Indole production by the tryptophanase TnaA in *Escherichia coli* is determined by the amount of exogenous tryptophan

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The signalling molecule indole occurs in significant amounts in the mammalian intestinal tract and regulates diverse microbial processes, including bacterial motility, biofilm formation, antibiotic resistance and host cell invasion. In *Escherichia coli*, the enzyme tryptophanase (TnaA) produces indole from tryptophan, but it is not clear what determines how much indole *E. coli* can produce and excrete, making it difficult to interpret experiments that investigate the biological effects of indole at high concentrations. Here, we report that the final yield of indole depends directly, and perhaps solely, on the amount of exogenous tryptophan. When supplied with a range of tryptophan concentrations, *E. coli* converted this amino acid into an equal amount of indole, up to almost 5 mM, an amount well within the range of the highest concentrations so far examined for their physiological effects. Indole production relied heavily on the tryptophan-specific transporter TnaB, even though the alternative transporters AroP and Mtr could import sufficient tryptophan to induce tnaA expression. This TnaB requirement proceeded via tryptophan transport and was not caused by activation of TnaA itself. Bacterial growth was unaffected by the presence of TnaA in the absence of exogenous tryptophan, suggesting that the enzyme does not hydrolyse significant quantities of the internal anabolic amino acid pool. The results imply that *E. coli* synthesizes TnaA and TnaB mainly, or solely, for the purpose of converting exogenous tryptophan into indole, under conditions and for signalling purposes that remain to be fully elucidated.

INTRODUCTION

Over 100 years ago, Theobald Smith discovered that *Escherichia coli* produces indole (Smith, 1897), and about 85 Gram-positive and Gram-negative species are now known to produce this compound (Lee & Lee, 2010). Although its presence has long been used to identify bacteria, indole has only recently been recognized as a signalling molecule that regulates diverse physiological processes, including motility (Bansal et al., 2007), biofilm formation (Bansal et al., 2007; Domka et al., 2006; Lee et al., 2007a, b, 2011; Di Martino et al., 2003), antibiotic resistance (Hirakawa et al., 2005), plasmid stability (Chant & Summers, 2007), persister cell formation (Vega et al., 2012) and virulence (Hirakawa et al., 2009). Indeed, indole produced from just a few cells can protect a large population of *E. coli* against several antibiotics (Lee et al., 2010). Indole-regulated behaviours also extend to inter-species communication by affecting efflux-mediated multidrug resistance, flagella synthesis, virulence factor expression and host cell invasion by indole-non-producers like *Salmonella enterica*, *Pseudomonas aeruginosa* and even the yeast *Candida albicans* (Lee et al., 2009; Nikaido et al., 2011, 2012; Oh et al., 2012; Raut et al., 2012). Finally, indole and other tryptophan-derived metabolites balance inflammation in the mammalian intestinal tract (Bansal et al., 2010; Keszthelyi et al., 2012; Nicholson et al., 2012) and strengthen the barrier function of epithelial tight junctions (Bansal et al., 2010; Keszthelyi et al., 2012; Nicholson et al., 2012). In short, indole and its derivatives strongly affect the physiology of a broad spectrum of bacteria and animals (Wikoff et al., 2009).

Despite the growing appreciation of its biological effects, it is not clear how much indole bacteria can produce or what determines the final yield. Indole is generated by the cytoplasmic enzyme, tryptophanase, which hydrolyses tryptophan to create indole, pyruvate and ammonia (Newton et al., 1965), a reaction that occurs exclusively in bacteria (Lee & Lee, 2010). In *E. coli*, this process is catalysed by the TnaA protein (Deeley & Yanofsky, 1981; Sarsero et al., 1991; Stewart & Yanofsky, 1985). It has been suggested that TnaA may degrade tryptophan synthesized by endogenous metabolic pathways (Yanofsky et al., 1991), but this would seem to be counterproductive because degrading too much of this amino acid would inhibit growth. Although indolediffuses across cell membranes without the aid of a specific transporter (Kamaraju et al., 2011; Piñero-Fernandez et al., 2011), tryptophan import is controlled by TnaB, though in its absence two other amino
acid transporters, AroP and Mtr, can import sufficient tryptophan to support cell growth (Brown, 1970; Yanofsky et al., 1991). How these importers contribute to indole production is unknown.

