Mammalian integral membrane receptors are homologous to facilitators and antiporters of yeast, fungi, and eubacteria

JONATHAN REIZER,¹ KIM FINLEY,² DONALD KAKUDA,² CAROL L. MACLEOD,²
AIALA REIZER,¹ AND MILTON H. SAIER, JR.¹

¹ Department of Biology and ² Cancer Genetics Program, Department of Medicine and Cancer Center,
University of California at San Diego, La Jolla, California 92093

(RECEIVED May 29, 1992; REVISED MANUSCRIPT RECEIVED September 15, 1992)

Abstract

We demonstrate that three integral membrane receptors of mammals—the ecotropic retroviral leukemia receptor (ERR), the human retroviral receptor (HRR), and the T-cell early activator (Tea)—are homologous to a family of transporters specific for amino acids, polyamines, and choline (APC), which catalyze solute uniport, solute:cation symport, or solute:solute antiport in yeast, fungi, and eubacteria. Interestingly, the ERR membrane protein was recently shown to function as a cation:amino acid cotransporter. A binary sequence similarity matrix and an evolutionary tree of the 14 members of this family, illustrating their sequence similarities and divergences, were constructed. Other proteins, including the developmentally controlled GerAII spore germination protein of *Bacillus subtilis* and the acetylcholine receptor of *Drosophila melanogaster* gave sequence comparison scores of a sufficiently large magnitude to suggest (but not to establish) a common evolutionary origin with members of the APC family.

We report an extended and corrected Tea cDNA sequence and show that the mammalian Tea and ERR encoding genes are differentially expressed in tissues and cell lines. Furthermore, the two mammalian cDNA sequences hybridize with other vertebrate and yeast genomic DNAs under stringent conditions. These observations support the notion that cell surface receptor proteins in mammals are transport proteins that share a common origin with transport proteins of single-celled organisms. Thus, permeases of essential metabolites may function pathologically as viral receptors.

Keywords: acetyl choline receptor; amino acids; antiport; choline; phylogenetic tree; polyamines; retroviral receptors; solute transporters; symport; uniport

Recently it was shown that transmembrane solute transporters fall into several families of homologous proteins (Henderson & Maiden, 1990; Higgins, 1990; Maloney, 1990; Saier, 1990). As an increasing number of sequences becomes available, analyses are revealing the existence of more superfamilies that extend across phylogenetic lines (Reizer et al., 1990, 1991). Thus, regardless of the organism in which a specific transport protein is found, it may be homologous to transport proteins in other organisms that function by similar, or even distinct, mechanisms (Saier & Reizer, 1991). Furthermore, recent work has shown that bacterial transport proteins may be the same as or homologous to receptor and regulatory proteins that

possess distinct functions (Vartak et al., 1991) as postulated previously (Saier & Jacobson, 1984). The present communication serves to extend earlier studies aimed at defining the evolutionary relationships among transport proteins and integral membrane receptor proteins possessing dissimilar functions. We extend previously reported analyses of amino acid transporters of bacteria, yeast, and fungi, showing that this family includes the arginine/or-nithine antiporter of *Pseudomonas aeruginosa*, the choline transporter of *Saccharomyces cerevisiae*, polyamine transporters of *Escherichia coli*, and three related mammalian integral membrane proteins: the T-cell early activation receptor (Tea; MacLeod et al., 1990), the human retroviral receptor (HRR; Yoshimoto et al., 1991), and the ecotropic retroviral leukemia receptor (ERR; Albritton et al., 1989). The latter protein functions both as a ret-

Reprint requests to: Jonathan Reizer, Department of Biology, University of California at San Diego, La Jolla, California 92093-0116.

roviral receptor and a cationic amino acid transporter (Kim et al., 1991; Wang et al., 1991). We also show that Tea and ERR are highly similar to one another, but their expression is differentially regulated in T-lymphoma cell lines. Evidence is presented that both of these genes are conserved in a variety of vertebrates as well as in yeast.

Additionally, the GerAII protein that is required for L-alanine-stimulated germination of *Bacillus subtilis* spores (Zuberi et al., 1987) and the acetylcholine receptor of *Drosophila melanogaster* exhibit degrees of sequence similarity to members of the APC (amino acid, polyamine, and choline) family of transporters that suggest, but do not establish, a common evolutionary origin. Finally, another viral receptor, specific for the gibbon ape leukemia virus, GALV, is shown to be homologous to a phosphate permease of *Neurospora crassa* and to an open reading frame (ORF) of unidentified function from the archaeobacterium, *Methanobacterium thermoautotrophicum*.

Results

Phylogenetic relationships among members of the APC family

The binary statistical analyses presented in Table 1 demonstrate that functionally characterized members of the APC transport family showed statistically significant sequence similarity with HRR (Yoshimoto et al., 1991) and with two murine proteins: Tea (MacLeod et al., 1990) and ERR (Albritton et al., 1989). Marked sequence divergence of these proteins was observed mainly in the hydrophilic N-termini that precede the first transmembrane helices and in the C-terminal regions. These 14 homologous proteins are of a similar size, although the prokaryotic transporters are substantially smaller than the eukaryotic proteins (Table 1). They all exhibit similar hydropathy profiles with 11–14 putative transmembrane α -helical segments (Kyte & Doolittle, 1982; unpubl.). It is of interest that all known transporters and the three mammalian proteins lack a characteristic N-terminal hydrophobic leader sequence (von Heijne, 1986). Possibly, one or more of the hydrophobic segments of these proteins can function as an internal signal sequence (Saier et al., 1989).

Examination of the binary statistical analyses summarized in Table 1 reveals that the seven recognized amino acid transporters of yeast, *E. coli*, and fungi, i.e., Hip1, Put4, Can1, Gap1, PutX, AroP, and PheP, which presumably function by solute uniport or solute:cation symport (Henderson & Maiden, 1990) are the most similar (26–62% identity; binary comparison scores of 21–99 standard deviations). In contrast, the ArcD protein, which specifies an arginine/ornithine antiporter of *P. aeruginosa* (Lüthi et al., 1990; Verhoogt et al., 1992) and falls into the class of bacterial precursor/product antiporters discussed by Poolman (1990), is more divergent. The ArcD antiporter of *P. aeruginosa* is most similar to the two *E.*

coli polyamine permeases, i.e., the putrescine/ornithine antiporter (PotE: Kashiwagi et al., 1991, 1992) and the putative cadaverine/lysine antiporter (CadB: Meng & Bennett, 1992; Watson et al., 1992) (24% identity; comparison scores of 23–33 standard deviations, see Table 1).

