Review Letter – Hypothesis

THE *tonB* GENE PRODUCT IN *ESCHERICHIA COLI*

Energy-coupling or molecular processing of permeases?

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1. Introduction

A single point mutation in the *tonB* gene of *Escherichia coli* results in an apparently diverse phenotype. Strains with a *tonB* gene lesion are simultaneously insensitive to phage T_1 and ϕ_{80} [1], and insensitive to colicins from group B [2]. They also lack specific iron-transport systems for ferric enterochelin [3], ferrichrome [4], ferric citrate [3], rhodoturulic acid [5] and an unidentified hydroxymate siderophore derived from a ColV plasmid [6,7], and a transport system for the cobalt complex, vitamin B_{12} [8] (table 1).

Receptors in the outer membrane of *E. coli* are involved in the transport of these ferric complexes [4,9–12] and vitamin B_{12} [13], and serve as specific binding sites for certain colicins and phage [10,14–17] (table 1). The specific binding of colicins or phage to their receptors is inhibited by the specific substrate (iron-siderophore or vitamin B_{12}) resulting in inhibition of the killing action of that colicin [18]. The irreversible binding of phage T_1 and ϕ_{80} required the membrane potential and TonB function [19] (TonB protein = *tonB* gene product). Thus, TonB function was associated with the outer membrane. However, not all functions associated with the receptors are TonB-dependent; these are summarised in table 1 and discussed below.

A defunct *tonB* leads to other pleiotropic effects derived from an inability to actively accumulate iron, and thus from iron starvation. Several enzymes in the biosynthetic pathway for aromatic amino acids and vitamins were de-repressed under conditions of iron tarvation in wild-type strains [20,21]. This effect is nediated by iron-dependent modification enzymes of tRNA [22] involved in attenuation control of genes coding for key enzymes of the aromatic biosynthetic pathway. In [20] a *tonB* strain had de-repressed levels of these enzymes resulting from iron starvation of the *tonB* strain by its inability to transport iron-chelated molecules. *tonB* (formerly *exbA*) strains hyper-excrete enterochelin [23], also a product of the aromatic biosynthetic pathway. In *tonB* strains, iron starvation also effects de-repressed levels of certain major proteins in the outer membrane [9,24] and minor proteins responsible for the binding of some group B colicins [25] including colicin I [26].

1.1. *Genetics*

The *tonB* allele has been mapped at min 27 on the chromosomal map of *E. coli* [27], close to the tryptophan operon. Between 10% [9] and 25% [28] of mutants resistant to the ferrichrome analogue and antibiotic, albomycin, are *tonB* strains. *tonB* strains have also been isolated by selection for resistance to colicins B [29] and I [52], and were resistant to phage T_1 [30]. Many *tonB* strains are deletions [23,30,31], the length and frequency of the deletion depending on the *tonB* strain and nearby chromosomal material.

Fine mapping of the *tonB* region using restriction enzyme analysis was presented in [27]. They mobilised the *tonB* region onto a ColE1 plasmid which complemented a *tonB* strain. In [32] a *tonB*$^+$ plasmid was employed in minicells and a 1730 basepair *Hin*dII fragment [27] defined which produced a 36 000 M_r protein responsible for the TonB function [32]. In [33] a *tonB*$^+$ plasmid was mobilised onto λ-transducing phage and a *tonB* gene product (TonB) of 40 000 M_r found; this protein was largely associated with the cytoplasmic membrane fraction [33]. The *tonB* operon is a single gene which is transcribed in a clockwise direction [32]. The complementation of a

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Table 1
Summary of the gross characteristics of TonB-dependent systems

System	Substrate	Map position on chromosome	Receptor	Permease	Factors which utilize system	
					TonB-dependent	TonB-independent
fep	Ferric enterochelin	13 min	FepA	FepB	Ferric enterochelin Colicin B Colicin D	None known
fhu	Ferrichrome	3 min	FhuA (formerly TonA)	FhuB	Ferrichrome Albomycin Colicin M Phage T_1 Phage ϕ_{80}	Phage T_5
btu	Vitamin B_{12} (cyanocobalamin)	89 min	BtuB	BtuA(?) BtuC(?)	Vitamin B_{12}	Colicin E_1 Colicin E_2 Colicin E_3 Phage BF23
fec	Ferric citrate	6 min	FecA	FecB	Ferric citrate	None known
?	Rhodoturulic acid	?	?	FhuB	Rhodoturulic acid	None known

tonB strain by a *tonB*$^+$ plasmid indicates that the *tonB*$^+$ gene is transdominant [32].