The question of how much indole bacteria can produce is important because a sufficiently high concentration (3–5 mM) reversibly inhibits cell growth, presumably because it reduces the electrochemical potential across the cytoplasmic membrane (Chimerel et al., 2012). When grown in LB medium, E. coli and Vibrio cholerae can raise the external indole concentration to 0.5–0.6 mM (Hirakawa et al., 2009; Li & Young, 2012; Mueller et al., 2009). Yet, despite this observation, potentially inhibitory concentrations (1–5 mM) have been added to cultures to determine how indole affects expression of multidrug exporters (Hirakawa et al., 2005; Nikaido et al., 2011; Nishino et al., 2005), bacterial virulence (Nikaido et al., 2012), plasmid stability (Chant & Summers, 2007; Field & Summers, 2012) and cell division (Chimerel et al., 2012). Whether bacteria can naturally produce these concentrations is unknown, so some of the effects observed in these previous studies could reflect the use of artificially high amounts of indole.

Here, we report that indole production by E. coli depends directly, and perhaps solely, on the amount of tryptophan in the external growth medium. Exogenous tryptophan was converted, virtually quantitatively, into indole, after which production ceased. Surprisingly, in the presence of sufficient amounts of tryptophan, E. coli produced and excreted indole to concentrations of 4–5 mM. This ability depended most strongly on the tryptophan importer protein, TnaB, and much less indole was produced by cells expressing the alternative transporters AroP and Mtr. Thus, E. coli can produce very high levels of indole, and the TnaA and TnaB proteins appear to have as their exclusive purpose the production of indole from exogenous tryptophan.

**METHODS**

*Strains and growth media.* Bacterial strains are listed in Table 1, and all strains were derivatives of E. coli MG1655. LB broth was used for routine strain construction and growth, and media were supplemented with kanamycin (Kan, 50 μg ml⁻¹) or ampicillin (Amp, 100 μg ml⁻¹) when required. For TnaA expression and indole production experiments, cells were grown in M9 minimal medium supplemented with 2 mM MgSO₄, 0.1 mM CaCl₂ and 33 μM thiamine, plus 1% Bacto Casamino acids or 1% of a 14 amino acid mixture (14 AA) that contained alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, serine, threonine and valine (0.71 mg amino acid ml⁻¹). Media were supplemented with tryptophan (0.0–5.0 mM), as indicated. All amino acids were from Sigma–Aldrich.

**Table 1. E. coli strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description*</th>
<th>Source or reference</th>
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<tbody>
<tr>
<td>MG1655</td>
<td>Wild-type F⁻ λ⁻ ilvG::rph-50 rph-1</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>GL38</td>
<td>MG1655 aec-sfGFP::kan</td>
<td>Li &amp; Young (2012)</td>
</tr>
<tr>
<td>GL40</td>
<td>MG1655 tnaA-sfGFP::kan</td>
<td>Li &amp; Young (2012)</td>
</tr>
<tr>
<td>GL69</td>
<td>MG1655 tnaA-sfGFP::frt</td>
<td>Li &amp; Young (2012)</td>
</tr>
<tr>
<td>GL607</td>
<td>MG1655 ΔtnaAB::frt</td>
<td>This study</td>
</tr>
<tr>
<td>GL611</td>
<td>GL607 ΔtuaB::frt Pₜₐ₇A::tnaA-sfGFP ΔtuaB::frt</td>
<td>This study</td>
</tr>
<tr>
<td>GL619</td>
<td>GL607 ΔtuaB::frt Pₜₐ₇A::tnaA-sfGFP tnaB::frt</td>
<td>This study</td>
</tr>
<tr>
<td>GL722</td>
<td>MG1655 tnaA-sfGFP ΔtnaB::kan</td>
<td>This study</td>
</tr>
</tbody>
</table>

*frt* indicates the presence of the following oligonucleotide scar that was left after removing the kan cassette: GAAGTCTTACATTCAGAAAT-AGGAACCTC.
Table 2. Oligonucleotides used to construct deletion and insertion mutations

