NOTES

Strong Function-Related Homology between the Pore-Forming Colicins K and 5

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Sequence determination of the *Escherichia coli* colicin K determinant revealed identity with the *E. coli* colicin 5 determinant in the immunity and lysis proteins, strong homologies in the pore-forming region (93.7%) and the Tsx receptor-binding region (77%) of the colicins, and low levels of homology (20.3%) in the N-terminal region of the colicins. This latter region is responsible for the Tol-dependent uptake of colicin K and the Ton-dependent uptake of colicin 5 in the respective colicins. During evolution, the DNA encoding colicin activity and binding to the Tsx receptor was apparently recombined with two different DNA fragments that determined different uptake routes, leading to the differences observed in colicin K and colicin 5 import.

The properties of colicin K (6, 10) and the mode of action of colicin K on cells and in artificial membranes (8, 24) have been studied in detail. Colicin K forms defined channels in membranes that destroy the electrochemical potential of the cytoplasmic membrane, and thus it kills cells. Uptake into sensitive cells requires the outer membrane proteins Tsx, OmpF, and OmpA (20) and the Tol system, composed of TolA, TolQ, TolR, and TolB (25). Colicin K has been purified from spent medium, and the amino acid composition has been determined (6). Although it was confused for some time with colicin A (11), colicin K is one of the well-characterized colicins.

We are interested in colicins because they are suitable tools to study the import of proteins through the outer membrane and the periplasmic space and into or across the cytoplasmic membrane. This process must be efficient since only a few molecules of colicin suffice to kill sensitive cells of Escherichia coli. Recently, we characterized colicins 5 (16) and 10 (15), which are unusual since they are taken up via the Ton system, composed of the TonB, ExbB, and ExbD proteins (2, 17), but their cognate Tsx receptor was thought not to belong to the Ton-dependent receptor proteins. Since colicin K binds to Tsx and requires the Tol system for uptake, we compared colicin K with colicins 5 and 10 to see whether their amino acid sequences reveal regions involved in the common Tsx dependence and the distinct Ton and Tol dependence. Furthermore, between colicins K and 5 we observed cross-immunity, which is rather unusual among colicins since even structurally and functionally closely related colicins display distinct immunities (3, 5, 12, 19).

The colicin K determinant was excised from plasmid pColK-K49 (Fig. 1) with *Asp*700I, and the 3.8-kb restriction fragment was cloned into the *Hin*dII site of pBCKS+. *E. coli* 5K transformed with the resulting plasmid pHP50 released colicin K and was immune to crude cell extracts obtained from *E. coli* K49(pColK-K49) and *E. coli* BZB2116(pColK-K235) containing colicin K. Both strands of a 2,161-bp fragment were completely sequenced (Fig. 2), revealing three encoded genes des-

ignated *cka* (colicin K activity), *cki* (colicin K immunity), and *ckl* (colicin K lysis). *cka* and *ckl* had the same transcription polarity, and *cki* had the opposite polarity (Fig. 1), an arrangement typical for genes of pore-forming colicins. *cka*, *cki*, and *ckl* encode open reading frames of 548, 96, and 43 amino acid residues, respectively. The colicin produced by transformants expressing the cloned colicin K determinant was active against the same cells as the colicin formed by *E. coli*(pColK-K49): wild-type cells were sensitive, while cells with mutations in *tsx*, *ompF*, *ompA*, or *tolA* were insensitive (Table 1).

Since the literature reports differing molecular weights of colicin K, ranging from 45,000 to 75,000 (9), we performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (16) with cell extracts of *E. coli* WM1576(pHP50) (Fig. 3). The molecular weight derived from the electrophoretic mobility was 66,000, which is close to the calculated molecular weight of 59,611. The amino acid composition derived from the nucleotide sequence is very close to the composition determined by amino acid analysis of isolated colicin K-K235 (Table 2).