1.2. *Physiology*

Dihydroxybenzoate (DHB), the metabolic precursor of enterochelin, stimulated growth of a *tonB aroB* strain at <5 μM but at higher concentrations progressively inhibited the growth rate during log phase [3]. DHB had similar effects on the incorporation of iron into a *tonB* strain [9]. According to [3], at low [DHB], small concentrations of enterochelin are excreted into the periplasmic space chelating available iron, which is then taken up via the *fep* system (review [34]) into the cytoplasm. As in the wild-type, ferric enterochelin is subsequently broken down by the ferric enterochelin esterase (*fes* gene product) into ferric-dihydroxy benzoyl serine, and the Fe^{3+} incorporated into proteins by ferrochelatase [35]. In the *tonB aroB* strain, higher [DHB] resulted in the biosynthesis of higher concentrations of enterochelin which was excreted into the extracellular medium chelating the available iron, and rendering it unavailable for uptake due to the putative block in the outer membrane. That the irreversible binding of phage T_1 was dependent on TonB function and on membrane potential [19], led to the proposal that the function of TonB was to energize transport across the outer membrane [36].

Not all transport systems which have a receptor in the outer membrane are TonB-dependent, e.g., uptake of maltose [8] and nucleosides via the receptors coded by the *lamB* [37–39] and *tsx* genes [40,41], respectively. λ-Phage requires the maltose receptor to infect the cell while phage T6 and colicin K use the *tsx* gene product in the outer membrane.

The function of TonB in energy transduction was supported by [42–44]. Activity of the vitamin B_{12} transport system declined rapidly when a *tonB* (Am) *supD* (Ts) strain was incubated at the non-permissive temperature [42]; this decline was associated with the energy-dependent phase of uptake rather than the initial binding to the receptor [42]. Loss of protein synthesis also resulted in a rapid decline in the energy-dependent phase of vitamin B_{12} uptake [43]. The rapid decline in TonB function as compared to the function of the vitamin B_{12} receptor implied that the *tonB* gene product may be an energy source for the relevant systems.

In [43,44] an *entA* strain grown with dihydroxybenzoate or with ferrichrome, had a reduced initial velocity of vitamin B_{12} uptake. In [18] ferric enterochelin or ferrichrome, but not the chromium derivative of ferrichrome, conferred resistance simultaneously to group B colicins, including colicins B, V and Ia. The basic mechanism clearly involves the avail-

ability of iron rather than inhibition at the receptor by the siderophore itself [18].

2. Discussion of existing hypotheses

Since the data in [3] were interpreted as indirectly supporting the hypothesis that a defective *tonB* gene product gave rise to a functional lesion in the outer membrane, most subsequent data were interpreted to fit a similar model. One variation of this model [8,42,45] envisaged the TonB function as coupling the activity of the receptor to the permease in the cytoplasmic membrane by promoting the release of substrates from the respective receptors into the periplasmic space thereby making it available to the permease on the cytoplasmic membrane [46]. The vitamin B_{12}-transport system has been researched most extensively. While BtuA phenotypes lacked the ability to actively accumulate vitamin B_{12}, BtuB phenotypes also lacked a receptor in the outer membrane capable of binding the substrate [47]. If these 2 phenotypes have separate genes, they are not separable by complementation analysis [48]. Characteristics of *btuB* and *btuA* mutants from [16,47,48] are listed in table 2.

btuA and *tonB* strains share characteristics which suggest that both possess a functional receptor in the outer membrane:

(i) Strains of both types are sensitive to BF23 and colicins of the E type;
(ii) Membrane preparations of both types 'neutralise' the effects of these antagonists;
(iii) Both lack the energy-dependent phase of vitamin B_{12} transport, although they bind the substrate, as does the parent.

The BtuB phenotype may arise from a defect of the receptor in the outer membrane while BtuA may arise from a defect in the permease. This contradicts [45] where 2 binding states are proposed for the receptor: (i) BtuA, responsible for the binding of vitamin B_{12}; (ii) BtuB, responsible for the attachment of colicins E and phage BF23. My interpretation agrees with that found in the ferrichrome transport system where the *fhuA* gene codes for the ferrichrome receptor in the outer membrane while the *fhuB* gene codes for the permease in the cytoplasmic membrane [28,50]. *fhuA* mutants are resistant to phage T_1, T_5 and ϕ_{80}, to colicin M and albomycin, and are unable to take up ferrichrome. *fhuB* mutants are sensitive to this phage and colicin M (with exceptions), insensitive to albomycin, and are unable to accumulate ferrichrome. This interpretation also agrees with that proposed for the *fep* system [51].

Indirect evidence for TonB funtion in energy coupling to conformational changes of receptors in the outer membrane includes observations on colicin M action [52–54] and the binding requirements of phage T_1, T_5 and ϕ_{80} and inhibition by ferrichrome [19,28,36].

An alternative hypothesis sees TonB function in terms of energy required for generation of sites of apposition between the outer and cytoplasmic membranes [55]. These sites are distinguished from those in [56]. However, there is little evidence for such a mechanism [55].