<table>
<thead>
<tr>
<th>Description</th>
<th>Oligonucleotides (5’–3’)*</th>
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<tr>
<td>ΔtnaAB</td>
<td>ACAGGGATCAGTGAATTTAAAAATGAAGGATATGATAATAGGACGGTCAAATTGGC H1P1</td>
</tr>
<tr>
<td>Pnad ΔtnaAB</td>
<td>TGCCGTGCCTTCTGGAAGATTGAGTACGTAACATGATATATCCTCCTTGTC H2P2</td>
</tr>
<tr>
<td>Pnad ΔtnaB</td>
<td>CTTGGGCCGGCGGTGTTGCTAAAGTTCTGGGCTAATTTAGAAGGAGATATAGTAATGAAACCTTTTAACATCTCCC H1P1</td>
</tr>
<tr>
<td>Pnad ΔtnaB</td>
<td>GCCCTTTTCAATTTCACAAAACTTAAATACGAGTTAGCCGTAATCTCTTTTGTC H2P2</td>
</tr>
<tr>
<td>Pnad ΔtnaB</td>
<td>GACGACGGTAATACCCGACCGGCATGTGAGGCTCTACAAAAATTTATATCATAACGAGTGTCG H1P1</td>
</tr>
<tr>
<td>Pnad ΔtnaB</td>
<td>CAGGCAATTTGACCGGTCTCTTTAGGCCTAAATTTAGGAACACG H1P1</td>
</tr>
<tr>
<td>Pnad ΔtnaB</td>
<td>TAGGACGGGTCAATTGGC H2P2</td>
</tr>
</tbody>
</table>

*Sequences homologous to target chromosomal locations are in italic type. H1 and H2 refer to homologous regions. P1 and P2 refer to priming sites.

and 510 nm emission. Indole was assayed as described previously (Li & Young, 2012), except that 100 μl Kovac’s reagent was added to each 10 μl sample. When the indole concentration exceeded 1 mM, samples were diluted so that the assay values fell within the linear range of the standard curve (0–1 mM). When applicable, samples were separated by electrophoresis in 12% SDS-PAGE gels, and TnaA–sfGFP was detected by in-gel GFP fluorescence imaging, as described previously (Li & Young, 2012).

**Indole production by disrupted cells.** *E. coli* cells continuously expressing TnaA–sfGFP and TnaB (strain GL619, Pnad ΔtnaA–sfGFP tnaB) or TnaA–sfGFP alone (GL611, Pnad ΔtnaA–sfGFP ΔtnaB) were grown in LB broth at 37 °C until they reached late-exponential phase. The following steps were performed at room temperature to avoid cold inactivation of TnaA (Kogan et al., 2009), unless otherwise indicated. Cells were washed and resuspended to OD600 1.0 in 100 mM potassium phosphate buffer, pH 7.8, after which the cells disrupted by passage through an LV1 high shear fluid processor (Microfluidics) at 20 000 p.s.i. (138 000 kPa). Indole production was initiated by adding 0.5 mM tryptophan to intact or disrupted cells and incubating with shaking at 37 °C.

**RESULTS**

**Indole accumulation is determined by the amount of exogenous tryptophan**

We wished, first, to determine the relationship between indole yield and the amount of exogenous tryptophan, which is known to induce the tna operon (Bilezikian et al., 1967). *E. coli* GL69, a strain carrying a chromosomal copy of TnaA–sfGFP, was grown in M9 minimal medium containing 1% Casamino acids, a mixture that lacks tryptophan, and supplemented with differing concentrations of tryptophan. Fusing sfGFP to TnaA does not affect the activity and stability of the protein (Li & Young, 2012), so the production of TnaA–sfGFP could be measured by measuring GFP fluorescence. Cultures incubated in the presence of 0, 0.25, 0.5 and 1.0 mM tryptophan grew at the same rate and to the same extent (Fig. 1a), indicating that neither the starting nutrients nor any accumulating metabolic products affected cell growth under these conditions. On the other hand, the amount of TnaA–sfGFP produced was directly proportional to the amount of exogenous tryptophan supplied (Fig. 1b), paralleling the reported behaviour of the tna operon (Yanofsky et al., 1991). Interestingly, the final indole concentration in the medium corresponded very closely to the starting concentration of tryptophan (Fig. 1c). Presumably, in the presence of 0.25 or 0.5 mM tryptophan, *E. coli* produced indole until this exogenous amino acid was depleted (Fig. 1c), at which time the production of TnaA–sfGFP slowed or stopped (Fig. 1b). Previous investigators found that *E. coli* and V. cholerae grown in LB medium excreted indole until it reached a final concentration of 0.5–0.6 mM (Hirakawa et al., 2009; Li & Young, 2012; Mueller et al., 2009). These figures are consistent with our present observations because LB contains approximately 0.51 mM tryptophan (Biosciences, 2006), meaning that the final amount of indole produced in LB also approximates to the amount of tryptophan available in the medium.