Comparison of the amino acid sequences of colicins K and 5 (Fig. 4) revealed a low level of homology in the N-terminal region (20.3%), which in all colicins contains the determinant for transport across the outer membrane. The different Nterminal sequences reflect the different uptake routes of colicins K and 5 via the Tol and the Ton systems, respectively, and they reflect the TolC dependence of only colicin 5 (16), as defined previously by comparison of colicins 10 and E1 (15). In contrast, the central region is highly homologous (77% identity), reflecting binding of both colicins to the common Tsx receptor. The level of homology in the pore-forming domains at the C-terminal end is even higher (93.7% identity). Since the C terminus is thought to interact with the immunity protein, cross-immunity between colicins K and 5 is based on the identical sequences in this region, and the distinct sequences of colicins 5 and 10 in this region confer the colicin-specific immunities (16) (underlined in Fig. 4). The 100% identity between the immunity proteins of colicins K and 5 further supports the argument for complete cross-immunity. Although there might be strict constraints on the structure of immunity proteins for specific interaction with their cognate colicins, it is

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FIG. 1. Arrangement of the colicin K activity (*cka*), immunity (*cki*), and lysis (*ckl*) genes on the natural plasmid pColK-K49. The arrows indicate the transcription polarity. Restriction sites indicated are those used for cloning.

<u>GTA</u>	<u>.C</u> AG1	TAT	ATTO	FTT	TA	\TT]	[AT]	IGT:	rtt <i>i</i>	\AT1	TAT	'A <u>A</u> 7	GAG	<u>iga</u> a D.	TT	TATO M	GCI A	AAAO K	GA E	60
														6	cka	3	-	•		
ACT	'AAGI	rgg <i>i</i>	ATAT	rgg <i>i</i>	ACCA	ACI	rgci	rgg:	I'GAG	TCT	TATC	GGG	GGA	ACA	GGC	GCC	AA'I	CTG	AA	120
L	S	G	Y	G	P	т	A	G	Е	s	М	G	G	т	G	A	N	L	N	
TCA	GCA	AGGT	rgg7	AA7	'AA'	'AA'	[AG]	raa:	FAGI	rgge	GTI	CAC	TGG	GGT	GGJ	rggī	TCT	GGGG	CA	1.80
Q	Q	G	G	N	N	N	S	N	ន	G	v	H	W	G	G	G	S	G	н	
TGG	TAAT	'AA'	rggi	rGGT	CAG	GGI	AAT	CTC3	AA7	TCF	TCI	rggj	TCT	'ACA	TCF	ACT	GTG	ATG	ΑA	240
G	N	N	G	G	Q	G	N	S	N	S	s	G	s	т	S	т	v	M	ĸ	
AAC	CGGI	GAG	STC7	ATA7	CTC	ACT	CCC	TG	GGT	GAI	GTT	GTC	'AT'I	AAT	'AA'	GAT	GGC	CTTC	C	300
т	G	Е	S	Y	г	т	P	W	G	D	v	v	I	N	N	D	G	L	P	
GGT	GATO	AAT	rgg1	rati	GTI	ATO	ACA	\GA/	AGAG	TAA	TCZ	ACI	CTG	GTT	'GA'I	TAAT	CCA	TTTC	GG	360
v	M	N	G	I	v	м	т	E	Е	N	S	т	L	v	D	N	Р	F	G	
AGG	GGTC	TCC	CAGA	\GT7	CTT	AAT	TCI	CTT2	ATT	AGT	GAT	TATG	CCA	TCA	CT1	TTT	GCA	GAAZ	٩G	420
G	v	S	R	v	L	N	S	L	I	s	D	M	P	S	L	F	A	E	s	
CTC	AGGZ	LAAT	TAAC	CAAC	'AAT	AAC	CACI	GC.	TCF	GTI	TAAT	ACT	GCA	CCA	ACG	AA	GCI	CAGO	ЭT	480
S	G	N	N	N	N	N	т	A	s	v	N	т	A	P	т	N	A	Q	v	
CAG	TGAI	TATO	GAT	TAAG	AGT	TCO	AAG	GTC	GTG	AGI	TAAT	GTA	ATT	'AAT	GAA	AAZ	CAA	AAGO	CA	540
S	D	М	D	K	s	S	ĸ	v	v	s	N	v	I	N	Е	ĸ	Q	ĸ	Q	
GAA	GAAI	AAA	ATT	rgci	ACA	CAG	ATI	TAG:	rga <i>f</i>	AAA	CAG	AAG	AAA	ATC	GAC	GAC	ATG	AAAA	٩A	600
K	N	ĸ	I	A	т	Q	I	s	Е	ĸ	Q	ĸ	к	I	Е	E	М	ĸ	ĸ	
AGT	ATTO	'AAG	CAT	CA7	TCT	TAT	CAT	rgg:	TATC	'ACT	GAT	CTT	'GAG	AGG	GAI	GTI	GAT	GAGI	гт	660
v	F	ĸ	н	н	S	Y	Ħ	G	I	т	D	г	Е	R	D	v	D	Е	г	
GCA	АААЛ		ATCA	LAAI	CAG	CTI	GAT	IGCI	AGAT	TAT	AGC		CTI	AAT	тст	TAT	AAA	AATA	٩C	720
Q	ĸ	ĸ	s	N	Q	L	D	A	D	I	s	ĸ	L	N	s	Y	ĸ	N	т	
ATT	GCAA	AGI		LATT	GGI	GAT	GTG	AA		CAG	AAG	;GA⊅	GCT	GAA	GAA	AAG	GCT	CGTO	ΞA	780
L	Q	s	ĸ	I	G	D	v	N	ĸ	Q	ĸ	E	A	E	Е	ĸ	A	R	Е	
ДДД	TGCZ	GAG	GTC	יקריז	'G & Z	CAT	'GA 2	ACT	rC TT	רעמי	YGAZ	GAG	מממ	CAC	CCC	CTC	aca	ദ്യം	10	840
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CGT	GAAC K	GTC; V	ACC. T	GGA: G	VITI F		GGP: G	A.LG(M	CGI R	GAC D	GTT: V	CAG	AAA K	AAA K	CTA	CAG	CGT R	CAGO O	CT L	1020
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TGA	AGCI	raac K	SCAG	GC1	GAG E	TAC Y	TCC	CGC2	AGTI V	GAA E	ראבי א	GAA E	CTI	'AAG K	LAA N	GCI A	GTI	AGT1	TT F	1080
10		10	~ ~	~	-	-	5	~	v	-	74			T.	14	~	v	þ	F	