The similarity of CadB, ArcD, and PotE is further reflected in the similarity of the hydropathy profiles of these three permeases (Fig. 1). The three polypeptides present almost superimposable hydropathy profiles with 12–14 putative membrane-spanning α -helices connected by short hydrophilic regions. It is interesting to note that although the family includes the proline facilitators of *S. cerevisiae* and *Aspergillus nidulans*, these proteins are not sufficiently similar to the Na⁺:proline symporter of *E. coli* to establish homology. Our previous study had shown that the latter protein belongs to a family of Na⁺:solute symporters, which includes both eukaryotic and prokaryotic transporters specific for sugars, amino acids, and vitamins (Reizer et al., 1990). Similarly, the putrescine permease (PotE) of *E. coli* is not similar to the recently described *E. coli* PotABCD system, which catalyzes spermidine-putrescine transport (Furuchi et al., 1991). The latter system consists of two integral membrane proteins (PotB and PotC), a membrane-associated protein that contains an ATP-binding consensus sequence (PotA), and a periplasmic polyamine binding protein (PotD; Furuchi et al., 1991). Thus, the PotABCD spermidine-putrescine transport system belongs to the ATP-driven pump and periplasmic binding protein-dependent family of integral membrane transport proteins (Saier & Reizer, 1991).

The unrooted phylogenetic tree for the 11 known transporters of the APC family and the three mammalian

Fig. 1. Hydropathy plots of the *Escherichia coli* CadB, *E. coli* PotE, and the *Pseudomonas aeruginosa* ArcD putative antiporters. Negative hydrophilicity index values indicate hydrophobic regions. Values were determined for a window of 15 residues using the algorithm of Kyte and Doolittle (1982).

Table 1. Binary comparisons of protein sequences of the APC transport family^a

	Hip1(S.c.) (532)	Put4(S.c.) (627)	Can1(S.c.) (590)	PutX(A.n.) (570)	Gap1(S.c.) (601)	PheP(E.c.) (458)	TEA(M.m.) (658)	ERR(M.m.) (622)	HRR(H.s.) (629)	Ctrl(S.c.) (563)	ArcD(P.a.) (482)	CadB(E.c.) (444)	PotE(E.c.) (439)
AroP(E.c.) (456)	28(450) [46]	37(271) [42]	38(285) [37]	32(265) [29]	33(425) [54]	62(439) [99]	23(329) [12]	20(279) [14]	24(207) [11]	27(71) [1]	17(362) [12]	20(341) [10]	18(362) [13]
Hip1(S.c.) (532)		33(494) [78]	36(464) [89]	31(419) [49]	55(440) [138]	31(430) [45]	17(177) [8]	23(367) [9]	23(170) [9]	20(445) [10]	19(441) [16]	20(286) [8]	18(394) [8]
Put4(S.c.) (627)			37(532) [89]	30(539) [90]	34(520) [94]	36(281) [35]	18(453) [5]	17(429) [6]	22(189) [9]	30(472) [4]	19(264) [0.1]	42(12) [0.5]	33(24) [2]
Can1(S.c.) (590)				33(518) [65]	36(527) [87]	39(285) [37]	21(315) [6]	21(337) [9]	21(344) [6]	24(102) [1]	16(413) [6]	20(395) [6]	19(417) [9]
PutX(A.n.) (570)					29(545) [53]	26(308) [21]	28(492) [4]	27(505) [6]	23(172) [5]	30(470) [3]	23(86) [0.2]	29(399) [4]	21(357) [6]
Gap1(S.c.) (601)						33(427) [49]	15(393) [3]	18(322) [4]	19(321) [6]	17(538) [4]	20(475) [15]	19(394) [8]	19(415) [9]
PheP(E.c.) (458)							22(169) [7]	23(169) [7]	23(170) [8]	22(270) [9]	19(367) [10]	20(379) [13]	18(435) [13]
TEA(M.m.) (658)								61(621) [200]	61(620) [193]	22(176) [4]	25(181) [9]	18(174) [5]	23(114) [1]
ERR(M.m.) (622)									87(629) [230]	18(426) [6]	28(80) [8]	20(192) [4]	23(193) [9]
HRR(H.s.) (629)										21(176) [5]	28(80) [6]	19(182) [5]	22(192) [9]
Ctrl(S.c.) (563)											16(408) [2]	19(317) [6]	18(284) [9]
ArcD(P.a.) (482)												24(378) [33]	24(392) [23]
CadB(E.c.) (444)													30(428) [53]

^a The value in parentheses below the protein designation refers to the number of residues in the intact protein. Values presented in the table that are not in brackets or parentheses represent percent identity for segments having the number of compared residues indicated in parentheses. The FASTA program (Pearson & Lipman, 1988) was used to assess similarities of the indicated proteins. Comparison scores in standard deviations, using the RDF2 program (Pearson & Lipman, 1988) and 200 shuffles, are given in brackets below the values for percent identity. Some values were determined using the ALIGN program (Dayhoff et al., 1983). Abbreviations used are as follows: AroP(E.c.), the general aromatic amino acid permease of *Escherichia coli* K-12 (Honore & Cole, 1990); Hip1(S.c.), the histidine permease of *Saccharomyces cerevisiae* (Tanaka & Fink, 1985); Put4(S.c.), the proline permease of *Saccharomyces cerevisiae* (Vandenbol et al., 1989); Can1(S.c.), the arginine permease of *S. cerevisiae* (Ahmad & Bussey, 1986); PutX(A.n.), the proline permease of *Aspergillus nidulans* (Sophianopoulou & Sczarcocchio, 1989); Gap1(S.c.), the general amino acid permease of *S. cerevisiae* (Jauniaux & Grenson, 1990); PheP(E.c.), the phenylalanine permease of *E. coli* (Pi et al., 1991); TEA(M.m.), *Mus musculus* (mouse) T-cell early activator (MacLeod et al., 1990); ERR(M.m.), *M. musculus* (mouse) ecotropic retroviral receptor (Albritton et al., 1989); HRR(H.s.), human retroviral receptor (Yoshimoto et al., 1991); Ctrl(S.c.), the choline permease of *S. cerevisiae* (Nikawa et al., 1990); ArcD(P.a.), the arginine/ornithine antiporter of *Pseudomonas aeruginosa* (Lüthi et al., 1990); CadB(E.c.), a putative cadaverine/lysine antiporter of *E. coli* (Meng & Bennet, 1992); and PotE(E.c.), a putrescine permease of *E. coli* (Kashiwagi et al., 1991).

receptors is shown in Figure 2. The proteins fall into four major clusters (Fig. 3). The first cluster consists of: Hip1(S.c.) and Gap1(S.c.), specific for histidine and general for amino acids, respectively; Put4(S.c.), PutX(A.n.), and Can1(S.c.), specific for proline, proline, and arginine, respectively; and AroP(E.c.) and PheP(E.c.), the only bacterial permeases of this group, which are specific for aromatic amino acids. The Tea, HRR, and ERR receptor proteins comprise the second cluster. The ArcD(P.a.), PotE(E.c.), and CadB(E.c.) facilitators, which most likely function in arginine/ornithine, putrescine/ornithine, and cadaverine/lysine antiport, respectively, comprise a third cluster, whereas the choline facilitator of *S. cerevisiae* (Ctr(S.c.)) comprises cluster 4. Clusters 2 and 3 are more closely related to each other than to clusters 1 and 4, and cluster 4 is most divergent among the sequences shown. The binary comparison scores tabulated in Table 1 substantiate these conclusions.