In the presence of 1 mM tryptophan, *E. coli* evidently converted most or all exogenous tryptophan into (Fig. 1c). When 5 mM tryptophan was added to the medium, approximately 4.5 mM and 4.7 mM indole was produced after 24 and 36 h of growth, respectively, even though the cells entered stationary phase at about 10 h (Fig. 2). However, when tryptophan was added to a final concentration of 10 mM, the level of indole excreted to the medium reached only 5–6 mM, even after extended incubation (3–5 days) (results not shown), suggesting that this was the maximum amount of indole that cells could accumulate. In any event, *E. coli* converted, almost quantitatively, exogenous tryptophan to indole, at least for exogenous tryptophan concentrations up to 5 mM. In addition, the presence of any concentration at any concentration did not affect the polar localization of TnaA–sfGFP (not shown) (Li & Young, 2012).

We were puzzled that TnaA stopped producing indole after intermediate amounts of tryptophan were depleted (Fig.
1c, 0.25 and 0.5 mM tryptophan). In these cases, cell mass continued to increase even after indole production had come to a halt, which indicated that the cells could still synthesize sufficient internal tryptophan to support growth and that these concentrations of indole were not inhibitory (Fig. 1a). In addition, there was plenty of TnaA–sfGFP present (Fig. 1b), which could presumably degrade any additional tryptophan synthesized internally. It was possible that the yield of indole was being controlled only by the amount of newly synthesized TnaA–sfGFP instead of by the total TnaA pool. In this situation, indole production would cease when the amount of inducing tryptophan fell so low that transcription of tnaA was terminated. To test this possibility, we inserted the tnaA–sfgfp and tnaB genes into the chromosome so as to place them under transcriptional control of the constitutive tufA promoter (strain GL619). tufA encodes elongation factor Tu, one of the most abundant proteins in E. coli (Ishihama et al., 2008), so TnaA–sfGFP should be produced in quantity, and the expression of the tnaA–sfgfp gene should no longer be affected by external concentrations of tryptophan. This was confirmed to be true because in this strain the amount of TnaA–sfGFP in the presence of 0 and 1 mM tryptophan was similar (Fig. 3a). However, even though TnaA–sfGFP was produced in quantity and continuously, indole accumulated only to a concentration that was roughly equal to the original amount of tryptophan in the medium (Fig. 3b). This was strong evidence that the final yield of indole was determined almost solely by the supply of exogenous tryptophan and not by the total amount of TnaA–sfGFP or the availability of newly synthesized enzyme.

**Indole production requires TnaB**

Because indole production depended mostly on the presence of exogenous tryptophan, the importation of this amino acid must be important. In E. coli, one or more of three tryptophan transporters could be responsible for this step: TnaB, AroP and/or Mtr (Yanovsky et al., 1991). By using mutants having different combinations of these transporters, Yanovsky et al. (1991) found that TnaB was most important for tryptophan import, at least at the level of inducing the tnaA operon as measured by the expression

![Fig. 1. Indole accumulation is determined by the amount of exogenous tryptophan in the growth medium. E. coli GL69 cells expressing TnaA–sfGFP from its original chromosomal locus were grown at 37 °C in M9-Casamino acids medium supplemented with 0–1 mM tryptophan (1c, 1 mM; ◆, 0.5 mM; △, 0.25 mM; ○, 0 mM). (a) Cell growth (OD600). (b) TnaA–sfGFP expression (GFP fluorescence). (c) Indole production. Graphs represent means ± SD from three independent cultures.](http://mic.sgmjournals.org)
of a tnaA–lacZ translational fusion. However, these authors did not measure indole production, so it is not clear which of the three transporters can sustain a tryptophan level sufficient for producing wild-type levels of indole. To clarify this issue, we deleted tnaB and expressed tnaA–sfGFP alone (in strain GL722). The mutant produced TnaA at 44 % of the level of wild-type cells (Fig. 4, ΔtnaB Casamino acids, white bar), which was consistent with observations that a tnaB mutant expresses tnaA–lacZ at 30 % of wild-type levels in a rich medium (Yanofsky et al., 1991). However, the amount of indole produced by the ΔtnaB strain was only 19 % that of wild-type, even though the other two tryptophan transporters were still active (Fig. 4, ΔtnaB Casamino acids, grey bar).