TABLE 1. Sensitivities of E. coli strains to colicins K and 5

Strain (genotype)	10-fold dilution resulting in clear (turbid) $zone^{a}$					
	Colicin K	Colicin 5				
5K (wild type)	2 (3)	2 (3)				
AB2847 (tsx)	(Ò)	(0)				
A592 (tolA)	r	2(3)				
HP45 $(ton \hat{B})$	2 (3)	r				
KB426 (<i>ompA</i>)	0 (2)	2 (3)				
MC513 (ompF)	0(1)	2 (3)				
C65 (tolC)	2 (3)	r				
5K, pHP51 (<i>cki</i> ⁺)	(1)	(1)				
5K, pHP40 (cfi ⁺)	(1)	(1)				

 a The last 10-fold dilutions which resulted in clear zones of growth inhibition and those resulting in turbid zones (in parentheses) are listed. For example, 2 indicates that the colicin suspension could be diluted 10^2 -fold to yield a clear zone. r, resistance.

surprising that no conservative amino acid substitutions and even no silent nucleotide replacements occurred in *cki* and *cfi*. The previous failure to recognize cross-immunity between colicins K and 5 (1) was probably due to the loss of pColK-K235 from *E. coli* BZB2116, since also in our hands, the same strain

CAGG	GAT	GGT.	AAG	GCC	GCT	'GAG	GTI	raa	AGAC	GCA	AGAG	CAC	GAAJ	ATT)	AAA	AGA.	AGC	TCA	GGA	1140
R	D	G	K	A	A	E	V	K	E	A	E	Q	K	L	K	E	A	Q	D	
TGCT	CTT	GAA.	AAG	AGC	CAG	ATA	AAZ	AGA:	rgct	rgt7	'GA'I	TAC	rato	GT	TGG2	ATT	TTA	TCA.	ATA	1200
A	L	E	K	S	Q	I	K	D	A	V	D	T	M	V	G	F	Y	Q	Y	
TATA I	ACC T	GAA E	CAA Q	TAT Y	GGA G	GAA E	AAZ K	ATA Y	GC7 A	AAAA K	ITA.	rgc". A	CAC Q	GA' D		AGC(A	GGA E	AAA K	GTC S	1260
ТААА	GGA	AAG.	AAA	ATT	CAA	.GGI	GTI	rgat	GA/	AGCT	CTC	GC/	AGC)	ATT	IGA	AAA(GTA	TAA	GAA	1320
К	G	K	K	I	Q	G	V	D	E	A	L	A	A	F	E	K	Y	K	N	
TGTA	CTG	GAT.	AAG	AAA	TTC	AGT	AAG	GTT	GAT	rcgo	GAT	rge:	TAT:	FTT	TAA'	rgc(GTT.	AGA	GTC	1380
V	L	D	K	K	F	S	K	V	D	R	D	A	I	F	N	A	L	E	S	
TGTT	AAT	TAT	GAT	GAG	TTA	TCT	AAG	BAAT	TTT	AACG	AAG	SAT/	ATC/	AAA(GTCC	GCT	ТАА	AAT	CAC	1440
