker is pBR322 digested with MspI.

### TABLE 1. Effect of various inducing conditions and regulatory mutations on expression of the nanC-lacZ fusion

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>Inducer added</th>
<th>Sp acta (nmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>None</td>
<td>Glucose</td>
<td>&lt;1</td>
</tr>
<tr>
<td>None</td>
<td>GlcNAc</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>Neu5Ac</td>
<td>240 ± 18</td>
</tr>
<tr>
<td>None</td>
<td>GlcNAc + Neu5Ac</td>
<td>105 ± 12</td>
</tr>
<tr>
<td>nanR</td>
<td>None</td>
<td>248 ± 12</td>
</tr>
<tr>
<td>nanR</td>
<td>Glucose</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>nanR</td>
<td>GlcNAc</td>
<td>223 ± 63</td>
</tr>
<tr>
<td>nanR</td>
<td>Neu5Ac</td>
<td>251 ± 21</td>
</tr>
<tr>
<td>nanR ompR</td>
<td>Glucose</td>
<td>1</td>
</tr>
<tr>
<td>nanR nagC</td>
<td>None</td>
<td>360 ± 19</td>
</tr>
<tr>
<td>nanR nagC</td>
<td>Glucose</td>
<td>281 ± 42</td>
</tr>
<tr>
<td>nanR nagC</td>
<td>GlcNAc</td>
<td>344 ± 36</td>
</tr>
<tr>
<td>nanR</td>
<td>M63 + glycerol</td>
<td>322 ± 45</td>
</tr>
<tr>
<td>nanR ompR</td>
<td>M63 + glycerol</td>
<td>128 ± 15</td>
</tr>
<tr>
<td>nanR cpxR</td>
<td>M63 + glycerol</td>
<td>153 ± 34</td>
</tr>
<tr>
<td>nanR cpxR ompR</td>
<td>M63 + glycerol</td>
<td>62 ± 12</td>
</tr>
</tbody>
</table>

* a Bacteria were grown in LB medium at 30°C or in M63 medium where indicated. Inducing carbon sources were added at 0.1%. Specific activities are expressed as nanomoles of o-nitrophenol formed per minute per milligram of bacteria (dry weight).
The tested compound was either superfused in the bath on the excised patch or present in the pipette and tested at different voltages at opposite polarities. Neu5Ac up to 50 mM did not induce any blockade of the channels when it was perfused in the bath \( n = 9 \) or present in the pipette \( n = 3 \). We also tested a possible effect of colominic acid, a mixture of polymers of Neu5Ac. Up to 50 mM (final concentration of the monomer), no effect was observed in the bath \( n = 5 \) or in the pipette \( n = 4 \). Finally, neither 70 mM GlcNAc \( n = 3 \) nor 50 mM trigalacturonate \( n = 3 \), which blocks KdgM porin channels, had any clear effect.

**DISCUSSION**

The results presented here suggest that NanC is an outer membrane channel protein allowing the entry of Neu5Ac into *E. coli*. In electrophysiological experiments, purified NanC reconstituted in pure lipid bilayers exhibited the classical properties of voltage-dependent porins. NanC proteins form high-conductance channels which are open at low membrane potentials. The conductance and the weak anion selectivity of NanC are similar to that of KdgM, which belongs to the same protein family (3). Unlike KdgM, which can be closed only at positive potential, NanC exhibited voltage-dependent closure at both polarities. In the case of KdgM, which is involved in oligogalacturonide transport, we showed that trigalacturonate was able to induce fast blockade of the channel (3). No effect of Neu5Ac was observed in the experiments performed with NanC.

Since the ability to detect blockade is dependent on the residence time of the blocker in the channel and hence on its size, we tested colominic acid, a mixture of polymers of Neu5Ac. The exact composition of this commercial mixture, which cannot be used as a carbon source for growth by *E. coli*, is not known, and thus its lack of effect is no proof that NanC channels cannot be blocked by long polymers of Neu5Ac. Clearly, our electrophysiological experiments cannot document a specificity of NanC porin channels for sialic acid.

