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CEA adhesion molecules: multifunctional proteins with signal-regulatory properties

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The carcinoembryonic antigen family comprises a large number of complex molecules, several of which possess cell adhesion activities. The primordial adhesion molecules of this family are the cell–cell adhesion molecules (C-CAMs), which have been found to be multifunctional, signal-regulatory proteins. C-CAMs inhibit tumor growth, interact with calmodulin, protein tyrosine kinases and protein tyrosine phosphatases, and are subject to specific dimerization reactions. These new insights indicate that C-CAMs are important regulators of cellular functions.

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Abbreviations

Bgp	biliary glycoprotein in mouse
BGP	biliary glycoprotein in man
C-CAM	cell–cell adhesion molecule
CEA	carcinoembryonic antigen
CGM	CEA gene family member
GPI	glycosylphosphatidylinositol
Ig	immunoglobulin
IgSF	Ig gene superfamily
ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibition motif
L	long
NCA	nonspecific cross-reactive antigen
PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
S	short
SH	Src homology
SHP	SH2-domain-containing PTP
SIRP	signal-regulatory protein

Introduction

The carcinoembryonic antigen (CEA), which was discovered in 1965 [1], has given its name to a large and complex family of abundantly expressed proteins. Available data indicate that these molecules are multifunctional, but largely their biological roles have remained elusive. One of the documented properties for many of the CEA family proteins is cell adhesion, and recently results have emerged from several laboratories that demonstrate that some of these adhesion molecules have important functions in signal transduction. This review will focus on recent progress in the study of the role of CEA-related adhesion molecules, particularly the phylogenetically conserved cell–cell adhesion molecules (C-CAMs), as signal-regulatory proteins, and on the possible molecular

mechanisms that are involved in signal transduction via CEA molecules. For background information and reference to earlier work, previous reviews [2–6] should be consulted.