V	N	Y	D	E	L	S	K	N	L	T	K	I	S	K	S	L	К	I	T	
CAGT S	CGA R	GTA' V	TCT S	TTT F	CTA L	TAT. Y	'GAT D	rGT1 V	GGT G	rtC1 S	GAI D	FTT:	ГААЛ К	AAA' N	IGCI A	AAT. I	AGA E	AAC T	IGG G	1500
TAAC	TGG	CGA	CCT	TTG	TTT	GTC	ACC	CTC	GAC	GAAA	AGI	rgco	GT(CGA'	IGT)	AGG'	TGT	GGC.	AAA	1560
N	W	R	P	L	F	V	T	L	E	K	S	A	V	D	V	G	V	A	K	
AATT	GTG	GCA'	TTA	ATG	TTT	AGT	TTI	TAT	GT.	rgg1	GTG	CC.	ICT:	rgg'	FTT(CTG	GGG	GAT	IGC	1620
I	V	A	L	M	F	S	F	I	V	G	V	P	L	G	F	W	G	I	A	
AATT	GTC	ACA	GGT.	ATT	GTT	TCT	тст	TTAC	TATA	AGGG	GAT	rga:	rgao	GTTC	GAG(CAA	ACT	таа'	IGA	1680
I	V	T	G	I	V	S	5	Y	I	G	D	D	E	L	S	K	L	N	E	
GTTG L	TTA L	GGG. G	ATT I	* TAA *	N TTT	R 'CTC	K TTT	L FAGA	T AGTO	¥ ₽TA₽	Y TAT	I TAT(F JAAJ	S ACT	P IGGG	L CAA'	G TCC	V TAC	P IGG	1740
I GATT	A GCC	M : ATT.	L AAT	L AAA	Y TAA	F AAC	L AAT	A rgcz	S AGAT	L FAAC	K TTC	C GCA	L FAAG	Y GTA	W	P GGG'	E TTC	K TTT	K TTT	1800
L TAAC	K TTT	T I GTA	N TTT	E TCT	K TTG	I ATA	Y .TA <u>1</u>	R FCTA Xbai	S AGAT	I FAT@	T GTI	L FAA'	K FTT2	S AGA(Y GTA:	P FGG.	Y ATA	L TAG	L CAA	1860
V	H	I	S	S	I	F	I	F	L	L	P	T	N	D	N	D	N	F	I	1920
TACA	TGT	ATT	GAT	GAT	ATA	.AAT	AT?	\AA1	TAAG	CAAA	AGGA	AGT(BTT2	ATC:	ATTO	GTC	GTT.	AAA'	TAT	
L TAAA	I ATA	L AGA	I ATC	W CAT S.D	P GGT	I 'ATA	L AGI	S IGA:	E TC2	P AGGT	L TAAZ	N ATT2	H ATG	L FAG	Y GTAC	Υ GTA	K TTT	L TAA	H ATG	1980
M ← CATA	C AGT	ki att	<u>A</u> TTT	<u>atg</u> ACT	<u>AAA</u> TTT	<u>A</u> GT <u>1</u>	<u>'GG</u> #	<u>AAA(</u> 5.D	نم <u>م:</u> م	ATA A	IGAJ	1AA' C :	FAA' I	ГАА' I	TTT I J	FTC F	TAA L	TTA I	CAA F	2040
TATT I L	ATT F	CTT L	GTC S	AGG G	ATG C	TCA			ACTZ	ATA1 Z I		GTGJ R J	ATG:	FAC.	AAGO Q (GAG G	GAA G	CCG	rtg V	2100
CACC A P	ATC S	TTC S	GTC S	TTC S	TGA E	ACT	GAG	CGG(r (GA:	rcgo I 7	GGT	PTC	AGTZ	AGA.	AAA	GAT	CAA	A <u>GG</u>	ATC:	<u>r</u> 2161

