The positive inside rule is not determined by the polarity of the Delta psi

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Published in:
Molecular Microbiology

DOI:
10.1046/j.1365-2958.1998.01001.x

Publication date:
1998

Molecular Microbiology (1998) **29**(4), 1125–1127

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Sir,

The transmembrane orientation of integral membrane proteins from many sources is described by the so-called *cis*-positive or positive-inside rule (Von Heijne, 1986, *EMBO J* **5**: 3021–3027; Von Heijne, 1992, *J Mol Biol* **225**: 487–494; Wallin and Von Heijne, 1998, *Prot Sci* **7**: 1029–1038). The positively charged amino acid residues arginine and lysine are enriched in cytoplasmic domains and extremely rare in extracytoplasmic domains. It has been suggested that the transmembrane electrical potential, $\Delta\psi$, is the main determinant of the topology of membrane proteins (Andersson and Von Heijne, 1994, *EMBO J* **13**: 2267–2272). In most organisms, the $\Delta\psi$ is inside negative and outside positive and is supposed to retard the transmembrane passage of positive charges. Here, we report data that contradict such an essential role for $\Delta\psi$ in determining the asymmetric distribution of positive charges of membrane proteins.

Obligate acidophiles, such as *Sulfolobus acidocaldarius*, are organisms that thrive at very low environmental pH values (pH 0.5–2.5). These organisms maintain their intracellular pH near neutrality. As a result of the massive transmembrane pH gradient, acidophiles have a reversed $\Delta\psi$, i.e. inside positive instead of negative (Moll and Schäfer, 1988, *FEBS Lett* **232**: 359–363). If the $\Delta\psi$ were a major cause of the positive inside rule, an inversed charge distribution is expected for membrane proteins of acidophiles. To test this prediction, we searched databanks for proteins with known topology that also exist in acidophiles. We focused on the homologues of SecY, a subunit of the precursor protein translocase, and on subunit 1 of cytochrome *c* oxidase. The transmembrane orientations of both proteins are established (Akiyama and Ito, 1989, *J Biol Chem* **264**: 437–442; Tsukihara *et al.*, 1996, *Science* **272**: 1136–1144). Homologues of these proteins were taken from representatives from all three kingdoms (Bacteria, Archaea and Eukarya) and were aligned using CLUSTALW (Thompson *et al.*, 1994, *Nucleic Acids Res* **22**: 4673–4680). The membrane segments were identified on the basis of hydrophobicity and sequence similarity. Subsequently, the number of charged residues in each hydrophilic loop was scored. Table 1 shows the results for more than nine homologues after assuming the same orientation for all of them ($N_{in} - C_{in}$ for SecY and Cox1 homologues with 10 and 12 hydrophobic segments respectively). The distribution of arginines and lysines over the hydrophilic loops is the same for all organisms including the acidophiles and agrees with the positive inside rule. In addition, the distribution of the negatively charged residues aspartate and glutamate was similar for all organisms (results not shown). These observations were confirmed for the bitopic SecE homologues from several acidophiles. It is concluded that the positive inside rule is not determined by the polarity of $\Delta\psi$, but is a result of the membrane protein insertion process. In this process anionic lipids, which could anchor the positive charges to the cytosolic leaflet of the membrane

Table 1. Charge distribution in integral membrane proteins.

		Arg + Lys			Arg + Lys	
Group	SecY/Sec61	In	Out	Oxidases	In	Out
Bacteria	*Escherichia coli*[a]	31	6	*Escherichia coli*[j]	26	4
	Bacillus subtilis[b]	26	1	*Bacillus subtilis*[k]	21	6
	Mycoplasma capricolum[c]	39	7	*Paracoccus denitrificans*[l]	16	6
Eukarya	*Rattus norvegicus*[d]	26	7	*Bos taurus*[m]	11	5
	Saccharomyces cerevisiae[e]	28	8	*Schizosaccharomyces pombe*[n]	11	5
	Pyrenomonas salina[f]	25	10	*Drosophila melanogaster*[o]	10	5
Archaea	*Methanococcus vannielli*[g]	29	2	*Halobacterium halobium*[p]	20	7
Non-acidophiles	*Haloarcula marismortui*[h]	21	4			
Acidophiles	*Sulfolobus acidocaldarius*[i]	23	1	*Sulfolobus acidocaldarius* QoxM[q]	22	4
				Sulfolobus acidocaldarius Qox1[r]	22	4
				Acidianus ambivalens[s]	18	5

Distribution of positively charged residues in SecY/Sec61 and oxidases belonging to the Cox1 family. Sequences were aligned using CLUSTALW. Positions of the transmembrane segments were determined by comparison with known structures. Based on the hydrophobicity profile, some oxidases contain more than 12 transmembrane segments. Only the first 12 transmembrane segments, which have high homology with the known structures, were used for the determination of the charge distribution.
SWISSPROT accession numbers are: **a.** P03844; **b.** P16336; **c.** P10250; **d.** P38378; **e.** P32915; **f.** P38397; **g.** P28541; **h.** P28542; **i.** P49978; **j.** P18401; **k.** P34956; **l.** P08305; **m.** P00396; **n.** P07657; **o.** P00399; **p.** P33518; **q.** P39481; **r.** P98008; **s.** P94117.

(Van Klompenburg *et al.*, 1997, *EMBO J* **16**: 4261–4266) may co-operate with proteinaceous components to establish membrane protein topology (Prinz *et al.*, 1998, *J Biol Chem* **273**: 8419–8424).