Three other proteins were found to exhibit significant degrees of sequence similarity with specific members of the APC family. Two of these proteins, the developmentally regulated GerAII spore germination protein of *B. subtilis* (Zuberi et al., 1987) and the acetylcholine receptor of *D. melanogaster* (Hermans-Borgmeyer et al., 1986; Sawruk et al., 1988), exhibit comparison scores of 8 standard deviations with ArcD (21% identity in 208 residues) and 7 standard deviations with Tea (24% identity in 80 residues), respectively. These comparison scores, particularly the former, suggest (but do not prove) that these proteins share a common origin with the APC family of transporters.

The third protein, which shows sequence similarity to

Fig. 2. Phylogenetic tree of homologous members of the amino acid, polyamine, and choline (APC) transport family. Evolutionary distances are provided adjacent to the branches. The tree was constructed using the progressive alignment method of Feng and Doolittle (1990) as described by Reizer and Reizer (1992). The abbreviations used are as indicated in Table 1.

members of the APC family of transporters, encoded by a partially sequenced ORF that is located downstream of the *Clostridium perfringens* histidine decarboxylase (*hdc*; van Poelje & Snell, 1990) may mediate heterologous or homologous exchange of histidine for histamine because it exhibits a comparison score of 13 standard deviations with ArcD of *P. aeruginosa* and 8 standard deviations with both CadB and PotE of *E. coli*. Interestingly, the metabolic enzymes that catalyze the formation of histamine, cadaverine, putrescine, and ornithine, i.e., histidine decarboxylase (Hdc; van Poelje & Snell, 1990), lysine decarboxylase (CadA; Meng & Bennett, 1992; Watson et al., 1992), ornithine decarboxylase (SpeF; Kashiwagi et al., 1991), and the arginine deiminase pathway enzymes (Lüthi et al., 1990), respectively, reside within operons that contain the genes encoding the putative histidine/histamine, cadaverine/lysine (CadB), putrescine/ornithine (PotE), and arginine/ornithine (ArcD) permeases. Conceivably, coordinate regulation of the synthesis of the precursor/product antiporter with the decarboxylase minimizes excessive precursor and/or product accumulation in the cells. In this regard it is tempting to suggest that synthesis of these antiporters, like expression of the genes that encode the corresponding decarboxylases, is induced at low external pH and/or during oxygen limitation (Gale, 1946; Auger et al., 1989; Gamper et al., 1991; Watson et al., 1992).

Since this manuscript was completed, three new members of the APC family have been identified. The first of these is LysP, the lysine-specific permease of *E. coli* (Steffes et al., 1992). LysP is most similar to the phenylalanine permease (PheP) of *E. coli* (37% identity) and the arginine permease (Can1) of *S. cerevisiae* (36% identity), exhibiting comparison scores with these proteins of 58 and 68 standard deviations, respectively. The second of these three proteins is GabP, the γ -amino butyrate permease of *E. coli* (Bartsch et al., 1992). This permease protein exhibits 36% identity, and a comparison score of 54 standard deviations with the phenylalanine permease (PheP) of *E. coli*. The third new member of this family is a putative permease designated YCL25c, which was recently identified on chromosome III of *S. cerevisiae* (Oliver et al., 1992). This putative permease is most similar to the general amino acid permease (Gap1) of *S. cerevisiae*, i.e., 47% identity and a comparison score of 147 standard deviations. Thus, these three new members of the APC family appear to belong to cluster 1 of the phylogenetic tree shown in Figure 2.

Experimental verification that Tea and Rec-1 (ERR) genes are differentially regulated and are conserved in evolutionarily divergent organisms

Figure 4 presents the corrected sequence of the hydrophobic Tea protein, which is 658 amino acids long. The

Fig. 3. Alignment of the homologous members of the APC transport family. Numbering above the aligned sequences corresponds to the number of residues that are not conserved in the aligned proteins. Numbers to the left of the sequences shown indicate the first amino acid in the row. Highlighted (black background) residues indicate residues that are conserved in all members of this family. Boxed residues are conserved within a specific subgroup within the family of amino acid transporters as follows: cluster 2, mammalian receptors; cluster 3, prokaryotic antiporters; cluster 1, prokaryotic and eukaryotic symporters. Because cluster 4 consists of a single protein, Ctr(S.c.), residues within this sequence are not boxed.