FIG. 2. Nucleotide sequences of cka, cki, and ckl. The predicted Shine-Dalgarno sequence (S.D.) is indicated. This sequence has been deposited in the EMBL nucleotide sequence data library (accession no. X87834).



FIG. 3. SDS-PAGE of $[^{35}S]$ methionine-labeled proteins of *E. coli* WM1576(pHP50 *cka*) and WM1576(pHP51 *cki*). The arrows indicate colicin K (lane 1) and the immunity protein (lane 2). The molecular masses (in kilodal-tons) of standard proteins (St.) are given.

TABLE 2. Amino acid composition of colicir	ı K
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	Daduaad na	% of total residues					
Amino acid(s)	of residues	Deduced	Chemically determined				
Lys	58	10.58	10.7				
His	6	1.09	1.1				
Arg	11	2.01	2.2				
Asn + Asp	42 + 30	7.66 + 5.47	13.5				
Thr	24	4.38	4.6				
Ser	50	9.12	9.1				
Gln + Glu	27 + 48	4.93 + 8.76	14.5				
Pro	9	1.64	1.7				
Gly	44	8.03	8.1				
Ala	47	8.58	8.7				
Cys	0	0	0				
Val	46	8.39	7.9				
Met	10	1.82	1.5				
Ile	25	4.56	4.2				
Leu	38	6.93	6.9				
Tyr	13	2.37	2.5				
Phe	15	2.73	2.8				
Trp	5	0.91	0.9				

^{*a*} The amino acid content, expressed both in absolute terms and as a percentage of the total number of amino acid residues, was determined from the nucleotide sequence and compared with the chemically determined composition of the purified protein reported by Goebel (6).