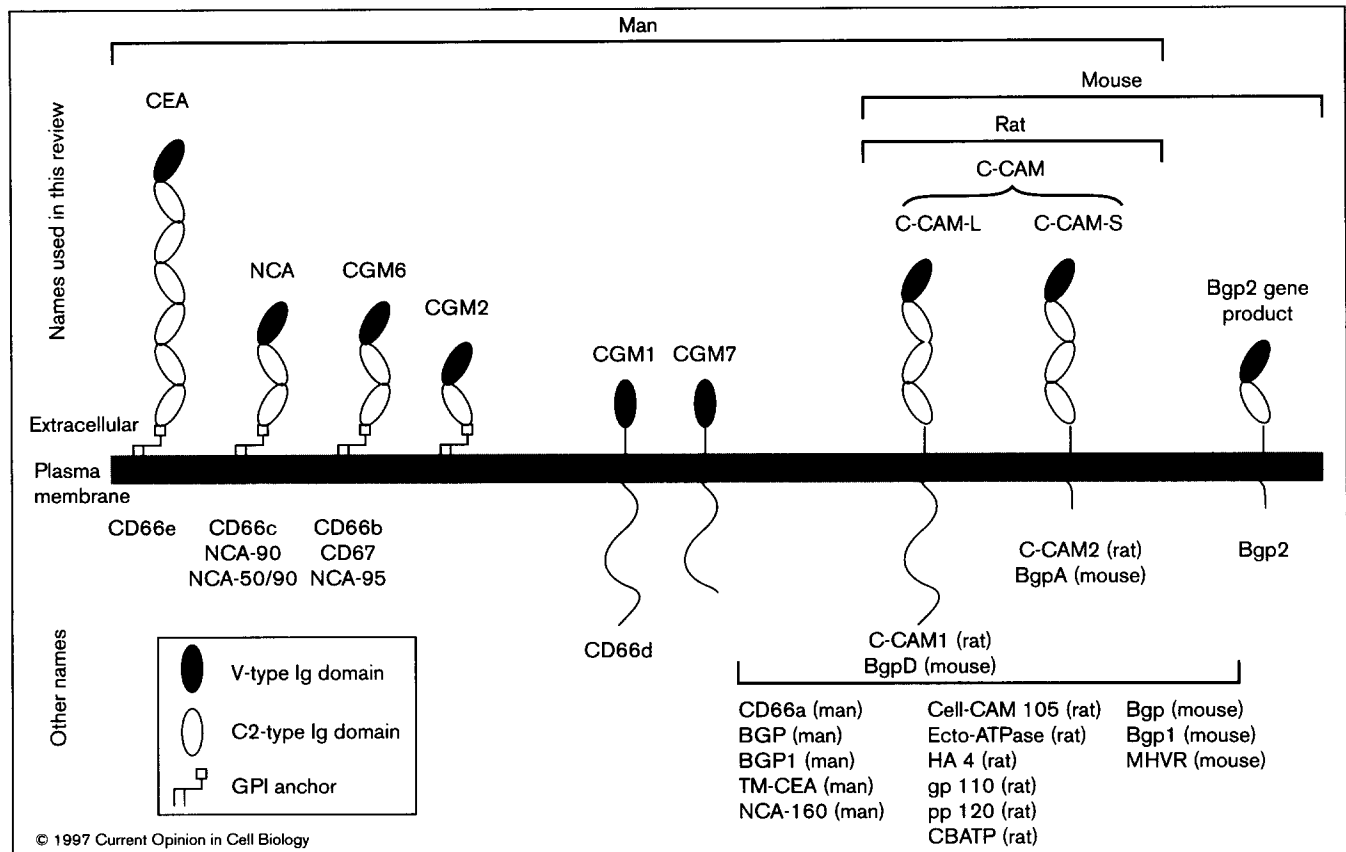
The CEA gene family

The CEA gene family belongs to the immunoglobulin gene superfamily (IgSF) and comprises a large number of genes [2,3]. Two major subgroups, designated the CEA and the pregnant-specific glycoprotein (PSG) subgroups, exist [2]. The majority of the proteins in the CEA subgroup are cell surface bound, whereas the PSGs are secreted. Only the cell surface bound molecules of the CEA subgroup will be considered here. Unfortunately, the nomenclature of the CEA family is complex and confusing, and several of the molecules are known under many different names. The cell surface bound CEA subgroup molecules and their names are shown in Figure 1.

The cell surface associated CEA family proteins are heavily glycosylated and can either be transmembrane or associated with the plasma membrane via a GPI (glycosylphosphatidylinositol) anchor. However, GPI-linked CEA family cell surface proteins are only found in humans (and probably in other primates as well), whereas the rodent cell surface molecules are exclusively transmembrane (Figure 1). In the rat, the transmembrane proteins have been called C-CAMs, while they are known as biliary glycoproteins in mice and man (abbreviated as Bgps and BGPs, respectively). In the following discussion, however, these homologous molecules will be referred to as rat C-CAMs, mouse C-CAMs, and human C-CAMs, respectively (Figure 1). All CEA-related cell surface molecules have an extracellular amino-terminal V-type Ig domain, which is followed by a variable number (0–6) of C2-type Ig domains.

The cell surface associated C-CAMs in rat, mouse and man are highly conserved with respect to both domain organization and amino acid sequence, suggesting that they originated from a common ancestor. Only one gene has been found in each of humans and rats, whereas mice have two related genes, *Bgp1* and *Bgp2* [7]. The number of Ig domains in C-CAMs varies from one to four as a result of differential splicing. The amino-terminal V-type domain is highly homologous to that of other CEA family members. Alternative splicing also gives rise to two forms of cytoplasmic domains containing either 10–12 or 71–73 amino acids [8–10]. The different cytoplasmic domains will be referred to as S (short) and L (long), respectively. In addition to glycosylation and splice variants, two allelic

Figure 1



Cell surface bound CEA-related molecules. The cell surface bound CEA-related molecules are either transmembrane proteins or bound to the plasma membrane via a GPI anchor. The GPI-linked molecules (CEA, NCA, CGM6 and CGM2) do not occur in rodents, nor do two transmembrane molecules, CGM1 and CGM7, but they are found in man. In contrast, the C-CAMs, which are phylogenetically conserved, are expressed in rats, mice and humans. Several of these molecules are known under a large number of names in the literature, and this confusion over nomenclature has contributed to the complexity of this field. In this review a simplified nomenclature, shown in the upper half of the figure, is used. Some of the many other names that are in use are shown in the lower part of the figure. In addition to the two C-CAM isoforms (C-CAM-L and C-CAM-S) shown in the figure, several alternatively spliced isoforms with fewer Ig domains occur. They have been given distinct names, and are not shown in the figure. Also CGM1 can be alternatively spliced, yielding either a soluble isoform or a transmembrane isoform with a shorter cytoplasmic domain; only the isoform with the long cytoplasmic domain is shown in the figure. CBATP, canalicular bile acid transport protein; CD, cluster of differentiation; gp, glycoprotein; HA 4, hepatocyte antigen 4; MHVR, mouse hepatitis virus receptor; pp, phosphoprotein; TM-CEA, transmembrane CEA.

variants, denoted *a* and *b*, occur in both rats and mice [8,9]. They are different from each other primarily in the amino-terminal Ig domain.

The GPI-linked CEA-related molecules, including CEA itself, represent the evolutionarily youngest members of the family. It is not clear why the human species contains GPI-linked cell surface bound CEA-related proteins, but it suggests that they may have replaced some of the functions of rodent C-CAMs. A comparison of the tissue expression of C-CAM and CEA in rat and human tissues gives some support for this idea [11,12]. Novel functions for the GPI-linked proteins may also have developed.

Cell surface CEA family molecules are multifunctional

In adult tissues the CEA-related cell surface molecules are expressed primarily in different epithelia, vessel

endothelia, and hematopoietic cells [11–15], but individual molecules show distinct expression patterns. The spatiotemporal expression pattern of C-CAMs suggests important functions during embryonic development; a highly dynamic expression has been seen in, for example, the trophoblasts of the placenta [16,17], the endothelial cells and pericytes during vascularization of the central nervous system [18], muscle development [16], and development and eruption of teeth [19].