That TonB function is unrelated to processes in the outer membrane and energization of transport is suggested by:

(i) Receptors of the vitamin B_{12}- (*btuB*) and ferrichrome-transport systems (*fhuA*) serve as recep-

Table 2
The characteristics of strains defective in vitamin B_{12} utilization

Relevant genotype	Presence or absence of vitamin B_{12}		Growth-limiting [vitamin B_{12}] (M)	Sensitivity to colicins E_1 and E_3	Sensitivity to phage BF23
	(i) Binding	(ii) Uptake			
Parent	Present	Present	5×10^{-11}	Sensitive	Sensitive
btuB(II)	Absent	–	5×10^{-10}	Resistant	Resistant
btuB(III)	Absent	–	5×10^{-7}	Resistant	Resistant
btuA	Present	Absent	5×10^{-7}	Sensitive	Sensitive
btuC	Present	Present	5×10^{-10}	Sensitive	Sensitive
tonB-451	Present	Absent	5×10^{-8}	Sensitive	Sensitive
tonB-452	Present	Absent	5×10^{-10}	Sensitive	Sensitive

tors for their substrates, for the infection and killing by phage BF23 and colicins E_1, E_2 and E_3 with *btuB*, and for phage T_5 with *fhuA* (table 1). Strains with *tonB* lesions continue to be sensitive to these antagonists although defective in their capacity to transport the corresponding ligands.

(ii) Cell suspensions treated to make their outer membrane more permeable to ferric enterochelin [51] and ferrichrome [50] remain dependent on the TonB function for these transport processes. Cell suspensions were made permeable to substances with an M_r-value about that of actinomycin by treatment with pronase. Strains lacking a functional receptor for ferrichrome (*fhuA*) were able to take up ferrichrome only after pronase treatment. Strains which were *tonB* or *fhuB* (lacking the permease for ferrichrome uptake) were both unable to transport ferrichrome before or after pronase treatment.

(iii) TonB function may not be related to energy transformation processes at all, at least in relation to the uptake of ferrichrome and ferric enterochelin (see below).

2.1. *TonB function in the energization of iron uptake?*

Genetic and physiological techniques have been used to determine at what stage during the transport process the TonB function is required for energization, if at all:

In the ferric enterochelin transport system a receptor in the outer membrane (FepA, [51]) serves for the uptake of ferric enterochelin and for binding of colicins B and D [10]. A permease (FepB) exists for transport across the cytoplasmic membrane [51]; and, based on enzyme yields from shocked cells, the ferric enterochelin esterase (*fes*) is thought to be localized solely in the cytoplasm (Braun, personal communication) and not in the cytoplasmic membrane as claimed in [55]. *fes* mutants cannot use ferric enterochelin as an iron source [35] although they can accumulate this substrate. In a *fes* strain, [^{55}Fe]ferric enterochelin, ferric [^{14}C]enterochelin and [^{14}C]enterochelin are accumulated to similar steady-state levels (fig.1). Since enterochelin contains no iron, its accumulation excludes the possibility that iron reduction is a prerequisite for transport [58].

Ferric enterochelin uptake is sensitive to energy

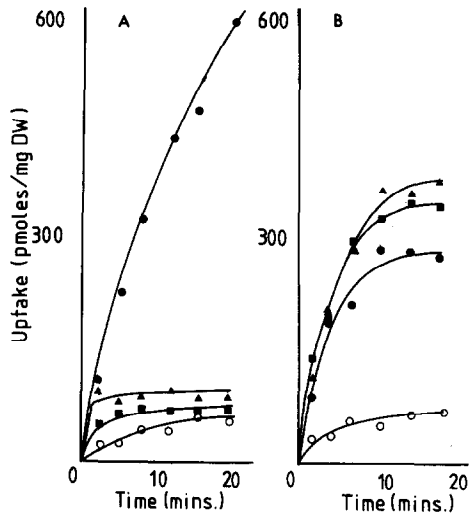

Fig.1. Uptake of iron and enterochelin by suspensions of (A) parent AN92 cells and (B) *fes* AN272 cells, after growth in 1 mM citrate and as in [3,57]. The uptake medium contained 100 μM nitrilotriacetic acid to inhibit the low affinity iron uptake system and either 2 μM [^{14}C]enterochelin (▲) or 2 μM [^{14}C]enterochelin + 1 μM Fe^{3+} (■) or 1 μM $^{55}Fe^{3+}$ (○) or 2 μM enterochelin + 1 μM $^{55}Fe^{3+}$ (●).

uncouplers [59]. A *fes* strain was used to characterize the energization of ferric enterochelin uptake [60]: both ferric enterochelin and proline accumulation required energy from the membrane potential under aerobic and anaerobic conditions. A striking exception [60] was the inability of dinitrophenol (DNP) to promote the efflux of accumulated [^{55}Fe]ferric enterochelin from a *fes* strain at the steady-state, in contrast to its effect on [^{14}C]proline. DNP added during this steady-state stage inhibited the efflux of [^{55}Fe]ferric enterochelin when a 10-fold excess of non-radioactive ferric enterochelin was added in a 'chase' experiment [60]. At pH 7 ferric enterochelin has 3 units of negative charge while proline has <1 unit. In line with the chemiosmotic theory, the component of the membrane potential utilized by the ferric enterochelin transport system may be different from that utilized by the proline system.

Ferrichrome uptake may be energized by the acetylation of the accumulated substrate before its rapid excretion [63] and not by the membrane potential (in contrast to the ferric enterochelin system). Cell smashes of *tonB* strains can acetylate ferrichrome, suggesting that the TonB function is not linked to this process. Thus, whether or not there is a common

process in the energization of ferric enterochelin and ferrichrome transport systems, which may be attributed to the TonB function, remains open.