In a medium lacking other aromatic amino acids, AroP and Mtr can transport enough tryptophan to induce expression of the tnaA–lacZ gene (Yanofsky et al., 1991). Thus, it was possible that under these conditions AroP and Mtr might support efficient indole production in the absence of TnaB. To test this, cells were grown in M9 medium supplemented with tryptophan plus 14 amino acids but lacking phenylalanine and tyrosine. As expected, TnaA–sfGFP production in the ΔtnaB mutant increased dramatically, reaching a level nearly equal to that produced by wild-type cells (Fig. 4, ΔtnaB 14 AA, white bar). This confirmed the observation of Yanofsky et al. (1991) that the activities of AroP and Mtr can support a wild-type level of expression of tnaA–lacZ if phenylalanine and tyrosine are absent. However, surprisingly, despite the near wild-type amount of TnaA–sfGFP, the ΔtnaB strain produced only 34 % of the amount of indole produced by the wild-type strain (Fig. 4, ΔtnaB 14 AA, grey bar). Thus, even though AroP and Mtr by themselves could transport enough tryptophan to induce near-normal expression of the tnaA operon, the amounts imported were evidently far below those that TnaB could make available for conversion to indole.

It was odd that AroP and Mtr, in the absence of TnaB, transported enough tryptophan to induce tnaA expression but that this produced only a minor amount of indole. One possibility was that TnaB was required to activate TnaA so that in the absence of TnaB very little indole could be produced. Another possibility was that even though tnaA transcription was normal, insufficient amounts of active TnaA were being produced for some other reason. To see if either alternative could explain the observation, we assayed for indole production in lysates prepared from cells making TnaA and TnaB and from cells producing TnaA alone. Breaking the cells would bring TnaA in direct contact with tryptophan without the need for transporters; if TnaB was required to produce or activate TnaA, then low indole activity would be observed in lysates lacking TnaB. TnaA and TnaB were expressed simultaneously and constitutively from the tufA promoter (strain GL619, P_{tufA}:: tnaA–sfGFP tnaB), and TnaA was expressed in the absence of TnaB in the same genetic background (strain GL611,
DISCUSSION

Prior to this work, it was unclear what determined the amount of indole produced by E. coli in culture. Here, we find that the final indole concentration depends almost exclusively on the availability of exogenous tryptophan, leading us to conclude that E. coli synthesizes TnaA (tryptophanase) and TnaB (a tryptophan importer) primarily so the organism can degrade exogenous tryptophan, either for metabolic purposes or for signalling. Furthermore, given enough tryptophan, we find that E. coli can produce up to 5–6 mM indole; an amount known to have important physiological effects on E. coli, other bacteria and mammalian cells.

In a way, the fact that external tryptophan drives indole accumulation is surprising because it has been generally thought that TnaA could degrade tryptophan synthesized internally and that by so doing could produce indole in amounts exceeding the availability of exogenous tryptophan (Lee & Lee, 2010; Yanofsky et al., 1991). However, E. coli grows normally when the amount of TnaA protein is increased by two- to threefold in an ibpAB mutant (Kuczyńska-Wiśnik et al., 2010), and we find that expressing tnaA constitutively from an unregulated promoter has no ill effects on cell growth. These results indicate that TnaA, even when present in large amounts, does not degrade enough internally generated tryptophan to adversely affect cell viability. Moreover, we find that when all or nearly all exogenous tryptophan is converted into indole, further production stops. This is consistent with E. coli being able to produce, at best, only 0.005–0.015 mM indole in the absence of external tryptophan (Vega et al., 2012). Therefore, the hydrolysis of anabolic tryptophan stores has a negligible effect on the amount of indole generated by E. coli.

Why does TnaA not degrade internally synthesized tryptophan and thus inhibit cell growth? We suggest the answer may lie in its relatively low affinity for tryptophan as compared with other enzymes. The measured $K_m$ of TnaA for tryptophan ranges from 0.30 to 0.60 mM (London et al., 1974; Newton et al., 1965; Scheer et al., 2011; Snell, 1975; Watanabe & Snell, 1977), while the $K_m$ of tryptophan-tRNA ligase for the same substrate is 0.017 mM (Zúñiga et al., 2002) and the $K_d$ of the trp operon repressor, TrpR, for tryptophan is 0.018 mM (Lane, 1986). Because the intracellular tryptophan concentration is about 0.012–0.024 mM when exogenous tryptophan is not available (Bennett et al., 2009), these enzyme characteristics mean that TnaA should be at a competitive disadvantage in the presence of tryptophan-tRNA ligase and TrpR. If so, then TnaA might access very little of the internal anabolic tryptophan pool. Degradation of this amino acid by TnaA would become important only if TnaB imported sufficient tryptophan from the surrounding medium, and when this amount was converted into indole, TnaA would once more compete poorly for internal stores.