Interest in this family also emanates from numerous reports showing that its members are associated with malignancy [20–27]. Alterations in expression patterns in various tumors have been reported for CEA, NCA (nonspecific cross-reactive antigen), CGM (CEA gene family member)2 and C-CAM. CEA has for a long time been the most widely used tumor marker in clinical

medicine. However, no clear picture of the functional roles of these molecules in cancer has yet emerged.

A number of functions have been ascribed to the membrane-bound CEA family molecules, including cell adhesion, ecto-ATPase activity, bile salt transport activity, receptor internalization, recognition of bacteria and viruses, blocking of cell differentiation, and regulation of signal transduction.

Cell adhesion

C-CAM and the GPI-linked molecules CEA and NCA mediate adhesion primarily by homophilic binding to molecules on adjacent cells, but heterophilic binding to produce the complexes CEA-NCA, CEA-BGP, and NCA-BGP has also been found [28]. The amino-terminal V-type Ig domains mediate these interactions; however, whereas C-CAM homophilic binding results from a reciprocal binding between the amino-terminal Ig domains of opposing molecules [29], the amino-terminal domain of CEA binds to another, more membrane-close Ig domain on its ligand CEA partner [30]. The three-dimensional structure of the amino-terminal domain seems to be important for adhesion, as mutation of Arg98 abrogates both rat C-CAM- and CEA-mediated adhesion [31]. This arginine residue is highly conserved in Ig domains and is essential for their conformation; it forms an intradomain salt bridge with a specific, highly conserved aspartate residue [32].

It has been reported that the cytoplasmic L domain is needed for C-CAM-mediated homophilic adhesion in insect Sf9 cells [31,33]. However, both the L and the S forms of C-CAM can mediate adhesion in mammalian CHO cells [34,35]; in these cells there are indications that the S form may even be more effective than the L form (K Wikström, B Öbrink, unpublished data). Thus, it seems as if the cellular background is essential for the manifestations of C-CAM adhesive activities, which may be a function of the supramolecular organization of C-CAM and its interactions with other molecules (see below).

In addition to homophilic and heterophilic binding between the CEA-related adhesion molecules themselves, human C-CAM and NCA mediate adhesion of granulocytes to E-selectin on vessel endothelial cells because they carry a specific carbohydrate structure known as the sialyl Le^x epitope which is a ligand for E-selectin [36,37]. This interaction triggers activation of β 2 integrins in the granulocytes, which leads to firm adhesion to the endothelial cells. Stimulation of granulocytes by antibodies to human C-CAM and NCA also induces β 2-integrin-mediated cell aggregation [38].

C-CAM is not present in intercellular junctions but is concentrated in areas where cells are more loosely associated with each other. A striking example is the junctional epithelium that forms a transition zone between the teeth

and the gingival epithelium. The junctional epithelial cells have no tight junctions, very few desmosomes, and express no E-cadherin (C Terling, J Wroblewski, B Öbrink, unpublished data), but they seem to be kept together by abundant microvillar projections [39]. The high concentration of C-CAM in these areas [19] indicates that it may have a major adhesive role.

Although C-CAM appears in intercellular contact areas in several locations, such as between stratified epithelial cells [11], in contact zones between endothelial cells and pericytes of vessel walls [18], and between hepatocytes in mature liver [40], it has also been found in high concentrations on microvillar-rich apical surfaces of simple epithelia and in bile canaliculi [11]. The GPI-linked molecules CEA and NCA are also found on apical surfaces and in the glycocalyx of colon epithelial cells [41]. Thus, in these locations the CEA-related molecules may have functions other than intercellular adhesion, although it is possible that they might mediate dynamic adhesive interactions between microvilli. However, one should consider the possibility that the adhesive properties of CEA-related molecules might reflect molecular interactions that are more important for signal transduction than for the formation of strong physical intercellular bonds.

Ecto-ATPase activity, bile salt transport activity, and receptor internalization

The cDNA encoding rat C-CAM was originally cloned as encoding a $\text{Ca}^{2+}/\text{Mg}^{2+}$ -dependent ecto-ATPase [42]. In agreement with this, increased ecto-ATPase activity was conferred on COS cells and Sf9 cells that were transfected with rat C-CAM-L [31,43]. The C-CAM-induced ecto-ATPase activity in Sf9 cells was abrogated by mutation of Arg98, which is believed to be part of an ATPase consensus sequence [31,42]. However, the agreement between the C-CAM sequence containing Arg98 and the ATPase consensus sequence is only partial. It seems more likely that this arginine residue affects the ATPase activity because of its essential role for the three-dimensional structure of the Ig domain, as discussed above. A truncation of the cytoplasmic L domain of C-CAM also destroyed the ATPase activity [31]. Thus, both an intact amino-terminal V-type Ig domain and an intact cytoplasmic L domain seem to be required for ecto-ATPase activity in these cells. However, efforts in a number of laboratories have failed to confirm any ATPase activity of purified C-CAM, and it has been found that the bulk of the ATPase activity can be chromatographically separated from C-CAM ([44]; I Hunter, B Öbrink, unpublished data). How could these apparently conflicting results be explained? There seem to be two possibilities: either the ecto-ATPase activity is not an intrinsic property of C-CAM, which instead might regulate the ATPase activity of another molecule; or the ATPase activity needs a particular C-CAM supramolecular organization, requiring both the amino-terminal Ig domain and the L domain, that is lost when C-CAM is solubilized from the membrane.