2.2. *An alternative rationale of DHB stimulation of* tonB *strains*

Dihydroxybenzoate (DHB) at <5 μM stimulated the growth of a *tonB aroB* strain in a substantially iron free medium (1 μM) [3]. Higher concentrations of DHB, progressively inhibited growth. The DHB effect was in fact an enterochelin effect, since a *tonB entF* strain did not respond to DHB [3]. An alternative hypothesis could be postulated when the growth rate was observed to be determined by the ratio of ferric enterochelin to enterochelin in the growth medium. In [3], the ferric enterochelin/enterochelin ratio increased to ~1 with 6 μM DHB (the medium contained ~1 μM Fe^{3+}, and 6 μM DHB, if fully used, is converted into 2 μM enterochelin). With increasing DHB levels the ratio approaches zero. Why the ferric enterochelin/enterochelin ratio is critical for the growth of *tonB* strains is explained by the following unpublished data:

(i) Enterochelin (10 μM) inhibits the uptake of [^{55}Fe]ferric enterochelin (1 μM) into parental strains by ~10%.

(ii) Enterochelin (10 μM) inhibits the incorporation of $^{55}Fe^{3+}$ from ferric enterochelin (1 μM) into a *tonB* strain.

(iii) Enterochelin (10 μM) inhibits the binding of [^{55}Fe]ferric enterochelin to FepA receptor sites in parental and *tonB* strains.

(iv) The growth rate of a *tonB aroE* strain could be manipulated by varying the ferric enterochelin/enterochelin ratio in the growth medium. The growth rate is dependent on this ratio and not on total [Fe^{3+}] of the medium (fig.2).

Why ferric enterochelin/enterochelin ratio is more critical in a *tonB* strain than in its parent may be explained by the relative rates of uptake of [^{55}Fe]ferric enterochelin. The rate of [^{55}Fe]ferric enterochelin uptake into a *tonB* strain is almost zero [3]. However, *tonB* (but not *fepB*) strain growth on ferric enterochelin and incorporation of $^{55}Fe^{3+}$ from [^{55}Fe]ferric enterochelin in a *tonB* strain, suggests that the FepB permease is present in lowered amounts or may operate with reduced efficiency. Reduction in this low rate by inhibition with enterochelin in a *tonB* strain would lead to iron deprivation and consequent inhibition of growth. The defect of the ferric enterochelin uptake system in a *tonB* strain is proposed to be in the lowered activity of the permease rather than a putative block in translocation across the outer membrane. In the vitamin B_{12} system *tonB* strains are 'leaky' to vitamin B_{12}. In comparison with a *btuB* strain which lacks a functional receptor in the outer membrane, *tonB* strains can grow on $10–10^3$-fold lower concentrations of vitamin B_{12}, albeit at levels higher than required for growth of the parent itself.

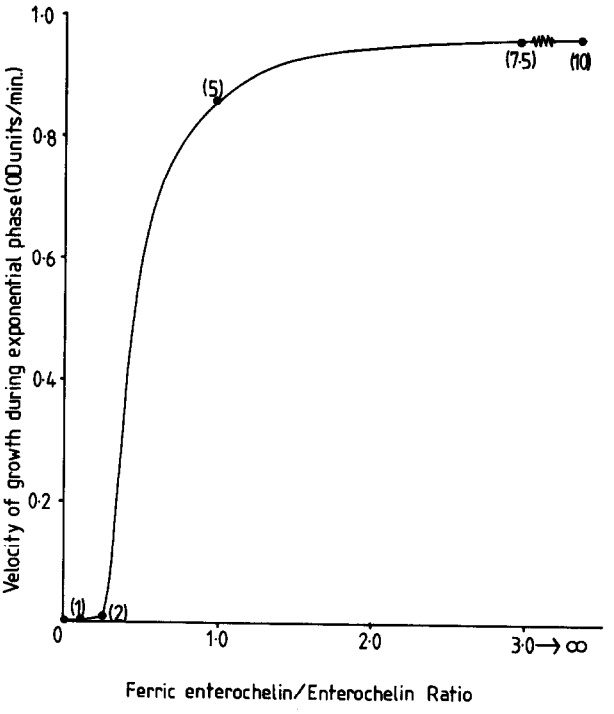

Fig.2. The effect of various ferric enterochelin/enterochelin ratios on the growth velocity of *tonB* strain BR158 during exponential phase. Enterochelin was estimated to be 10 μM [82]. Added iron (μM) is shown in parentheses at each point on the curve.

Since TonB-dependent transport systems may not contain a common energy-dependent step which could be attributed to the TonB function, and *tonB* strains 'leak' TonB-dependent substrates like ferric enterochelin and vitamin B_{12}, an hypothesis is now presented which explains both the genotypic and phenotypic characteristics of *tonB* strains.