cggccgcttttccgtgcttttgcctgaacactgaacacttaccataagcaacct 60
 ctgctacaagagcctggtagcaacacccctattttcggtttctgttgcctcttccacc 120
 ATGATTCCTGCAGAGCAGTGTCTGCTTTTCGCGCATGCTGATCCGGAGAAAAATGTC 180
 M I P C R A V L T F A R C L I R R K I V
 ACATGGACAGCCTTGAAGATTCAAACCTGCGCGTGTCTAACACCGTGGACCTCATC 240
 T L D S L E D S K L C R C L T T V D L I
 GCTTTGGGGTGGAGCACTCTGGGCGTGGGGTCTACGCTCTGGTGGGGAAGTGGC 300
 A L G V G S T L G A G V Y V L A G E V A
 AAAGCCGATTTCGCGCCAGTATCTGGTGTCTTTCTCATGCTGCGCTGGCCTCGGT 360
 K A D S G P S I V V S F L I A A L A S V
 ATGGCGCGCTTTGCTATGCTGAATTTGGGCGCCAGTACCAAGAGCTGGATCGGTAT 420
 M A G L C Y A E F G A R V P K T G S A Y
 CTATACACTTACGTCAGGTGGAGAGCTGTGGCGCTTACCTACCTGGTGAATCTCATC 480
 L Y T Y V T V G E L W A F I T G W N L I
 CTGTCTATGTCATAGGTACGTCAGTGTGCAAGAGCATGGATGGCACCTTTGACGAA 540
 L S Y V I G T S S V A R A W S G T F D E
 CTCTTAATAAACAGATTGGCCAGTTTTTCAAAACGTACTTCAAAATGAATTACCTGGT 600
 L L N K Q I G Q F F K T Y F K M N Y T G
 CTGGCAGATATCCAGACTTCTTTGCCGTGTGCTTGTATTACTCTGGCAGGTCTTTTA 660
 L A E Y P D F F A V C L V L L L A G L L
 TCTTTGGAGTAAAGAGTCTGCTTGGTGAATAAAATTTTACAGCTATTAATATCTG 720
 S F G V K E S A W V N K F F T A I N I L
 GTCTTCTCTTTGTCATGGTGGTGGTGTGTAAGGAAATGTGGTAACTGGAAGATC 780
 V L L F V M V A G F V K G N V A N W K I
 AGTGAAGAGTTTCTCAAAATATATCAGCAAGTGTAGAGAACCACCTTCTGAGAACGGA 840
 S E E F L K N I S A S A R E P P S E N G
 ACAAGCATCTACGGGGTGGCGGCTTTATGCCCTATGGCTTTACAGGACGTTGGCTGGT 900
 T S I Y G A G G F M P Y G F T G T L A G
 GCTGCAACGTGCTTTTATGCCCTTGTGGCTTTGACTGCATTGCAACAACCGGTGAAGAG 960
 A A T C F Y A F V G F D C I A T T G E E
 GTTCGGAATCCACAAAAGCGATCCCCATCGGAATAGTGACGTCCTTACTTGTCTGCTTT 1020
 V R N P Q K A I P I G I V I S L L V C F
 ATGGCTTACTTTGGGTTTCTGAGCTTTAACGCTTATGATGCTTACTACCTCTGGAT 1080
 M A Y F G V S A A L T L M M P Y Y L L D
 GAGAAAAGTCCACTCCAGTCGCGTTTGTAGTATGTCAGATGGGCCCCGCCAAATACGTT 1140
 E K S P L P V A F E Y T V R W G P A K Y V
 GTCGCAGCAGGCTCCCTCTGCGCTTATCAACAAGTCTTCTGGATCCATTTTCCCAATG 1200
 V A A G S L C A L S T S L L G S I F P M

CCTCGTGAATCTATGCTATGGCGAGGATGGGTTCCTTTTCAATGTCTAGCTCAAATC 1260
 P R V I Y A M A E D G L L F K C L A Q I
 AATTCAAAACGAAGACACCAGTAATTGCTACTTTGTCATCGGGTGCAGTGGCAGCTGTG 1320
 N S K T K T P V I A T L S S G A V A A V
 ATGGCCITTTCTTTTACCTGAAGGCCCTCGTGGACATGATGCTATTGGCAACCTCATG 1380
 M A F L F D L K A L V D M M S I G T L M
 GCCTACTCTCTGGTGGCAGCTGTGCTTATTCTCAGGTACCAACCTGGCTTGTGTAC 1440
 A Y S L V A A C V L I L R Y Q P G L C Y
 GAGCAGCCCAATACACCCCTGAGAAAGAACTCTGGAATCATGTACCAATGGCACTTTG 1500
 E Q P K Y T P E K E T L E S C T N A T L
 AAGAGGAGTCCAGGTACCATGCTGCAAGGACAGGTTTCAGCTACGAACCTCTTC 1560
 K S E S Q V T M L Q G Q G F S L R T L F
 AGCCCCCTGCGCTGCCACACGACAGTGGCTTCCCTTGTGAGCTTTCTGGTGGATTC 1620
 S P S A L P T R Q S A S L V S F L V G F
 CTGGCTTCTCATCTGGGTGAGTATTCTAACACGATGCGCTCCAGGCCATTGCC 1680
 L A F L I L G L S I L T T Y G V Q A I A
 AGACTGGAAGCTGGAGCCTGGCTCTTCTCGCCCTGTCTCTGCTCTGCGCTGCCGTC 1740
 R L E A W S L A L L A L F L V L C A A V
 ATTCTGACCATTTGGAGGACGCCACAGAATCAGCAAAAAGTAGCCTTACGTCGCCGTT 1800
 I L T I W R Q P Q N Q Q K V A F M V P F
 TTACCGTTTCTGCGGCTTCCAGCATCTGGTCAACATTACTTATGTTCCAGTTAAGT 1860
 L P F L P A F S I L V N I Y L M V Q L S
 GCGGACACTGGATCAGATTCAGCATCTGGATGGCGCTTGGCTTTCTGATCTATTTCGCC 1920
 A D T W I R F S I W M A L G F L I Y F A
 TATGGCATTAGACAGCTTGGAGGTAACCCAGGGACGAAGAAGACGATGAGGATGCC 1980
 Y G I R H S L E G N P R D E E D D E D A
 TTTTCAGAAAACATCAATGTAGCAACAGAAAGATCCGCTATGCAAGCAAATGACCAT 2040
 F S E N I N V A T E E K S V M Q A N D H
 CACCAAGAAACCTCAGCTTACCTTTTCACTTCTATGAAAGACAAGTGAATGTTGATGC 2100
 H Q R N L S L P F I L H E K T S E C
 tggccctcggcttaccacgcataccttaacatgagtagctgtggccggaatgccacca 2160
 tctgtctgggctgtcgtgggtctgtctgtggacatggcttgccaaactgtacttctcct 2220
 ccagacagcttctctcagatgggtgattctgtgtctgaggagactgacctgagagcactc 2280
 ctacgctatatgtatcccaaacagtagtgcgtgtgcgtacatgtatgtctgcagatg 2340
 gagtgtcaatgttgcgttattagctgtgacataattccagcatggtaattgggtggc 2400
 atatactgcacacactagtaaacagtagtattgctgaatagagatgtattctgtatatgtc 2460
 ctagggtggctggggaaatagtggtgttctttattaggtatgatgacctcagtttgagc 2520
 atactgaatgccatccctgtcaggatgttttaacagtggtcatgggtggggaggggata 2580
 aggaatgggcatgtctataaattgtaatgcataatcttctctacttgcgaagacag 2640
 ctttcttaaacggccaggagagtggttcttctctgtatgacaagatgaagaggtagt 2700
 ctgtggctggagatggccaatcc 2720

Fig. 4. Upper Panel: Extended and revised nucleotide sequence of Tea cDNA and deduced amino acid sequence. One of seven cDNA sequences isolated from an SL12.4 T-lymphoma cDNA library is shown with the predicted amino acid sequence. The arrow indicates the 5' end of the original cDNA clone; the asterisk marks the previously reported methionine start of the predicted protein (MacLeod et al., 1990). The corrected sequence has been deposited in GENBANK with the accession number M32485. **Lower panel:** Hydrophilicity profiles of the Tea and ERR proteins. The predicted protein sequences of Tea and ERR were compared using a window of 15 residues with the algorithm of Kyte and Doolittle (1982).