In experiments with reconstituted purified C-CAM [45] or with C-CAM-transfected COS cells [43], it has been observed that rat C-CAM-L can mediate ATP-driven efflux of taurocholate. Site-directed mutagenesis demonstrated that this activity required phosphorylation of Ser503 and was regulated by phosphorylation of Tyr488; both of these amino acid residues are located in the cytoplasmic domain of C-CAM-L [43]. The transport activity was completely abrogated by truncation of the cytoplasmic domain. However, it is not easy to see how a single-pass membrane protein can act as a transmembrane, taurocholate-transporting molecule, unless specific oligomerization of C-CAM-L occurs. The ileal and hepatocyte sinusoidal bile acid transporters are multipass membrane-spanning proteins [46,47]. A possibility is that C-CAM may interact with a bile acid transporter and thus act as a tissue-specific regulator of bile acid transport.

C-CAM also has receptor-regulating effects as observed from its effect on the insulin receptor. A two- to threefold increase in the rate of receptor internalization, triggered by insulin stimulation, was observed in cells that were transfected with C-CAM [48].

Microbial receptors

CEA-related surface proteins act as receptors for both bacteria and viruses. Fimbrial proteins of several strains of *Escherichia coli* and *Salmonella* bind to D-mannosyl residues on CEA, NCA and human C-CAM [49,50]. A different mode of bacterial binding was recently described for *Neisseria gonorrhoeae* and *Neisseria meningitidis*, which bind specifically to the amino-terminal Ig domains of human C-CAM, NCA, CGM1 and CEA via their virulence-associated Opa proteins [51,52].

Like several other cell adhesion molecules of the IgSF, C-CAM can also function as a virus receptor. Thus, the mouse hepatitis virus (MHV), a murine coronavirus, utilizes mouse C-CAM as its receptor [53]. Several splice variants coded by the *Bgp1^a* gene act as MHV receptors, but mice having the allelic variant *Bgp1^b* are resistant to MHV. However, in transfected cells one of the *Bgp1^b*-coded isoforms could also act as a receptor [53], although the efficiency of the two allelic variants differs significantly [54]. A protein product from the murine C-CAM gene *Bgp2* can also act as receptor for MHV [7]. Rat coronaviruses do not use rat C-CAM as a receptor.

Block of cell differentiation

It has been argued that overexpression of CEA and NCA in human carcinomas interferes with cell differentiation [55,56]. This suggestion is supported by findings that ectopic expression of CEA and NCA in rat myoblasts blocks biochemical and morphological differentiation [55]. CEA can cooperate with Myc and Bcl-2 and cause complete cellular transformation [56]. The differentiation-blocking effect of CEA depends on its adhesive activity, but it is not known if specific signaling mechanisms are involved.

Regulation of signal transduction

There are now convincing data showing that C-CAMs and several of the human GPI-linked CEA-related molecules participate in signal transduction. In human granulocytes, which express C-CAM-L, CGM6, NCA and CGM1, treatment with monoclonal antibodies against these molecules stimulates the induction mediated by the tripeptide N-formyl-Met-Leu-Phe of both a respiratory burst and activation of $\beta 2$ integrins [57*,58*]. This effect depends on the presence of extracellular calcium ions and involves transient tyrosine phosphorylation of the cytoplasmic domain of C-CAM-L [58*]. Antibodies against all four CEA-related molecules can trigger respiratory burst and $\beta 2$ -integrin-mediated adhesion, but there is evidence that the stimulatory effect mediated by CGM6 and NCA requires cooperation with C-CAM [57*]. This is supported by the finding that C-CAM can form complexes with CGM6 and NCA, but not with CGM1, in granulocytes (B Singer, personal communication), and by the demonstration of activation-dependent, conformation-specific epitopes in granulocyte C-CAM [59]. Thus, the signaling effects in granulocytes may require a cytoplasmic domain, such as those carried by C-CAM-L and CGM1.

Perhaps the most spectacular demonstration of CEA-related molecules as important regulators of signal transduction comes from the recent findings that the transmembrane C-CAMs can inhibit growth of malignant tumors. The first indication that C-CAM may be important for tumor development was the observation that rat transplantable hepatocellular carcinomas expressed either no C-CAM or only low amounts of chemically altered C-CAM [20]. Recently, downregulation of C-CAM expression has also been reported during prostate carcinogenesis [26], in colorectal carcinomas in mice [23] and man [22], and in human hepatocellular carcinomas [27], but the picture is complex as significant C-CAM expression has been demonstrated in other neoplasms [21,25]. However, none of these reports has presented any quantitative analyses of the expression patterns of the various C-CAM isoforms. Recent observations indicate that co-expression of C-CAM-L and C-CAM-S, and the ratios of these two isoforms, are crucial for regulation of the functional activities of C-CAM [60**].