3. Hypothesis

(1) The *tonB* gene product acts in the molecular processing of the relevant permeases, either:

(a) As a positive regulator of the permease genes for the transport systems of ferric enterochelin, ferrichrome, ferric citrate, rhodoturulic acid, vitamin B_{12} and any other TonB-dependent substrate;

or

(b) The *tonB* gene product acts as a specific protease which modifies the proteins FepA, BtuB, FecA and FhuB to act as permeases in the cytoplasmic membrane rather than receptors in the outer membrane (except FhuB).

(2) Permeases of TonB-dependent systems are functionally unstable. The activity of these transport systems requires continuous protein synthesis.

(3) The action of group B colicins requires functional cytoplasmic membrane proteins, some or all of which may be TonB-dependent permeases, which require the *tonB* gene product for their expression.

(4) The irreversible binding of phage T_1 and ϕ_{80} [19] requires a cytoplasmic membrane protein which may be the *tonB* gene product or some protein produced as a result of positive regulation by the TonB protein.

3.1. *The* tonB *gene product as a positive regulator* (1(a))

Integrated metabolic systems for sugars [65], amino acids [66,67] and phage replication [68,69] in bacteria contain control mechanisms capable of positively regulating gene expression.

The main criteria for establishing the existence of positive regulators of structural genes have been discussed in [70]. Those for which evidence is available TonB are discussed below.

(i) The occurrence of pleiotropic-negative mutants at high frequency similar to that found in a structural gene, may be taken as presumptive evidence for a positive regulator

Of strains selected for resistance to albomycin, an antibiotic of similar structure to ferrichrome, between 10% [9] and 25% [28] of resistant strains mapped close to the tryptophan region indicating that they are probably *tonB*. These figures represent a high frequency of *tonB* strains. The pleiotropic nature of *tonB* strains has been discussed.

(ii) The isolation of a deletion mutation (or a few nonsense mutations) in the proposed regulatory gene whose phenotypic expression results in the failure to induce or derepress structural genes in one or more operons (pleiotropic-negative mutants, R^-*)*

A large number of *tonB* strains are deletions [23,30]. The length of the deletion varies and depends on the nature of the chromosome surrounding this region [31]. These mutants cannot accumulate various iron-chelators and vitamin B_{12} for which distinct transport systems exist (see above) and hence fail to induce the structural genes.

(iii) Demonstration that the regulatory gene is not part of the operon(s) that it is proposed to control

The map position of the *tonB* allele (min 27) on the chromosome of *E. coli* is quite different from those operons that it is proposed to control; i.e., operons responsible for the transport of ferric enterochelin (min 13), ferrichrome (min 3), ferric citrate (min 6) and vitamin B_{12} (min 89).

(iv) Test for dominance: R^+ *(wild-type, inducible allele) and* R^c *alleles should be dominant to* R^-

The $tonB^+$ gene on a plasmid is dominant over a *tonB* lesion on the chromosome [31].

Although these conditions are largely fulfilled by the characteristics of the *tonB* gene, an important criterion remains unsubstantiated: constitutive strains able to accumulate ferrichrome (for example) but remaining *tonB*, should be isolated. Such strains would have a mutation in the *fhuB* operator region which allows expression of that gene without positive regulation by the TonB protein. A number of the conditions outlined in [70] are fulfilled suggesting that the TonB protein acts as a positive regulator (activator) of permease genes involved in iron and vitamin B_{12} transport.

Little is known of the number and fine structure of *fhu*, *fep*, *fec* and *btu* genes in terms of promoter and operator regions, and base sequences. With *fhu* genes, merodiploid analysis [28] showed that *fhuA* and *fhuB* genes are necessary for the uptake of ferrichrome. In [50] a functional *fhuA* gene product was shown necessary for the receptor function in the outer membrane while *fhuB* is required for transport across the cytoplasmic membrane, as predicted in [28]. The *fhuB* gene should contain a specific operator sequence to accommodate the hypothesized function of the TonB protein as its positive regulator.

Although 2 different classes of phenotype have been shown for *fep* (A + B) mutants [51], *btu* (B + A) mutants [71] and *fec* (A + B) mutants [72] and these classes have different cotransduction frequencies with neighbouring genetic markers, complementation analysis has yielded only single classes with *fep* [73] and *btu* mutants [48]. While 2 *fhu* genes have been shown by merodiploid analysis, only negative complementation results have been obtained with *fep* and *btu* mutants.

3.2. *The* tonB *gene product involved in post-transcriptional modification* (1(b))

In view of the contradictions to (1(a)) presented by the putative single cistron, a function of TonB protein in post-translational modification of transport proteins can be envisaged. Thus in *fep*, *btu* and perhaps *fec* systems, the TonB function would involve modification of the receptor protein so that it remained functional within the cytoplasmic membrane as the permease. This could occur by a specific cleavage of the receptor protein during its extrusion through the membrane. A mutant defective in the activity of a TonB-dependent permease would also have a corresponding defunct receptor activity; however, the reverse would not necessarily occur. Thus mutants that were *fepB* could also be *fepA* with respect to ferric enterochelin transport. But strains which were *fepA* may have a functional FepB permease, since the defective part of the FepA may be cleaved off by the *tonB* gene product. A similar argument could apply to the *btu* system. In this case no genetic complementation of *fepA* and *fepB* would be anticipated.