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Received 11 April, 1998; revised 1 June, 1998; accepted 5 June, 1998.

Competence regulons, genomics and streptococci

Sir,

Recently, Campbell *et al.* (1998, *Mol Microbiol* **27:** 929–939) reported a very elegant study combining a genetic screen with genomic analysis, which led to the identification of six loci belonging to the competence regulon of *Streptococcus pneumoniae*. Starting from the first identified competence-induced (*cin*) operon, initially described as containing two (*exp10-recA*; Pearce *et al.*, 1995, *J Bacteriol* **177:** 86–93) or three genes (*cinA-recA-dinF*; Martin *et al.*, 1995, *Mol Microbiol* **15:** 367–379) and recently shown to also contain the *lytA* gene (Mortier-Barrière *et al.*, 1998, *Mol Microbiol* **27:** 159–170) (Fig. 1), the authors used a *exp10::lacZ* transcriptional fusion to establish a genetic screen for *cin* promoters on solid media. Then, out of 1029 random *lacZ* chromosomal insertion mutants screened, Campbell *et al.* (1998, ibid.) retained one mutant, TYG, that showed a fivefold induction during competence in liquid medium. The *tyg* transcription start site was mapped. In parallel, a 52 bp (−38 to +14) minimal *cin* promoter sequence was defined by deletion analysis of the *exp10* upstream region. The −14 to −7 region of *tyg* was found to be identical to the corresponding region of *exp10* (Fig. 1). The authors hypothesized that this sequence (TACGAATA), referred to hereafter as the *cin* box, was critical for *cin* expression. A search of the pneumococcal genome uncovered six contigs containing the *cin* box. Further analysis revealed that five contained loci essential for genetic transformation. These loci encoded proteins with strong similarity to proteins involved in DNA processing during transformation in other bacteria (Campbell *et al.*, 1998, ibid.). Despite its key role in this study, the TYG mutant was apparently not characterized further.

We were curious to see whether the *tyg* promoter controlled a genetic transformation locus and whether other specific transformation loci (e.g. the homologue of the *B. subtilis comF* operon) might be present in this regulon. We first searched the TIGR *S. pneumoniae* genome database (http://www.ncbi.nlm.nih.gov/BLAST/tigrbl.html) with the published *tyg* promoter sequence. Much to our surprise, the *tyg* promoter was found to be located within the previously identified *dinF* gene and oriented so as to produce an antisense RNA (Fig. 1). As this raised the question of the function of this promoter, we searched for potential orf(s) expressed under its control. This search did not

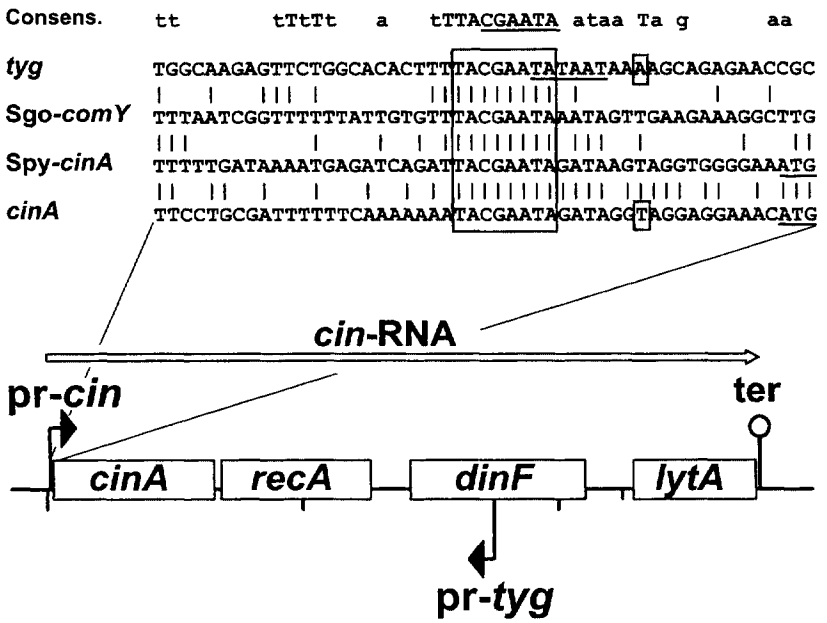

Fig. 1. Location of the *tyg* promoter (pr-*tyg*) within the *cinA–recA* operon (bottom) and alignment of regions containing putative or proven *cin* promoter sequences (top). The *cin*-box identified by Campbell *et al.* (1998, ibid.) as well as the transcription start of the *cinA-recA* operon (Pearce *et al.*, 1995, ibid.) and that of *tyg* are boxed. Sequences corresponding to 17 putative competence loci (nine from *S. pneumoniae*, i.e. those identified by Campbell *et al.* (1998, ibid.), including *tyg* and *cinA* [*cinA*], plus *comF* and *addAB*; seven from *S. pyogenes*, namely homologues of *S. pneumoniae cinA* [Spy-*cinA*], *dprA* and *ssb*, and homologues of the four *B. subtilis* late *com* operons; and *comY* from *S. gordonii* [Sgo-*comY*]) were aligned using Multalin (Corpet, 1988, *Nucleic Acids Res* 16: 10881–10890). The consensus sequence (top line) was based on the frequency of a nucleotide in each position being greater than 60% (lower case) or 75% (capital); invariant positions are underlined. The translation start codon of the *S. pneumoniae* and *S. pyogenes cinA* genes is underlined.