Inhibition of tumor growth by C-CAMs

A direct approach to investigating the effects of C-CAM on tumor growth has recently been taken. In one study it was demonstrated that transfection of mouse C-CAM-L (the *Bgp1^a* gene product) into mouse colon carcinoma cells inhibited tumor development in mice of the same genetic background as the carcinoma cells [61]. In another study it was found that transfection of rat C-CAM1^a-L into human prostate carcinoma cells caused inhibition of tumor development caused by injection of these cells into nude mice [62]. In the latter study it was furthermore demonstrated that transfection of an antisense construct decreased C-CAM expression in benign rat prostate cells

and made these cells tumorigenic in nude mice. Rat C-CAM-L can also prevent tumor growth of bladder carcinomas [63•] and breast carcinomas [64•] in nude mice. The inhibitory effect resides in the cytoplasmic L domain, as transfection with the S isoform alone did not cause inhibition of tumor growth [61]. In a preclinical study it was demonstrated that delivery of rat C-CAM-L, by means of an adenovirus vector, to tumor-carrying animals also prevented tumor growth [65]. Efforts are now being made to develop C-CAM-based clinical treatment of cancer, but clearly much work remains to be done to clarify the basic mechanisms of the tumor-inhibitory effects of C-CAM.

That the tumor-inhibiting effect depends on the cytoplasmic domain of C-CAM-L was further substantiated by deletion of this domain; deletion abrogated the tumor growth inhibitory effect [64•]. However, the tumor-inhibitory effect is not a simple function of expression of C-CAM-L; upon increasing the expression of C-CAM-L above physiological levels, the tumor inhibitory effect disappeared [60••]. Thus, there seems to be a window of C-CAM-L expression levels in which its inhibitory effects become visible. Interestingly, the settings of this window seem to be regulated by the expression of C-CAM-S [60••]. Upon simultaneous expression of C-CAM-L and C-CAM-S the tumor-inhibitory effect was observed also at much higher expression levels. This was dependent on the ratio of the expression levels of C-CAM-L to C-CAM-S. At L:S levels of ≤ 1.3 tumor inhibition occurred, even at very high total expression levels of C-CAM. At higher L:S ratios, there was no tumor inhibition, at either high or moderate total C-CAM expression levels. Thus, the ratio of the expression levels of the L and S isoforms is critical for regulating the tumor-inhibitory effect of C-CAM-L.

In most tissues and cells there is a simultaneous expression of C-CAM-L and C-CAM-S [66], and regulation of the L:S ratios as a means of regulating signal transduction by C-CAM is an intriguing possibility. Support for this idea comes from the finding that the expression levels of the two isoforms can indeed be regulated independently of each other in an epithelial rat cell line, NBT-II [67]. In these cells fibroblast growth factor (FGF)-1 (acidic FGF) selectively upregulates the expression of C-CAM-L, and this is correlated with an epithelial-to-mesenchymal transition.

Molecular mechanisms of signaling by C-CAMs

The downstream events that are involved in signal transduction by C-CAMs are not known, but inspection of the structure of the cytoplasmic L domain (Figure 2) and studies of the interactions of C-CAM both with itself and with other proteins give exciting clues to the mechanisms that might be involved. As will be seen, C-CAMs participate in a complex network of molecular interactions that may be essential both for the effects on signaling and for the regulation of enzymatic and transport activities.

Binding of protein tyrosine kinases and protein tyrosine phosphatases

The cytoplasmic L domain contains two tyrosine residues in phosphorylatable motifs (Figure 2). The membrane-proximal tyrosine residue (Tyr488 in rat) has been found to be phosphorylated by the insulin receptor kinase in hepatocytes [68], by c-src in epithelial cells [69], and by c-src [69] and lyn and hck [70] in granulocytes. Convincing data about phosphorylation of the membrane-distal tyrosine (Tyr513 in rat) are lacking. These two tyrosine residues are part of a modified ITAM (immunoreceptor tyrosine-based activation motif), although the similarity is imperfect, as the spacing between the two tyrosines (24–26 amino acids) is greater than the spacing of 10 amino acids found in classic ITAMs [71•]. However, the proximal tyrosine is part of a perfect ITIM (immunoreceptor tyrosine-based inhibition motif) [72]. ITAMs and ITIMs are present in a number of membrane receptors, such as T-cell receptor complexes, B-cell receptor complexes, Fc γ RIIB, CD22, interleukin receptors, and natural killer cell inhibitory receptors (KIRs) [72,73•]. Upon phosphorylation ITAMs and ITIMs bind protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs), respectively, whose actions lead to stimulation or termination of signaling with profound effects on cellular proliferation. Interestingly, different splice variants of KIRs having short or long cytoplasmic domains occur [73•]; the spacing between the two tyrosine residues in the long KIR cytoplasmic domain is 29 amino acids, which is similar to that found in the long cytoplasmic domain of C-CAMs.