In the *fhu A/B* system TonB function would involve the post-translational modification of the *fhuB* gene product. Proteases, like the *recA* gene product, specifically cleave certain proteins like the λ immunity repressor [75,76]. The observation that the TonB protein was associated with the cytoplasmic membrane [33] would fit with its proposed function in the post-translational modification of transport proteins as they pass through the membrane.

3.3. *Permeases of TonB-dependent systems are functionally unstable* (2)

Two sources of information support this proposition:

(i) When a *tonB* (Am) mutation was introduced into a *supD*^{ts} background and incubated at non-permissive temperature, the uptake of vitamin B_{12} and the sensitivity to colicin D (both TonB-dependent) decreased at a rate far greater than the sensitivity to phage BF23 which is dependent on the vitamin B_{12} receptor but is TonB-independent [42]. This was interpreted as meaning that the TonB function is relatively unstable. Following this hypothesis, the TonB protein would not be synthesized at the non-permissive temperature and the permease function would decline rapidly due to the lack of positive regulation of the permease gene and the proposed inherent instability of the permease protein.

(ii) Vesicles which accumulate ferric enterochelin are extremely difficult to prepare (unpublished) although good proline transport is possible. Limited uptake of ferrichrome into vesicles has been reported [77] although accumulation rates were very low. If the permeases are unstable continuous protein synthesis would be required to maintain the functional stability of the transport systems [44]. Some membrane-bound proteins are unstable [78].

3.4. *The action of group B colicins requires functional cytoplasmic membrane proteins, some or all of which, may be TonB-dependent permeases* (3)

Many *fepB* and *fhuB* strains are tolerant to colicin B [51] and colicin M [50], respectively. Colicin M-tolerant strains were selected on the basis of their albomycin resistance, mapped in the *dapD* region [9] and were unable to accumulate ferrichrome after the outer membrane had been made permeable by prior pronase treatment [50]. The frequency of colicin-tolerant *fepB* strains or *fhuB* strains is low.

3.5. *The irreversible binding of phage* T_1 *and* ϕ_{80} *requires a cytoplasmic membrane protein which may be the* tonB *gene product or some protein produced as a result of the proposed positive regulator activity of the TonB protein* (4)

The N protein, which is a positive regulator protein for some genes of λ phage, increases the interaction between λ and the membranes of the host cell [81]. Whether this is a result of insertion of the N protein into the membrane or indirectly by the insertion of another protein, itself regulated by the N protein, is unclear. The TonB protein is present in the cytoplasmic membrane fraction after sarkosyl treatment [33].

4. Conclusion

This hypothesis accommodates the observation that a large number of *tonB* strains, many of which are deletions, are isolated from strains resistant to albomycin and unable to utilise ferrichrome as an iron source. It explains why the uptake of ferric enterochelin, ferrichrome, ferric citrate and vitamin B_{12} is not observed in *tonB* strains, without invoking an energy coupling argument. The energy-dependent phase of transport is absent in *tonB* strains, due to the low expression of cytoplasmic permeases, rather than the coincident ability to couple energy from the membrane potential.

Low levels of FepB permease can be produced in a *tonB* strain, allowing limited accumulation ('leak') and growth to occur in the presence of ferric enterochelin. The data in [3] were explained by the ferric enterochelin/enterochelin ratio being rate limiting for growth when low levels of FepB permease were biosynthesized in a *tonB* strain.

The instability of TonB-dependent permeases had been attributed to the TonB function [42,44], on the finding that vitamin B_{12} uptake decreases at a greater rate than the sensitivity to phage BF23 after the *tonB* gene had been made non-functional. Since the TonB protein would be involved in the expression of the relevant permeases, in this case the vitamin B_{12} permease, and since this permease is relatively unstable, the hypothesis accommodates the finding. The requirement of continuous protein synthesis for vitamin B_{12} uptake is also explained by the instability of the TonB-dependent permeases. This instability may be an adaptation in *E. coli* to protect itself against antagonists like group B colicins and certain phage, after the supply of iron for cell growth has been satisfied.

That ferrichrome and ferric enterochelin can induce resistance to group B colicins, and that this resistance occurs by specific inhibition at the level of the receptor and by the availability of iron [18], can be explained by the inactivation (or repression of the *tonB* gene) of the function of TonB protein and the consequent lack of expression of cytoplasmic membrane proteins necessary for group B colicin action. That both ferrichrome and dihydroxybenzoate, the precursor of ferric enterochelin, decrease the uptake of vitamin B_{12} [43], is similarly explained: iron in sufficient quantities decreases the expression of the permease for vitamin B_{12} transport, via a decrease in the activity of the TonB protein.

This hypothesis postulates that facilitated diffusion takes place at the outer membrane whereas active accumulation is restricted to processes across and surrounding the cytoplasmic membrane. The cytoplasmic membrane is unique for its coupling of energy production from respiration and ATP hydrolysis to energy utilization (transport and oxidative phosphorylation) in a chemiosmotic manner. As this hypothesis assumes accepted theories of regulation and post-transcriptional modification it should be readily corroborated.