However, strong indications that Neu5Ac could be a NanC substrate come from the facts that Neu5Ac induces NanC synthesis and that NanC is required for the entry of Neu5Ac in
the absence of the general porins OmpC and OmpF. Neu5Ac has a molecular mass (309 Da) that allows its entry into bacteria through these nonspecific porins (exclusion size, 600 Da). Why should *E. coli* possess a specific channel for this compound? The concentration of free sialic acid is generally low in most animal tissues (29), and a specific channel would allow efficient uptake of this good carbon and nitrogen source that would otherwise diffuse too slowly by the general porins because of their low concentration. Neu5Ac can also be found as a homopolymer (polysialic acid) at the surface of *E. coli* K1 strains or as the terminal sugar of polysaccharidic chains at the surface of eukaryotic cells. Intestinal mucus is rich in these sialic acid-containing carbohydrates (4). A specific channel could be useful for the uptake of these oligomeric sugars.

*nanC* forms a putative operon with two genes, *yjhS* and *yjhT*. These genes encode proteins with a possible sequence signal that could be involved in the periplasmic degradation of Neu5Ac-containing oligomers. The fact that a *nanC* mutant is still able to grow on Neu5Ac indicates that the products of these two genes are not required for the metabolism of this compound.

The identified or predicted substrates of the KdgM family members are oligogalacturonate, oligorhamnogalacturonate, alginate, and Neu5Ac (3, 22). The common feature of these compounds is the presence on the sugars of an acidic function. The marine bacterium *Vibrio furnissii* is able to metabolize chitin, a polymer of GlcNAc, a nonacidic derivative of Neu5Ac. The chitooligosaccharides enter the bacteria not through a KdgM family channel protein but through a general porin belonging to the PhoE family (11). Determination of the three-dimensional structure of KdgM or another member of the family should clarify why a specific family of channel proteins is required for acid sugars and how these compounds cross the channel.

*E. coli* *yshA* (*ompL*), the closest *nanC* homologue, belongs to a group of eight genes that probably form an operon. All these genes present homologies with genes involved in sugar metabolism: two genes of the family of sodium:galactoside symporters (*yihP* and *yihO*), a glycoside hydrolase (*yihQ*), an aldose epimerase (*yihR*), a sugar epimerase (*yihS*), an aldolase (*yihT*), and an oxidoreductase (*yihU*). Thus, *yshA* is also probably involved in the transport of a sugar, probably acidic.

NanC regulation seems to be quite complex. NanR and NagC are two regulators that control *nanC* expression by binding to or in the vicinity of its promoter. They also regulate *fimB* expression in a still unexplained way, since their binding sites are located more than 500 bp upstream of the *fimB* promoters (27). However, it is clear that they allow coordination of *nanC* and *fimB* expression, since NanR and NagC repress one and activate the other of these divergently arranged genes. The presence of sialic acid, an indicator of the presence of an animal host, induces the sialic acid degradation pathway (the first step of which is NanC) and inhibits *fimB*, which regulates *E. coli* adhesion by type I fimbriae. Two other regulators, OmpR and CpxR, activate *nanC* expression, although a direct control has not been proven.

Regulation of porin gene expression by osmolarity via OmpR is a well-studied phenomenon (8). Control of *fimB* expression by osmolarity has recently been described, and OmpR has the role of repressor (26). Thus, it has opposite actions on...
nanC (activation) and fimB (repression). Such differential actions are also observed for NanR and NagC. CpxR and OmpR control the formation of adhesion structures such as curli and Pap pili (9, 17, 21). It would not be surprising if, like NanR, NagC, and OmpR, CpxR plays a role in the control of type 1 fimbiosis.

Additional regulatory proteins controlling nanC probably exist: introduction in a strain bearing a nanC-lacZ fusion of a plasmid containing the regulatory region of nanC led to a level of expression of the fusion 18-fold higher than in the nanR mutant (data not shown). This suggests the existence of an additional repressor(s) controlling nanC expression that would be titrated by the presence of the plasmid. A role of these regulators in the control of fimB expression remains to be proven, but its complex regulation by far upstream sequences that control nanC transcription shows that nanC and fimB expression is highly intertwined. These intricate regulations may be the reason for the exceptional length of the nanC-fimB intergenic region. Through these common regulatory mechanisms, sialic acid appears to be not just a carbon source, but also a complex environmental signal controlling the way of life of E. coli.

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