C-CAM can bind both PTKs and PTPs (Figure 3). Phosphorylation of the membrane-proximal tyrosine leads to binding of c-src in colon carcinoma cells [69], and of lyn and hck in granulocytes [70]. C-CAM-L can also bind the PTP Src homology (SH)2-domain-containing PTP-1 (SHP-1) in colon carcinoma cells [71•]. This binding requires the presence of both tyrosine residues although only the membrane-proximal tyrosine has been found to be phosphorylated. C-CAM-L is also able to bind the PTP SHP-2, and tyrosine-phosphorylated C-CAM-L is a substrate for both SHP-1 and SHP-2 (N Beauchemin, M Huber, personal communication). However, at present it is not clear if the binding of SHP-1 or SHP-2 is involved in the tumor-inhibitory effect, because mutation of Tyr488 to phenylalanine does not abrogate the tumor-inhibitory effect of rat C-CAM-L, at least not in breast carcinoma cells [64•].

Binding of calmodulin

Another signal-regulating protein that can bind to the cytoplasmic domains of C-CAM is calmodulin [74•]. Both C-CAM-L and C-CAM-S contain a calmodulin-binding site which is found close to the lipid bilayer (Figure 2). Rodent C-CAMs also have a calmodulin-binding site in the distal portion of the cytoplasmic domain, close to the carboxy-terminal end of the cytoplasmic L domain.

Figure 2

ITAM consensus sequence	
D/EXXXXXD/EXXYXXLXXXXXXXXYXXL/I	
ITIM consensus sequence	
I/VXYXXL	
Mouse C-CAM-L cytoplasmic domain	
445 -	<u>YFLYSRKSGGGSDQRDLTEHKPSTSNHNLAPSDNSPNKVD</u> DVA <u>YTVLNFNSQQPNRPTSAPSSPRATETV</u> YSEV <u>KKK</u> - 521
Rat C-CAM-L cytoplasmic domain	
445 -	<u>YFLYSRKTGGGSDHRDLTEHKPSTSSHNLGSPDDSPNKVD</u> DVS <u>YSVLNFNAQQSKRPTSASSP</u> --TETV YSV <u>VKKK</u> - 519
Human C-CAM-L cytoplasmic domain	
446 -	<u>CFLHFGKTGRASDQRDLTEHKPSVSNHTQDHSNDPPNKM</u> EV <u>TYSTLNFEAQQPTQPTSASPSLTATEII</u> YSE <u>VKKQ</u> - 522
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Amino acid sequences and binding motifs in the C-CAM-L cytoplasmic domain in mouse, rat and man. The amino acid sequences of the cytoplasmic domains of mouse, rat and human C-CAM-L are highly homologous. The numbers to the left and to the right of the sequences indicate amino acid positions in the intact proteins. The consensus sequences of ITAMs and ITIMs are shown at the top, with critical amino acids shown in large bold type, and the corresponding critical amino acid residues in the C-CAM sequences are also indicated by large, bold letters. The ITAM consensus sequence contains two tyrosine residues with a spacing of 10 amino acids between them. Note that the distance between the two tyrosines in the C-CAM sequences is larger than that of the ITAM consensus sequence. However, the most amino-terminal of the two tyrosines in C-CAM is part of a perfect ITIM consensus sequence in all three organisms. Binding sites for calmodulin are underlined. The single-letter amino acid code is used, where X represents any amino acid. Dashes indicate where gaps were introduced to maximize alignment of the C-CAM-L sequences. As rat C-CAM-L is shorter, Tyr513 in rat corresponds to Tyr515 in mouse and Tyr516 in man.

Binding of calmodulin to C-CAM is regulated by the intracellular calcium concentration and leads to downregulation of C-CAM self-association [74*].

Dimerization of C-CAMs

Another feature of C-CAMs, which might be important for the signaling mechanisms of C-CAM, is that both the L and the S isoforms can dimerize [75*] (Figure 3a). This has been demonstrated by the use of chemical cross-linkers on both intact epithelial cells and purified C-CAM in solution. The dimers are in equilibrium with the monomers, but the extent of dimerization can be controlled by the cells. Increase of the intracellular calcium concentration leads to dissociation of C-CAM dimers. This dissociation might be mediated by calmodulin, as it was found that calcium-activated calmodulin caused dissociation of C-CAM dimers *in vitro* [75*] (Figure 3b).

The C-CAM dimers are preferentially homodimers, that is, C-CAM-L dimerizes with itself and C-CAM-S dimerizes with itself [75*]. Both the ectodomain alone and the L domain alone can dimerize (I Hunter, B Öbrink,

unpublished data), which implies that the affinity of C-CAM-L for itself is higher than the affinity of C-CAM-S for itself or the affinity of C-CAM-L for C-CAM-S. As the dimerization process is in chemical equilibrium, both a change in the overall expression level of C-CAM and a shift of the ratio between the L and the S forms would alter the monomer:dimer ratio and the concentration of heterodimers.