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References

[1] Fredericq, P. and Sinarda, J. (1970) Ann. Inst. Pasteur 118, 767.
[2] Pugsley, A. P. and Reeves, P. (1976) J. Bacteriol. 127, 218–228.
[3] Frost, G. E. and Rosenberg, H. (1975) J. Bacteriol. 124, 704–712.
[4] Hantke, K. and Braun, V. (1975) FEBS Lett. 49, 301–305.
[5] Pugsley, A. P. and Reeves, P. (1976) J. Bacteriol. 126, 1052–1062.
[6] Williams, P. H. (1979) Infect. Immun. 26, 925–932.
[7] Stuart, S. J., Greenwood, K. T. and Luke, R. K. (1980) J. Bacteriol. 143, 35–42.
[8] Bassford, P. J., Bradbeer, C., Kadner, R. J. and Schnaitman, C. A. (1976) J. Bacteriol. 128, 242–247.
[9] Braun, V., Hancock, R. E. W., Hantke, K. and Hartmann, A. (1976) J. Supramol. Struct. 5, 37–58.
[10] Pugsley, A. P. and Reeves, P. (1977) Biochem. Biophys. Res. Commun. 74, 903–911.

[11] Hollifield, W. C. and Neilands, J. B. (1978) Biochemistry 17, 1922–1928.
[12] Wayne, R. and Neilands, J. B. (1975) J. Bacteriol. 121, 497–503.
[13] White, J. C., Di Girolamo, P. M., Fu, M. L., Preston, Y. A. and Bradbeer, C. (1973) J. Biol. Chem. 248, 3978–3986.
[14] Braun, V., Schaller, K. and Wolff, H. (1973) Biochim. Biophys. Acta 323, 87–97.
[15] Luckey, M., Wayne, R. and Neilands, J. B. (1975) Biochem. Biophys. Res. Commun. 64, 687–693.
[16] DiMasi, D. R., White, J. C., Schnaitman, C. A. and Bradbeer, C. (1973) J. Bacteriol. 115, 506–513.
[17] Bradbeer, C., Woodrow, M. L. and Khalifah, L. I. (1976) J. Bacteriol. 125, 1032–1039.
[18] Wayne, R., Frick, K. and Neilands, J. B. (1976) J. Bacteriol. 126, 7–12.
[19] Hancock, R. E. W. and Braun, V. (1976) J. Bacteriol. 125, 409–415.
[20] McCray, J., Schoner, R. and Herrmann, K. M. (1973) Fed. Proc. FASEB 32, 464.
[21] McCray, J. W. and Herrmann, K. M. (1976) J. Bacteriol. 125, 608–614.
[22] Eisenberg, S. P., Yarus, M. and Soll, L. (1979) J. Mol. Biol. 135, 111–126.
[23] Guterman, S. K. and Dann, L. (1973) J. Bacteriol. 114, 1225–1230.
[24] McIntosh, M. A. and Earhart, C. F. (1977) J. Bacteriol. 131, 331–339.
[25] Pugsley, A. P. and Reeves, P. (1976) Biochem. Biophys. Res. Commun. 70, 846–853.
[26] Konisky, J., Soucek, S., Frick, K., Davies, J. K. and Hammond, C. (1976) J. Bacteriol. 127, 249–257.
[27] Postle, K. and Reznikoff, W. S. (1978) J. Bacteriol. 136, 1165–1173.
[28] Kadner, R. J., Heller, K., Coulton, J. W. and Braun, V. (1980) J. Bacteriol. 143, 256–264.
[29] Gratia, J. P. (1964) Ann. Inst. Pasteur 107 suppl. 5, 132–151.
[30] Gratia, J. P. (1966) Biken. J. 9, 77–87.
[31] Coukell, M. B. and Yanofksy, C. (1971) J. Bacteriol. 105, 864–872.
[32] Postle, K. and Reznikoff, W. S. (1979) J. Mol. Biol. 131, 619–636.
[33] Plastow, G. S. and Holland, I. B. (1979) Biochem. Biophys. Res. Commun. 90, 1007–1014.
[34] Rosenberg, H. and Young, I. G. (1974) in: Microbial Iron Metabolism: A comprehensive treatise, ch. 3, Academic Press. New York.
[35] Porra, R. J., Langman, L., Young, I. G. and Gibson, F. (1972) Arch. Biochem. Biophys. 153, 74–78.
[36] Hantke, K. and Braun, V. (1978) J. Bacteriol. 135, 190–197.
[37] Randall-Hazelbauer, L. and Schwartz, M. (1973) J. Bacteriol. 116, 1436–1446.
[38] Szmelcman, S. and Hofnung, M. (1975) J. Bacteriol. 124, 112–118.
[39] Braun, V. and Krieger-Brauer, H. J. (1977) Biochim. Biophys. Acta 469, 89–98.
[40] Hantke, K. (1976) FEBS Lett. 70, 109–112.
[41] Krieger-Brauer, H. J. and Braun, V. (1980) Arch. Microbiol. 124, 233.
[42] Bassford, P. J., Schnaitman, C. A. and Kadner, R. J. (1977) J. Bacteriol. 130, 750–758.
[43] Kadner, R. J. and McElhaney, G. (1980) J. Bacteriol. 143, 135–141.
[44] Kadner, R. J. and McElhaney, G. (1978) J. Bacteriol. 134, 1020–1029.
[45] Bassford, P. J., Kadner, R. J. and Schnaitman, C. A. (1977) J. Bacteriol. 129, 265–275.
[46] Reynolds, P. R., Mouttur, G. P. and Bradbeer, C. (1980) J. Biol. Chem. 255, 4313–4319.
[47] DiGirolamo, P. M., Kadner, R. J. and Bradbeer, C. (1971) J. Bacteriol. 106, 751–757.
[48] Bassford, P. J. and Kadner, R. J. (1977) J. Bacteriol. 132, 796–805.
[49] Sabet, S. F. and Schnaitman, C. A. (1973) J. Biol. Chem. 248, 1797–1806.
[50] Wookey, P. J., Hussein, S. and Braun, V. (1981) J. Bacteriol. 146, 1158–1161.
[51] Wookey, P. J. and Rosenberg, H. (1978) J. Bacteriol. 133, 661–666.
[52] Davies, J. K. and Reeves, P. (1975) J. Bacteriol. 123, 96–101.
[53] Braun, V., Schaller, K. and Wabl, M. R. (1974) Antimicrob. Agents Chemother. 5, 520–533.
[54] Braun, V., Frenz, J., Hantke, K. and Schaller, K. (1980) J. Bacteriol. 142, 162–168.
[55] Konisky, J. (1980) in: Bacterial outer membranes: Biogenesis and function (Inouye, M. ed) p. 319, Wiley, New York.
[56] Bayer, M. E. (1968) J. Virol. 2, 346–356.
[57] Frost, G. E. (1974) PhD Thesis, Australian National University, Canberra, ACT.
[58] Leong, J. and Neilands, J. B. (1976) J. Bacteriol. 126, 823–830.
[59] Pugsley, A. P. and Reeves, P. (1977) J. Bacteriol. 130, 26–36.
[60] Wookey, P. J., Rosenberg, H., Young, I. G. and Braun, V. (1982) in preparation.
[61] Lieberman, M. A. and Hong, J. S. (1976) J. Bacteriol. 125, 1024–1031.
[62] Lieberman, M. A., Simon, M. and Hong, J. S. (1977) J. Biol. Chem. 252, 4056–4067.
[63] Hartmann, A. and Braun, V. (1980) J. Bacteriol. 143, 246–255.
[64] Weaver, C. A. and Konisky, J. (1980) J. Bacteriol. 143, 1513–1518.
[65] DeCrombrugghe, B. and Pastan, I. (1978) in: The Operon (Miller, J. H. and Reznikoff, W. S. eds) pp. 303–324, Cold Spring Harbor Laboratory, New York.
[66] Yanofsky, C. (1981) Nature 289, 751–758.
[67] Whipp, M. and Pittard, A. J. (1977) J. Bacteriol. 132, 453–461.
[68] Rosenberg, M., Court, D., Shimatake, H., Brady, C. and Wulff, D. (1978) in: The Operon (Miller, J. H. and Reznikoff, W. S. eds) pp. 345–371, Cold Spring Harbor Laboratory, New York.