originally published sequence (MacLeod et al., 1990) predicted a shorter protein that was truncated at the N-terminus due to a probable reverse transcriptase error in the cDNA sequence. The sequence data shown in Figure 4, obtained from an additional, independently derived cDNA clone, reveal that the cDNA clone encodes a much longer protein, which is even more closely related to the ERR protein than previously reported (MacLeod et al., 1990). This new sequence information extends the ORF by an additional 615 bp, corresponding to 205 additional amino acids at the N-terminus of the predicted protein. The corrected Tea and ERR protein sequences exhibit overall sequence identity of 61% (Table 1). The hydropathy profiles for the two murine proteins are nearly identical, and both proteins are predicted to contain 12 transmembrane spanning domains (Fig. 4). The amino acid sequences of the murine integral membrane proteins are recognizably similar to established transporters of the APC family (Table 1); their overall structure and the number of transmembrane spanning regions are also similar.

To directly compare the gene expression patterns of the Rec-1 and Tea genes, a Northern blot of SL12.3 and SL12.4 cell line mRNA was probed sequentially with Tea and then Rec-1 random-primed labeled cDNA. The transcript sizes are clearly distinct (Fig. 5). The data show that the Tea and Rec-1 genes are both expressed in the SL12.4 cell line, but only Rec-1 transcripts are detectable in the SL12.3 cell line. The two genes also show differential expression in a variety of tissues (Albritton et al., 1989; MacLeod et al., 1990). The coexpression of Tea and Rec-1 in the SL12.4 cell line may reflect similar but nonidentical functions for their gene products. The differential expression of the genes reveals a common theme of complex regulation of transport proteins in higher eukaryotes (Bell et al., 1990; Readhead, 1990; Thorens et al., 1990).

Fig. 5. Northern analysis of Tea and Rec-1 gene expression in two related T-lymphoma cell lines SL12.3 and SL12.4. Total cellular RNA (10 μ g) from two related T-lymphoma cell lines was analyzed on a Northern blot probed sequentially with labeled Tea and Rec-1 cDNA probes as described in Materials and methods. Even loading was assessed based on acridine orange staining of rRNA as described in Materials and methods.

To assess whether other species have sequences similar to the genes encoding Tea or ERR, a Southern "zoo-blot" was probed sequentially with Tea and Rec-1 cDNA. The genomic Southern blot (Fig. 6) revealed that all vertebrates examined in this study, i.e., DNA from birds, human, monkey, rat, mouse, dog, cow, rabbit, as well as yeast show distinct hybridization patterns. For example, the 8.1- and 3.0-kb DNA fragments from rat, which hybridize to the Tea probe, are distinct from the 10.4- and 7.8-kb fragments, which hybridize to the Rec-1 probe. Similarly, distinctly different hybridization patterns are also observed in DNA from mouse, dog, cow, rabbit, chicken, and yeast. These distinct patterns of hybridization indicate that the probes are not cross-reacting.

Interestingly, the intensity of the hybridization signal on the blot probed with Tea cDNA is very similar among the vertebrate species examined. This observation provides strong evidence that the Tea homologues are highly conserved, similar to the reported conservation of the ERR protein sequence between human and mouse, which is 87.6% identical (Yoshimoto et al., 1991). These related gene sequences hybridize to different DNA sequences in each species tested under stringent hybridization and washing conditions, which were designed to reveal only the

Fig. 6. Southern analysis of DNA from a variety of species. A "zoo-blot" containing DNA from the indicated species was prepared as described in Materials and methods. The blot was first probed with Tea cDNA, stripped, and reprobed with Rec-1 cDNA. The numerals to the left of the panels indicate the positions of molecular weight markers (HindIII-digested lambda DNA) given in kilobase pairs.

equivalent isoform(s) among genes of this family. These findings indicate that the Tea and Rec-1 genes are distinct from each other and that each is conserved across wide evolutionary boundaries.

Homology of the GALV receptor with a fungal phosphate permease and an archaeobacterial ORF

As has been noted previously (B. O'Hara, pers. comm. cited in Vile and Weiss, 1991), the GALV receptor (O'Hara et al., 1990) is similar to the pho-4+-encoding phosphate permease of the fungus *N. crassa* (Mann et al., 1989). The two proteins exhibit 32% identity in an overlapping region of 247 amino acid residues (comparison score of 35 standard deviations). Additionally, we found that a much smaller ORF (211 amino acid residues long) of unknown function, i.e., the hydrophobic ORF2 of the archaeobacterium *M. thermoautotrophicum* (Ostergaard et al., 1987), is homologous to the GALV receptor (32% identity in an overlapping region of 154 amino acid residues; comparison score of 15 standard deviations). The hydropathy plots of the GALV receptor and the *N. crassa* phosphate transporter (Fig. 7) reveal that each of these integral membrane proteins possesses two distinct clusters of hydrophobic regions located at the N- and C-termini, respectively, separated by a large hydrophilic domain (ca. 200–250 amino acid residues). Interestingly, the similarity between these two membrane proteins is mainly confined to the N-terminal cluster of the membrane-spanning helices, which precede the long hydrophilic middle domain. These observations provide further support to the notion that transport proteins and viral receptors are homologous, i.e., derived from common ancestors, and that permeases of essential metabolites in mammals may have pathological functions as receptors for cellular infection by viruses.

Fig. 7. Hydropathy plots of the gibbon ape leukemia virus (GALV) receptor and Pho-4+. Hydrophobic regions correspond to negative index numbers. Values were determined for a window of 15 residues using the algorithm of Kyte and Doolittle (1982).

Discussion

The analysis of genomic DNA from various vertebrates and yeast reveals that these organisms have DNA sequences sufficiently homologous to allow detection by Southern analysis. This fact suggests that genes encoding proteins similar to members of the APC family are widespread in nature.

The alternate expression patterns of the Rec-1 and Tea genes (vide infra, Albritton et al., 1989; MacLeod et al., 1990) are similar to those that have been seen in the glucose facilitator family (GLUT). To date, five different mammalian GLUT genes have been isolated and sequenced (reviewed by Bell et al., 1990; Readhead, 1990; Thorens et al., 1990). These GLUT isoforms share between 39 and 65% amino acid identity within a given species, while each isoform has a much higher degree of identity with its counterpart across species. Each of these isoforms shows a distinct pattern of tissue-specific transcriptional regulation, suggesting that each isoform has evolved a distinct function, and that together they accomplish glucose homeostasis. A similar consideration may apply to HRR, ERR, and Tea. Their primary functions may be amino acid transport, but they may also be involved in the regulation of amino acid metabolism. The differential regulation of Tea and Rec-1 gene expression in different tissues (Albritton et al., 1989; MacLeod et al., 1990) and in the SL12 cell line is similar to the complex regulation observed in the GLUT system. Additional studies directed at defining the function of the Tea gene product are currently underway and will further clarify this point.