The regulation of C-CAM expression and variation in the monomer:dimer ratio of C-CAM-L in epithelial cells could have profound influences on the regulatory and signaling activities of C-CAM, as it might affect the binding of PTKs, PTPs and other proteins (Figure 3c). In addition to influences by calmodulin, the monomer:dimer ratio might also be regulated by phosphorylation. Both the S and the L cytoplasmic domains contain several serine/threonine residues that can be phosphorylated. Protein kinase C catalyzed phosphorylation of Ser503 in rat C-CAM-L [43], Ser449 in rodent C-CAMs and Thr453 in human C-CAM-L (M Edlund, K Wikström, R Toomik, P Ek, B Öbrink, unpublished data) has been demonstrated. These phosphorylation sites are

Figure 3

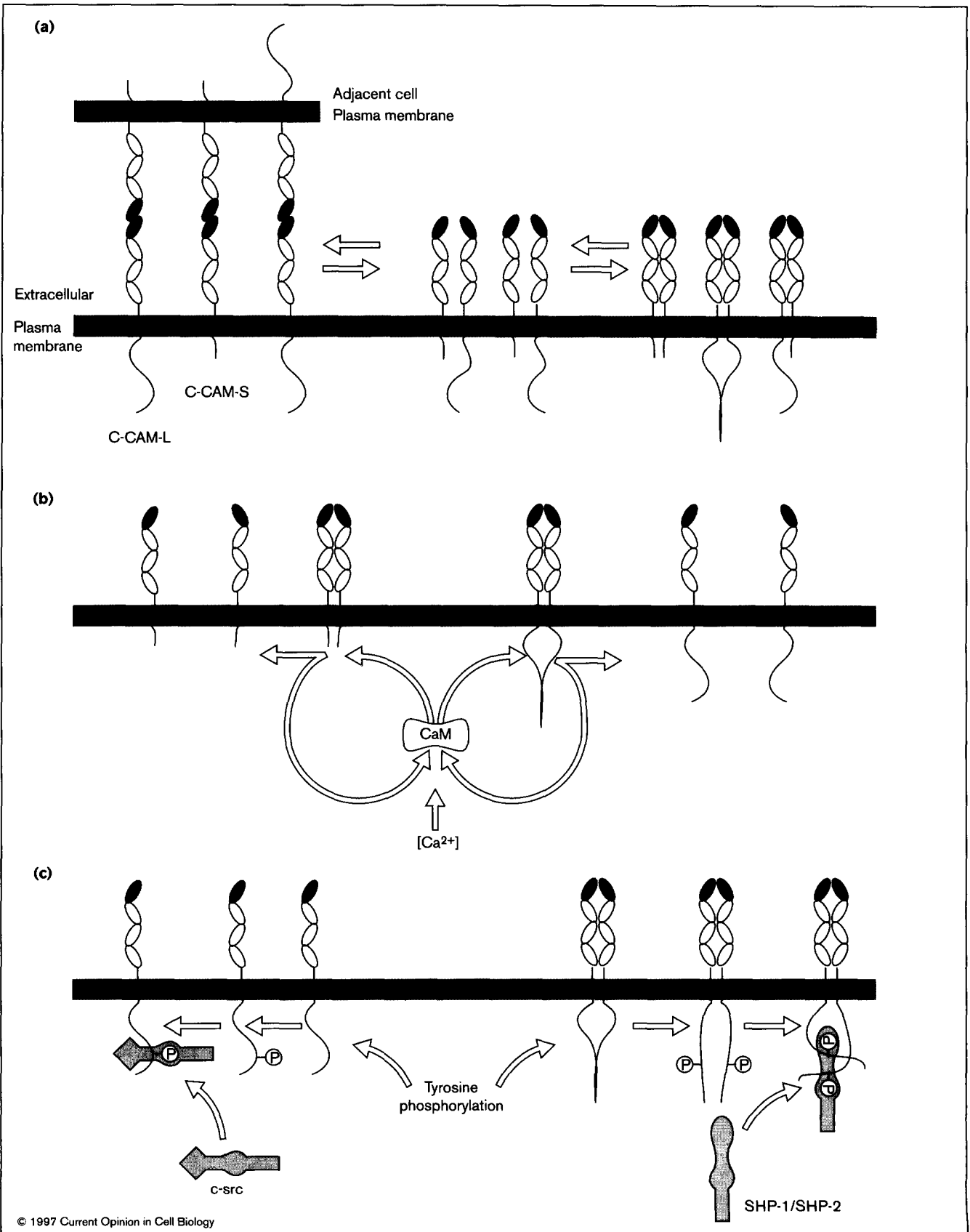


Figure 3

Protein-binding patterns of C-CAM. C-CAM can participate in a complex, dynamic network of simultaneously occurring binding interactions with itself and with other proteins. However, in order to describe these interactions in a simple way, three modes of interactions are illustrated separately here. **(a)** It has been demonstrated that both C-CAM-L and C-CAM-S can form homodimers. Heterodimer formation between C-CAM-L and C-CAM-S has not been demonstrated, but is likely to occur to some extent. Monomers (center) and dimers (right) are in equilibrium with each other. Both the ectodomains and the long cytoplasmic domain of C-CAM-L are believed to contribute to dimerization. In addition, both C-CAM isoforms can mediate cell adhesion by reciprocal binding between the amino-terminal Ig domains of opposing C-CAM molecules on adjacent membranes (left). As illustrated, it has been demonstrated that monomeric C-CAM can mediate cell adhesion, but it is not known whether dimeric C-CAM mediates adhesion or not. **(b)** Both C-CAM-L and C-CAM-S can bind calmodulin (CaM) in a Ca^{2+} -dependent manner. Increase of the calcium concentration leads to calmodulin binding to C-CAM; this binding causes dissociation of C-CAM dimers. Calmodulin can be recycled when the calcium concentration decreases. **(c)** The cytoplasmic domain of C-CAM-L can be tyrosine phosphorylated by src-family kinases and by the insulin receptor kinase (not shown). This can lead to binding both of src-family kinases (e.g. c-src), which contain one SH2 domain (a tyrosine-phosphate-binding domain) per molecule, and of the protein tyrosine phosphatases SHP-1 and SHP-2, which contain two SH2 domains per molecule. The binding modes that are illustrated here are speculative; it is not known whether it is monomeric or dimeric C-CAM-L, or both, that binds the kinases and phosphatases. Circled P, phosphorylation.

located within the calmodulin-binding sites in the C-CAM cytoplasmic domains [74*] (Figure 2).

C-CAMs and other signal-regulatory proteins

C-CAMs and some of the other CEA-related adhesion molecules constitute a family of signaling and signal-regulating proteins that affect growth and other cellular activities. It is thus of particular interest to compare them with another family of signal-regulatory proteins (SIRPs) that has been identified quite recently [76**,77**]. SIRPs share a number of characteristics with the C-CAMs. They are transmembrane proteins, belonging to the IgSF, with three Ig domains in the extracellular portions. Short and long cytoplasmic domains exist, the long domains having four tyrosine residues that can be phosphorylated. The first tyrosine residue is part of an ITIM, and the spacing between this and the following tyrosine, as well as between