COL	5	MDKVTDNSPDVESTESSEGSFPTVGVDTGDTITATLATGTENVGGGGGGAF	-50
COL	ĸ	MAKELSGYGPTAG-ESMGGTGANLNQQGGNNNSNSGVHWGGGSGHGNNGG	-49
COL	5	GGASESSAAIHATAKWSTAQLKKHQAEQAARAAAAEAALAKAKSQRDALT	-100
COL	ĸ	QGNSNSSGSTSTVMKTGESYLTPWGDVVINNDGLPVMNGIVMTEENSTLV	-99
COL	5	QRLKDIVNDALRANAARSPSVTDLAHANNMAMQAEAERLRLAKAEQKARE	-150
COL	ĸ	DNPFGGVSRVLNSLISDMPSLFAESSGNNNNNTASVNTAPTNAQVSDMDK	-149
COL	5	EAEAAEKALREAERQRDEIARQQAETAHLLAMA	-183
COL	к	SSKVVSNVINEKQKQKNKIATQISEKQKKIEEMKKVFKHHSYHGITDLER	-199
COL	5	EAAEAEKN	-191
COL	ĸ	** * ** DVDELQKKSNQLDADISKLNSYKNTLQSKIGDVNKQKEABEKARENAEVA	-249
		Tay	7
COL	5	RQDSLDEEHRAVEVAEKKLAEAKAELAKAESDVQSKQAIVSRVAGELENA	-241
COL	к	EHETLNEEKQAVAEAEKRLAEAKAELAKAESDVQSKQATVSRVAGELENA	-299
COL	5	QKSVDVKVTGFPGWRDVQKKLERQLQDKKNEYSSVTNALNSAVSIRDAKK	-291
COL	к	**************************************	-349
		·	
COL	5	TDVONAETKI.KRAKDALEKSOVKDSVDTMVGEVOVITEOVGEKVSBIAOD	-341
00.3	-	.* ** ***** *****	1
COL	ĸ	AEVKEAEQKLKEAQDALEKSQIKDAVDTMVGFYQYITEQYGEKYAKIAQD	- 399
COL	5	LAEKAKGSKFSSVDEALAAFEKYKNVLDKKISKVDRDAIFNALESVNYDE	-391
COL	ĸ	LAEKSKGKKIQGVDEALAAFEKYKNVLDKKFSKVDRDAIFNALESVNYDE	-449
COL	5	LSKNLTKISKSLKITSRVSFLYDVGSDFKNAIETGNWRPLFVTLEKSAVD	-441
COL	ĸ	**************************************	499
]
COL	5	VGVAKIVALMFSFIVGVPLGFWGIAIVTGIVSSYIGDDELNKLNELLGI	-490
COL	ĸ	VGVAKIVALMFSFIVGVPLGFWGIAIVTGIVSSYIGDDELSKLNELLGI	-548
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FIG. 4. Comparison of the colicin K and colicin 5 amino acid sequences. Identical residues are marked by asterisks, similar residues are indicated by dots, and dashes indicate sequence gaps that were introduced to optimize the homology. The region proposed to be involved in the Tsx-dependent uptake across the outer membrane and the proposed pore-forming region in the cytoplasmic membrane are boxed.

from the same source (T. Pugsley, Institut Pasteur, Paris, France) did not confer immunity to colicins 5 and K and also did not produce colicin K. This conclusion is supported by the phenotype of strain BZB2116 pColK-K235, obtained from E. Bremer, University of Marburg, Marburg, Germany, that conferred immunity to colicins 5, K-K235, and K-K49.

The lysis proteins that facilitate release of colicins generally display a high degree of homology but are not identical. However, Ckl and Cfl are identical, and the nucleotide sequences of *ckl* and *cfl* differ only by a single base pair. Since Ckl contains a cysteine residue in the consensus sequence of lipoproteins of the murein-lipoprotein type (7), it is likely that Ckl is modified by the same type of lipid as are the other lysis proteins (22).