The Tea and ERR proteins are encoded by distinct genes that are highly related to one another, and their sequences hybridize to distinctly different size classes of DNA from birds, mammals, and yeast under stringent hybridization and washing conditions, which were designed to distinguish related members of this putative family. Under these conditions, the hybridization pattern should detect only the equivalent isoform for each gene in several other species. It is possible that Tea and ERR are members of a subfamily of proteins that transport basic amino acids, and that their sequences have been conserved over extended evolutionary periods.

In a larger context, four findings resulted from the computer analyses reported here. First, it is clear that antiporters share a common origin with uni/symporters, and that there consequently may be no fundamental mechanistic difference between these transport modes as suggested previously (Saier & Reizer, 1991; Saier, 1992). Second, the three mammalian cell surface proteins—Tea, HRR, and ERR (Albritton et al., 1989; MacLeod et al., 1990; Yoshimoto et al., 1991)—are members of the APC family, suggesting that their primary cellular function involves amino acid, polyamine, or choline transport. Evidence that ERR is actually involved in amino acid transport has

recently been presented (Kim et al., 1991; Wang et al., 1991). Third, the GerAII protein is known to be required for L-alanine-stimulated germination of *B. subtilis* spores, but its biochemical function is not known. A role as a cell surface receptor has been proposed (Zuberi et al., 1987). The finding reported here, i.e., that GerAII exhibits sequence similarity with members of the APC family, suggests that GerAII functions as an L-alanine transport protein. In this regard, it is interesting to note that the oligopeptide permease of *B. subtilis* has recently been shown to play a role in the initiation of sporulation, whereas the dipeptide permease of this organism may play a role in maintenance of the vegetative state under conditions of nutrient shift-down (Perego et al., 1991). Finally, limited sequence similarity was noted between members of the APC family and the acetylcholine receptor protein of *D. melanogaster* (Sawruk et al., 1988). The latter protein is a member of an extensive family of receptor-ion channel proteins not previously believed to be related to the family of solute transporters possessing two to eight segments, each containing six transmembrane spanners, transmembrane segments of the type represented by the APC family (Saier & Reizer, 1991). If the latter result is reflective of a common evolutionary origin, these two major families of integral membrane proteins will be represented by a single superfamily. However, insufficient data are currently available to establish this possibility, and the reported similarity could have arisen by convergent evolution. Further studies will be required to define the structural and evolutionary relationships of these two groups of proteins.

Materials and methods

Computer-aided analysis

The FASTA program (Pearson & Lipman, 1988) was used to search the current PIR protein databank (version 30) and to compare the percent identity of one protein to another. The RDF2 (Pearson & Lipman, 1988) and the ALIGN (Dayhoff et al., 1983) programs were used to calculate the comparison scores shown in Table 1. Construction of the phylogenetic tree was performed using the progressive alignment method (Feng & Doolittle, 1990) essentially as described (Reizer & Reizer, 1992). The hydropathy plots were created using the algorithm of Kyte and Doolittle (1982).

Isolation of Tea cDNA clones

To verify the Tea cDNA sequence previously reported (MacLeod et al., 1990), a cDNA library was prepared using RNA from the SL12.4 T-lymphoma cell line from which the original clone was isolated. The library was probed with a BalI fragment prepared from the original cDNA clone. Seven independent clones were identified,

restriction mapped, and sequenced by the chain termination method of Sanger et al. (1977). Initial sequencing primers for Bluescript (T7 and T3) and subsequent oligonucleotide primers (17mers) were custom synthesized by the Molecular Biology Core Laboratory of the University of California, Cancer Center. Reagents for sequencing were obtained from Pharmacia.

Gene expression studies

Total RNA was isolated from murine SL12.3 and SL12.4 T-lymphoma cell lines by the guanidine isothiocyanate method (Davis et al., 1986) as previously reported (MacLeod et al., 1990). The Northern blot was prepared using 10 µg of total cellular RNA after electrophoresis on 1% agarose denaturing formaldehyde gel, subsequently stained with acridine orange, and photographed to confirm even loading and locate the 18S and 28S rRNA. The blot was sequentially hybridized with random-primed labeled cDNA probes (Tea and Rec-1) as noted in the figure legends. The Tea probe extends from 560 to 2,716 bp on the sequence reported in this paper. The Rec-1 probe extends from 1 to 2,425 bp (Albritton et al., 1989). Although the Tea and Rec-1 cDNA clones are 56.3% identical, the longest stretch of sequence identity within the known sequence is 23 bp (found at position 445–468 on the Tea cDNA clone). The Tea probe does not include that region. The blot was hybridized using standard methods at 42 °C, and washed with a final stringency of 0.1× SSPE (1× SSPE = 0.18 M NaCl, 1 mM NaH₂PO₄, 1 mM EDTA, pH 7.4), 0.1% sodium dodecyl sulfate (SDS) at 42 °C.

Genomic Southern analysis of Tea and Rec-1 (ERR) genes

Each lane in a Southern "zoo-blot" contained 5 µg of genomic EcoRI-digested DNA from human (placenta), rhesus monkey (liver), Sprague-Dawley rat (liver), Balb/c mouse (liver), canine (kidney), bovine (liver), rabbit (liver), chicken (liver), and yeast (total). The digested samples were electrophoresed on a 0.7% agarose gel, blotted onto an Amersham Hybond nylon membrane, prehybridized for 1 h at 65 °C in 6× SSC (1× SSC = 0.15 M NaCl, 15 mM Na citrate, pH 7.0) (Sambrook et al., 1989), and hybridized for 18 h with 2 × 10⁶ cpm of random-primed labeled Tea cDNA probe, stripped, and subsequently re-probed with Rec-1 cDNA. The blot was washed in 2× SSC, 0.1% SDS at room temperature for 15 min and at 65 °C for an additional 15 min.

Acknowledgments

We thank Jeanine Kleeman for excellent technical assistance in recloning the Tea gene and Phuoc Huynh for determining the sequence of the new cDNA clones. This work was supported by Public Health Service grants 5R01AI21702 and 2R01AI14176

from the National Institute of Allergy and Infectious Diseases, grant 8RO137778 from the National Cancer Institute, and grant IM-653 from the American Cancer Society.