[69] Herskowitz, I. and Hagen, D. (1980) Annu. Rev. Genet. 14, 399–445.
[70] Englesberg, E. and Wilcox, G. (1974) Annu. Rev. Genet. 8, 219–242.
[71] Kadner, R. J. and Liggins, G. L. (1973) J. Bacteriol. 115, 514–521.
[72] Husscin, S., Hantke, K. and Braun, V. (1981) Eur. J. Biochem. 117, 431–437.
[73] Laird, A. J., Ribbons, D. W., Woodrow, G. C. and Young, I. G. (1980) Gene 11, 347–357.
[74] Oeschger, M. P. and Berlyn, M. K. B. (1974) Mol. Gen. Genet. 134, 77–83.
[75] Roberts, J. W., Roberts, C. W. and Craig, N. L. (1978) Proc. Natl. Acad. Sci. USA 75, 4714–4718.
[76] Roberts, J. W. and Roberts, C. W. (1975) Proc. Natl. Acad. Sci. USA 72, 147–151.
[77] Negrin, R. S. and Neilands, J. B. (1978) J. Biol. Chem. 253, 2339–2342.
[78] Schroer, D. W. and St. John, A. C. (1981) J. Bacteriol. 146, 476–483.
[79] Larrabee, K. L., Phillips, J. O., Williams, G. J. and Larrabee, A. R. (1980) J. Biol. Chem. 255, 4125.
[80] Mount, D. W. (1980) Annu. Rev. Genet. 14, 279–319.
[81] Hallick, L., Boyce, R. P. and Echols, H. (1969) Nature 223, 1239–1242.
[82] Young, I. G. (1976) Prep. Biochem. 6, 123–131.