Colicins display the same multidomain structure regardless of whether they form channels, act as nucleases, or inhibit lipid carrier regeneration (3, 19). The functional domains are responsible for binding to the receptor (central region), for uptake via the Ton or Tol system (N-terminal region), and for killing cells and binding to the immunity protein (C-terminal region). The uptake region of colicin 10 has been further subdivided into sites involved in Ton- and TolC-dependent transport (15). The data on colicin K support our previous domain assignments in that colicin K shows only a very low level of homology to colicin 5 in the N-terminal region, because it does not require the Ton system and TolC, as does colicin 5. In addition, our proposal that colicin genes are assembled from DNA fragments that encode functional domains



FIG. 5. Differences in the nucleotide sequences, indicated by vertical bars, of the genes encoding the activity, immunity, and lysis genes of colicins K and 10, compared with those of colicin 5. The genes encoding the activity proteins were divided into gene fragments coding for domains binding to the Tsx receptor and pore formation. The 3' part of *cka* contains so many nucleotide replacements that they were no longer resolved (black box).

(15, 23) is fully supported by the sequences of the colicin K determinant. If one relates the nucleotide sequences of colicins K and 10 with those of colicin 5, the differences are centered in the 3' half of cta, in cti, in ctl, and in the 5' half of the cka gene (Fig. 5). This pattern of nucleotide substitutions suggests that the DNA fragment encoding the N-terminal half of colicin K is derived from a source different from that of colicins 5 and 10 and that the DNA fragment encoding the C-terminal half of colicin 10 and the immunity and lysis proteins must have an origin other than that of colicins K and 5. The number of amino acid replacements in the N-terminal part of colicin K, which is much higher than that in the central part, suggests different origins of these two DNA fragments. It is remarkable that the various DNA pieces encode rather precise functional domains which when recombined within the homologous regions in different combinations give rise to new colicins.

The plasmids encoding the determinants of colicins K, 5, 10, and E1 are similar in size, and the colicin activity, immunity,



FIG. 6. (A) Sequence similarity between colicins K (residues 13 to 53) and E3 (residues 31 to 71) in the regions presumably involved in Tol-dependent uptake across the outer membrane. The previously proposed TolA box (15) is underlined. (B) The sequence similarity between colicins K and A that presumably specifies interaction with the OmpF protein in the outer membrane. Asterisks in both panels denote identical residues; dashes indicate similar residues.

and lysis genes are arranged in the same manner as that shown in Fig. 1 for the colicin K determinant. The sequences flanking the colicin determinants are homologous (data not shown), and the common EcoRI site of the pCol5, pCol10, and pColK plasmids is located in a gene homologous to the exc2 gene of pColE1 (4) (data not shown). These features and the amino acid sequences assign colicin K to the group of pore-forming colicins to which colicins E1, Ia, Ib, 5, and 10 belong, as opposed to the class of pore-forming colicins composed of colicins A, B, and N.

We tentatively proposed a TolA box in colicin E1 (15). A similar box is contained in colicin K (underlined in Fig. 6A) located at the beginning of the glycine-rich sequence in the N terminus, which occurs in many colicins (21). Colicin K exhibits an additional high level of sequence homology with a short segment of colicin E3 (13) (Fig. 6A). Furthermore, colicin K displays a strong sequence homology with a small portion of colicin A (14) (Fig. 6B), with which it shares the OmpF and Tol requirement for uptake. After binding to the same Tsx receptor, colicins K, 5, and 10 are translocated across the outer membrane by distinct transport systems. How these transport systems differ mechanistically can only be assessed when the modes of action of the Ton and the Tol systems are understood. The colicins may be suitable tools to unravel these systems.

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REFERENCES

- Bradley, D. E., and S. P. Howard. 1992. A new colicin that adsorbs to outer membrane protein Tsx but is dependent on the *tonB* instead of the *tolQ* membrane transport system. J. Gen. Microbiol. 138:2721–2724.
- Braun, V. 1995. Energy-coupled transport and signal transduction through the Gram-negative outer membrane via TonB-ExbB-ExbD-dependent receptor proteins. FEMS Microbiol. Rev. 16:295–307.
- 3. Braun, V., H. Pilsl, and P. Groß. 1994. Colicins: structures, modes of action,

transfer through membranes, and evolution. Arch. Microbiol. 161:199–206. 4. Chan, T. C., H. Ohmori, J. I. Tomizawa, and J. Lebowitz. 1985. Nucleotide