References

- Ahmad, M. & Bussey, H. (1986). Yeast arginine permease: Nucleotide sequence of the CAN1 gene. *Curr. Genet.* 10, 587-592.
- Albritton, L.M., Tseng, L., Scadden, D., & Cunningham, J.M. (1989). A putative murine ecotropic retrovirus receptor gene encodes a multiple membrane-spanning protein and confers susceptibility to virus infection. *Cell* 57, 659-666.
- Auger, E.A., Redding, K.E., Plumb, T., Childs, L.C., Meng, S.-Y., & Bennett, G.N. (1989). Construction of *lac* fusions to the inducible arginine- and lysine decarboxylase genes of *Escherichia coli* K12. *Mol. Microbiol.* 3, 609-620.
- Bartsch, K., von Johnn-Marteville, A., & Schulz, A. (1992). The *E. coli* gab cluster: Nucleotide sequence of the structural genes gabDP, molecular organization of the cluster and characterization of the GABA permease. (GENBANK; accession number M88334.)
- Bell, G.I., Kayano, T., Buse, J.B., Burant, C.F., Takeda, J., Lin, D., Fukumoto, H., & Seino, S. (1990). Molecular biology of mammalian glucose transporters. *Diabetes Care* 13, 198-208.
- Davis, L., Dibner, M.D., & Battey, J.F. (1986). *Basic Methods in Molecular Biology*, pp. 129-146. Elsevier, New York.
- Dayhoff, M.O., Barker, W.C., & Hunt, L.T. (1983). Establishing homologies in protein sequences. *Methods Enzymol.* 91, 524-545.
- Feng, D.-F. & Doolittle, R.F. (1990). Progressive alignment and phylogenetic tree construction of protein sequences. *Methods Enzymol.* 183, 375-387.
- Furuchi, T., Kashiwagi, K., Kobayashi, H., & Igarashi, K. (1991). Characteristics of the gene for a spermidine and putrescine transport system that maps at 15 min on the *Escherichia coli* chromosome. *J. Biol. Chem.* 266, 20928-20933.
- Gale, E.F. (1946). The bacterial amino acid decarboxylases. *Adv. Enzymol.* 6, 1-32.
- Gamper, M., Zimmermann, A., & Haas, D. (1991). Anaerobic regulation of transcription initiation in the *arcDABC* operon of *Pseudomonas aeruginosa*. *J. Bacteriol.* 173, 4742-4750.
- Henderson, P.J.F. & Maiden, M.C.J. (1990). Homologous sugar transport proteins in *Escherichia coli* and their relatives in both prokaryotes and eukaryotes. *Philos. Trans. R. Soc. Lond. B* 326, 391-410.
- Hermans-Borgmeyer, I., Zopf, D., Ryseck, R.-P., Hovemann, B., Betz, H., & Gundelfinger, E.D. (1986). Primary structure of a developmentally regulated nicotinic acetylcholine receptor protein from *Drosophila*. *EMBO J.* 5, 1503-1508.
- Higgins, C.F. (1990). The role of ATP in binding-protein-dependent transport systems. *Res. Microbiol.* 141, 353-360.
- Honore, N. & Cole, S.T. (1990). Nucleotide sequence of the *aroP* gene encoding the general aromatic amino acid transport protein of *Escherichia coli* K-12: Homology with yeast transport proteins. *Nucleic Acids Res.* 18, 653.
- Jauniaux, J.-C. & Grenson, M. (1990). GAP1, the general amino acid permease gene of *Saccharomyces cerevisiae*. Nucleotide sequence, protein similarity with the other bakers yeast amino acid permeases, and nitrogen catabolite repression. *Eur. J. Biochem.* 190, 39-44.
- Kashiwagi, K., Miyamoto, S., Suzuki, F., Kobayashi, H., & Igarashi, K. (1992). Excretion of putrescine by the putrescine-ornithine antiporter encoded by the *potE* gene of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 89, 4529-4533.
- Kashiwagi, K., Suzuki, T., Suzuki, F., Furuchi, T., Kobayashi, H., & Igarashi, K. (1991). Coexistence of the genes for putrescine transport protein and ornithine decarboxylase at 16 min on *Escherichia coli* chromosome. *J. Biol. Chem.* 266, 20922-20927.
- Kim, J.W., Closs, E.I., Albritton, L.M., & Cunningham, J.M. (1991). Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. *Nature* 352, 725-728.
- Kyte, J. & Doolittle, R.F. (1982). A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157, 105-132.
- Lüthi, E., Baur, H., Gamper, M., Brunner, F., Villeval, D., Mercenier, A., & Haas, D. (1990). The *arc* operon for anaerobic arginine catabolism in *Pseudomonas aeruginosa* contains an additional gene, *arcD*, encoding a membrane protein. *Gene* 87, 37-43.
- MacLeod, C.L., Finley, K., Kakuda, D., Kozak, C.A., & Wilkinson, M.F. (1990). Activated T cells express a novel gene on chromosome 8 that is closely related to the murine ecotropic retroviral receptor. *Mol. Cell. Biol.* 10, 3663-3674.
- Maloney, P.C. (1990). A consensus structure for membrane transport. *Res. Microbiol.* 141, 374-395.
- Mann, B.J., Bowman, B.J., Grotelueschen, J., & Metzenberg, R.L. (1989). Nucleotide sequence of *pho-4+*, encoding a phosphate-repressible phosphate permease of *Neurospora crassa*. *Gene* 83, 281-289.
- Meng, S.-Y. & Bennett, G.N. (1992). Nucleotide sequence of the *Escherichia coli* *cad* operon: A system for neutralization of low extracellular pH. *J. Bacteriol.* 174, 2659-2669.
- Nikawa, J.I., Hosaka, K., Tskukagoshi, Y., & Yamashita, S. (1990). Primary structure of the yeast choline transport gene and regulation of its expression. *J. Biol. Chem.* 265, 15996-16003.
- O'Hara, B., Johann, S.V., Klinger, H.P., Blair, D.G., Robinson, H., Dunn, K.J., Sass, P., Vitek, M., & Robins, T. (1990). Characterization of a human gene conferring sensitivity of infection by gibbon ape leukemia virus. *Cell Growth Differ.* 1, 119-127.
- Oliver, S.G., van der Art, Q.J.M., Agostoni-Carbone, M.L., Aigie, M., Alberghina, L., et al. (1992). The complete DNA sequence of yeast chromosome III. *Nature* 357, 38-46.
- Ostergaard, L., Larsen, N., Leffers, H., Kjems, J., & Garrett, R. (1987). A ribosomal RNA operon and its flanking region from the Archaeobacterium *Methanobacterium thermoautotrophicum*, Marburg strain: Transcription signals, RNA structure and evolutionary implications. *Syst. Appl. Microbiol.* 9, 199-209.
- Pearson, W.R. & Lipman, D.J. (1988). Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci. USA* 85, 2444-2448.
- Perego, M., Higgins, C.F., Pearce, S.R., Gallagher, M.P., & Hoch, J.A. (1991). The oligopeptide transport system of *Bacillus subtilis* plays a role in the initiation of sporulation. *Mol. Microbiol.* 5, 173-185.
- Pi, J., Wookey, P.J., & Pittard, A.J. (1991). Cloning and sequencing of the *pheP* gene, which encodes the phenylalanine-specific transport system of *Escherichia coli*. *J. Bacteriol.* 173, 3622-3629.
- Poolman, B. (1990). Precursor/product antiport in bacteria. *Mol. Microbiol.* 4, 1629-1636.
- Readhead, S. (1990). Glucose transporter defects underlie insulin resistance. *J. NIH Res.* 2, 58-62.
- Reizer, A., Pao, G.M., & Saier, M.H., Jr. (1991). Evolutionary relationships between the permease proteins of the bacterial phosphoenolpyruvate:sugar phosphotransferase system. Construction of phylogenetic trees and possible relatedness to proteins of eukaryotic mitochondria. *J. Mol. Evol.* 33, 179-193.
- Reizer, A. & Reizer, J. (1992). Progressive multiple alignment of protein sequences and the construction of phylogenetic trees. In *Methods in Molecular Biology: Computer Analysis of Sequence Data* (Griffin, A.M. and Griffin, H.G., Eds.). Humana Press, Totowa, New Jersey, in press.
- Reizer, J., Reizer, A., & Saier, M.H., Jr. (1990). The Na⁺/pantothenate symporter (PanF) of *Escherichia coli* is homologous to the Na⁺/proline symporter (PutP) of *E. coli* and the Na⁺/glucose symporters of mammals. *Res. Microbiol.* 141, 1069-1072.
- Saier, M.H., Jr. (1990). Evolution of permease diversity and energy-coupling mechanisms: An introduction. *Res. Microbiol.* 141, 281-286.
- Saier, M.H., Jr. (1992). Mechanisms of carbohydrate transport in bacteria and comparisons with those in eukaryotes. In *The Structure of Biological Membranes* (Yeagle, P., Ed.), pp. 833-891. CRC Press, Boca Raton, Florida.
- Saier, M.H., Jr. & Jacobson, G.R. (1984). *The Molecular Basis of Sex and Differentiation: A Comparative Study of Evolution, Mechanism and Control in Microorganisms*. Springer-Verlag, New York.
- Saier, M.H., Jr. & Reizer, J. (1991). Families and superfamilies of transport proteins common to prokaryotes and eukaryotes. *Curr. Opin. Struct. Biol.* 1, 362-368.
- Saier, M.H., Jr., Werner, P.K., & Muller, M. (1989). Insertion of proteins into bacterial membranes: Mechanism, characteristics, and comparisons with the eucaryotic process. *Microbiol. Rev.* 53, 333-366.
- Sambrook, J., Fritsch, E.F., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