the third and the fourth tyrosines, is 23 amino acids. Both SHP-1 and SHP-2, as well as Grb2 (growth factor receptor binding protein 2), bind to the long cytoplasmic domain upon tyrosine phosphorylation of the long domain, which can be catalyzed by the insulin receptor and various growth factor receptors and can be induced by cell attachment to fibronectin. SIRPs have negative effects on cellular proliferation induced by insulin and growth factors. A large number of genes and/or allelic variants for SIRPs exist, and there are indications of various alternatively spliced products.

The structural and functional similarities between C-CAMs and SIRPs are overwhelming, and it will be interesting to see if yet other related families of signal-regulatory proteins exist. A candidate would be platelet/endothelial cell adhesion molecule-1 (PECAM-1), another cell adhesion molecule of the IgSF. PECAM-1 can possess one of several differently spliced cytoplasmic domains that regulate its ligand-binding properties [78]. In two of these alternative cytoplasmic domains there are two tyrosine residues that bind SHP-2 upon phosphorylation [79*]. The first of these tyrosines is part of an ITIM and the spacing between the two tyrosines is 22 amino acids, which is similar to the spacing in C-CAMs, natural killer cell inhibitory receptors,

and SIRPs. Stimulation of PECAM-1 with antibodies leads to upregulation of integrin function in leukocytes and platelets which results in increased adhesion to endothelial cells [79*].

Conclusions and future perspectives

The CEA family is a large and complex family containing several cell surface bound proteins with cell adhesion activity and signal-regulatory properties. Accumulating data show that these adhesion molecules are multifunctional and participate in the regulation of various cellular activities. The phylogenetically original cell adhesion molecules in the CEA family, the C-CAMs, affect ecto-ATPase activity, bile acid transport and receptor internalization. In addition, they function as receptors for various bacteria and for mouse hepatitis virus. Considerable progress has been made concerning the role of C-CAMs as important negative regulators of cellular and tumor growth, and much information has been collected on the supramolecular organization of C-CAMs and their interactions with various signaling molecules. The challenging tasks now will be to define how C-CAMs regulate cellular activities, which signaling mechanisms are affected, and how the various molecular interactions in which C-CAMs participate are related to these events. A central problem will be to understand the role of the different C-CAM isoforms and how they interact in the regulation of the signaling activities. It will be interesting to see if the activities of other signal-regulatory proteins with alternative cytoplasmic domains, such as SIRPs, natural killer cell inhibitory receptors, and platelet/endothelial cell adhesion molecules, may be regulated by similar interactions between different isoforms of these proteins.

Another important task for future analysis will be to decipher the functional roles of the GPI-linked CEA-related molecules. This might be done by comparative analysis of the cellular responses of granulocytes from rodents (which do not have GPI-linked CEA-related molecules) and man (who do have GPI-linked CEA-related molecules). Could it be that the granulocyte GPI-linked molecules have replaced the short cytoplasmic domain isoform of C-CAM?

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