- sequence and gene organization of ColE1 DNA. J. Biol. Chem. 260:8925– 8935.
- De Graaf, F. K., and B. Oudega. 1986. Production and release of cloacin DF13 and related colicins. Curr. Top. Microbiol. Immunol. 125:183–205.
- Goebel, W. F. 1973. The nature of colicin K of *Escherichia coli* K235. Proc. Natl. Acad. Sci. USA 70:854–858.
- Hantke, K., and V. Braun. 1973. Covalent binding of lipid to protein. Diglyceride and amide-linked fatty acid at the N-terminal end of the mureinlipoprotein of the *Escherichia coli* outer membrane. Eur. J. Biochem. 34: 284–296.
- Jetten, A. M., and M. E. R. Jetten. 1975. Energy requirement for the initiation of colicin action in *E. coli*. Biochim. Biophys. Acta 387:12–22.
- Konisky, J. 1978. The bacteriocins, p. 71–136. In L. N. Ornston and I. R. Sokatch (ed.), The bacteria, a treatise on structure and function, vol. 6. Academic Press, New York.
- Kunugita, K., and M. Matsuhashi. 1970. Purification and properties of colicin K. J. Bacteriol. 104:1017–1019.
- Luria, S. E. 1982. The mistaken identity of colicin A. J. Bacteriol. 149:386.
 Mankovich, J. A., C.-H. Hsu, and J. Konisky. 1986. DNA and amino acid
- sequence analysis of structural and immunity genes of colicins Ia and Ib. J. Bacteriol. 168:228–236.
 13. Masaki, H., and T. Ohta. 1985. Colicin E3 and its immunity gene. J. Mol.
- Biol. 182:217–227.
 14. Morion, J., R. Lloubes, S. Varenne, M. Chartier, and C. Lazdunski. 1983. Complete puedestide sequence of the structural gene for collisin A. a gene
- Complete nucleotide sequence of the structural gene for colicin A, a gene translated at a non-uniform rate. J. Mol. Biol. **170**:271–285.

- Pilsl, H., and V. Braun. 1995. Novel colicin 10: assignment of four domains to the TonB- and TolC-dependent uptake via the Tsx receptor and to pore formation. Mol. Microbiol. 16:57–67.
- Pilsl, H., and V. Braun. 1995. Evidence that the immunity protein inactivates colicin 5 immediately prior to the formation of the transmembrane channel. J. Bacteriol. 177:6966–6972.
- Postle, K. 1993. TonB protein and energy transduction between membranes. J. Bioenerg. Biomembr. 25:591–601.
- Pugsley, A. P. 1984. The ins and outs of colicins. Part I. Production and translocation across membranes. Microbiol. Sci. 1:168–175.
- Pugsley, A. P. 1984. The ins and outs of colicins. Part II. Lethal action, immunity and ecological implications. Microbiol. Sci. 1:203–205.
- Pugsley, A. P. 1985. *Escherichia coli* K12 strains for use in the identification and characterization of colicins. J. Gen. Microbiol. 131:369–376.
- Pugsley, A. P. 1987. Nucleotide sequencing of the structural gene for colicin N reveals homology between the catalytic C-terminal domains of colicin A and N. Mol. Microbiol. 1:317–325.
- Pugsley, A. P. 1988. The immunity and lysis genes of ColN plasmid pCHAP4. Mol. Gen. Genet. 211:335–341.
- Roos, U., R. E. Harkness, and V. Braun. 1989. Assembly of colicin genes from a few DNA fragments. Nucleotide sequence of colicin D. Mol. Microbiol. 3:891–902.
- Schein, S. J., B. L. Kagan, and A. Finkelstein. 1978. Colicin K acts by forming voltage-dependent channels in phospholipid bilayer membranes. Nature (London) 276:159–163.
- Webster, R. E. 1991. The tol gene products and the import of macromolecules into Escherichia coli. Mol. Microbiol. 5:1005–1011.