- Sanger, F., Nicklen, S., & Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
- Sawruk, E., Hermans-Borgmeyer, I., Betz, H., & Gundelfinger, E.D. (1988). Characterization of an invertebrate nicotinic acetylcholine receptor gene: The *ard* gene of *Drosophila melanogaster*. *FEBS Lett.* 235, 40-46.
- Sophianopoulou, V. & Scazzocchio, C. (1989). The proline transport protein of *Aspergillus nidulans* is very similar to amino acid transporters of *Saccharomyces cerevisiae*. *Mol. Microbiol.* 3, 705-714.
- Steffes, C., Ellis, J., Wu, J., & Rosen, B.P. (1992). The *lysP* gene encodes the lysine-specific permease. *J. Bacteriol.* 174, 3242-3249.
- Tanaka, J. & Fink, G.R. (1985). The histidine permease gene (*HIP1*) of *Saccharomyces cerevisiae*. *Gene* 38, 205-214.
- Thorens, B., Charron, M.J., & Lodish, H.F. (1990). Molecular physiology of glucose transporters. *Diabetes Care* 13, 209-217.
- Vandenbol, M., Jauniaux, J.-C., & Grenson, M. (1989). Nucleotide sequence of the *Saccharomyces cerevisiae* PUT4 permeases. *Gene* 83, 153-159.
- van Poelje, P.D. & Snell, E.E. (1990). Cloning, sequencing, expression, and site-directed mutagenesis of the gene from *Clostridium perfringens* encoding pyruvoyl-dependent histidine decarboxylase. *Biochemistry* 29, 132-139.
- Vartak, N.B., Reizer, J., Reizer, A., Gripp, J.T., Groisman, E.A., Wu, L.-F., Tomich, J.M., & Saier, M.H., Jr. (1991). Sequence and evolution of the FruR protein of *Salmonella typhimurium*: A pleiotropic transcriptional regulatory protein possessing both activator and repressor functions which is homologous to periplasmic sugar binding proteins. *Res. Microbiol.* 142, 951-963.
- Verhoogt, H.J.C., Smit, H., Abee, T., Gamper, M., Driessen, A.J.M., Haas, D., & Konings W.N. (1992). *arcD*, the first gene of the *arc* operon for anaerobic arginine catabolism in *Pseudomonas aeruginosa*, encodes an arginine-ornithine exchanger. *J. Bacteriol.* 174, 1568-1573.
- Vile, R.G. & Weiss, R.A. (1991). Virus receptors as permeases. *Nature* 352, 666-667.
- von Heijne, G. (1986). Towards a comparative anatomy of N-terminal topogenic protein sequences. *J. Mol. Biol.* 189, 239-242.
- Wang, H., Kavanaugh, M.P., North, R.A., & Kabat, D. (1991). Cell-surface receptor for ecotropic murine retroviruses is a basic amino-acid transporter. *Nature* 352, 729-731.
- Watson, N., Dunyak, D.S., Rosey, E.L., Slonczewski, J.L., & Olson, E.R. (1992). Identification of elements involved in transcriptional regulation of the *Escherichia coli* *cad* operon by external pH. *J. Bacteriol.* 174, 530-540.
- Yoshimoto, T., Yoshimoto, E., & Meruelo, D. (1991). Molecular cloning and characterization of a novel human gene homologous to the murine ecotropic retroviral receptor. *Virology* 185, 10-17.
- Zuberi, A.R., Moir, A., & Feavers, I.M. (1987). The nucleotide sequence and gene organization of the *gerA* spore germination operon of *Bacillus subtilis* 168. *Gene* 51, 1-11.