The question of how much indole E. coli can produce is important because this compound affects different aspects of bacterial physiology in a concentration-dependent manner. For example, 0.5 mM indole influences bacterial motility (Bansal et al., 2007), biofilm formation (Domka et al., 2006; Lee et al., 2007a; Di Martino et al., 2003), persist cell formation (Vega et al., 2012) and the secretion of certain virulence factors (Hirakawa et al., 2009); but

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Fig. 5. TnaB is required for efficient tryptophan uptake but not for TnaA activation. GL619 cells continuously expressing TnaA–sfGFP and TnaB (PtnaA::tnaA–sfGFP tnaB; □) or GL611 cells expressing TnaA–sfGFP alone (PtnaA::tnaA–sfGFP ΔtnaB; ○) were washed and resuspended in potassium phosphate buffer to OD<sub>600</sub> 1.0. Intact (a) or mechanically disrupted (b) cells were assayed for indole production after adding 0.5 mM tryptophan. Means ± SD of three independent experiments are presented.
higher concentrations (1–2 mM) are required to affect the expression of multidrug exporters (Hirakawa et al., 2005; Nikaido et al., 2011; Nishino et al., 2005), the BaesR stress response (Leblanc et al., 2011), and a range of virulence factors (Nikaido et al., 2012). Even higher indole concentrations (3–5 mM) inhibit cell division (Chimerel et al., 2012) and affect plasmid stability (Field & Summers, 2012). The lingering question, then, has been whether these effects are physiologically relevant or whether they are artefacts of non-physiological indole concentrations. Here, we show that with adequate amounts of tryptophan E. coli can produce and excrete indole to an external concentration of ~5 mM, which means it is possible to achieve the high concentrations required to influence certain biological processes. It is not yet clear why indole does not accumulate to even greater levels when 10 mM tryptophan is supplied. One possibility is that excess indole inhibits TnaA enzymic activity. Alternatively, these levels of indole might inhibit tryptophan transport by dissipating the proton motive force, which may be required for TnaB activity (Sarsero et al., 1991).

What are the relative contributions of the various transporters for delivering tryptophan to TnaA? The AroP and Mtr transporters import enough tryptophan to induce the expression of the tna operon when phenylalanine and tyrosine are not present in the growth medium (Yanofsky et al., 1991). Although we confirm this to be true, we find that even under these conditions TnaB remains essential for maximum indole production. TnaB does not seem to activate TnaA directly because TnaA by itself converts tryptophan into indole, provided the cells are disrupted to allow access to its substrate. Overall, then, though AroP and Mtr may import enough tryptophan to induce the tna operon, large-scale indole production depends on the activity of TnaB. Interestingly, tryptophan import into enteropathogenic E. coli leading to the death of Caenorhabditis elegans also depends on TnaB (Bhatt et al., 2011), implying that this phenomenon might be driven by the accumulation of excess indole.

Finally, where might E. coli encounter an environment that contains enough exogenous tryptophan so that the organism can produce high concentrations of indole? The simple answer seems to be the normal intestinal tract. For example, in the pig gut, the concentration of tryptophan is 0.7 mM) and lower in the large intestine (Knarreborg et al., 2002). In human faeces, indole has been measured to be between 0.25 and 1.1 mM (Karlin et al., 1985; Zuccato et al., 1993), amounts that are in line with levels that are produced easily by E. coli when cultured in a rich medium. So far, indole concentrations have been measured only for bulk intestinal contents. But these concentrations may be even greater in the circumscribed intestinal niches in which E. coli resides, so that very high local concentrations of indole may accumulate. Also, tryptophan accounts for up to 0.23 % of the weight of animal muscle tissue (US Department of Agriculture, 2011), equal to ~11 mM if the tissue were to be degraded fully. Although this tryptophan is not generally available to intestinal flora, large amounts might accumulate under the right conditions (e.g. during a severe necrotic infection or during decomposition of an animal carcass). Also, the amount of tryptophan in a host can vary greatly depending on diet (Wesoly & Weiler, 2012) and antibiotic exposure (Zheng et al., 2011), so it is not unthinkable that indole concentrations in portions of the mammalian gut might reach levels as high as the maximum that can be produced in vitro.

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REFERENCES


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