Minireview

Calmodulin signaling via the IQ motif

Martin Bähler[a], Allen Rhoads[b,*]

[a] *Institut für Allgemeine Zoologie und Genetik, Westfälische Wilhelms Universität, Münster, Germany*
[b] *Department of Biochemistry and Molecular Biology, Howard University College of Medicine, Washington, DC 20059, USA*

Received 19 October 2001; revised 6 November 2001; accepted 14 November 2001

First published online 6 December 2001

Edited by Gianni Cesareni and Mario Gimona

Abstract The IQ motif is widely distributed in both myosins and non-myosins and is quite common in the database that includes more than 900 Pfam entries. An examination of IQ motif-containing proteins that are known to bind calmodulin (CaM) indicates a wide diversity of biological functions that parallel the Ca^{2+}-dependent targets. These proteins include a variety of neuronal growth proteins, myosins, voltage-operated channels, phosphatases, Ras exchange proteins, sperm surface proteins, a Ras Gap-like protein, spindle-associated proteins and several proteins in plants. The IQ motif occurs in some proteins with Ca^{2+}-dependent CaM interaction where it may promote Ca^{2+}-independent retention of CaM. The action of the IQ motif may result in complex signaling as observed for myosins and the L-type Ca^{2+} channels and is highly localized as required for sites of neuronal polarized growth and plasticity, fertilization, mitosis and cytoskeletal organization. The IQ motif associated with the unconventional myosins also promotes Ca^{2+} regulation of the vectorial movement of cellular constituents to these sites. Additional regulatory roles for this versatile motif seem likely.

Key words: IQ motif; Calmodulin; Calcium regulation; Myosin

1. Introduction

1.1. Calcium-dependent calmodulin (CaM) regulation

CaM mediates the effects of Ca^{2+}, a major cellular second messenger, promoting highly localized calcium signaling that regulates molecular motor activity, cytoskeletal organization, mitosis and many other aspects of cell function [1–3]. The discovery of CaM and many of its subsequent target proteins was based on activation by Ca^{2+} and inhibition by EGTA [4,5]. Ca^{2+}-dependent signaling depends on increases in the Ca^{2+}/CaM complex that produces activation by increasing the binding affinity or catalytic potency of target proteins. The Ca^{2+}-dependent CaM-binding proteins often possess a region that is characterized by a basic, often amphipathic, helix consisting of approximately 20 amino acids. These regions possess critical hydrophobic residues at positions 1, 8 and 14 and frequently an additional hydrophobic contact at position 5 [6–9]. Tryptophan residues are sometimes present, but are not essential. Basic amino acids are distributed throughout the motif and often flank the critical hydrophobic residues. A completely new form of partially Ca^{2+}-dependent and -independent CaM interaction with the gating domain of the Ca^{2+}-activated K^+ channel has recently been described [10], and certainly other binding arrangements remain to be defined. The protein sequence of CaM is highly conserved and identical among vertebrates, but the Ca^{2+}/CaM-binding regions of its targets exhibit low homology and little sequence dependence. This probably reflects individual evolutionary routes to acquisition of CaM regulation with each target protein displaying variants of a fundamentally common binding region.

1.2. Ca^{2+}-independent CaM regulation

Although the first CaM-dependent proteins to be identified were Ca^{2+}-dependent, other proteins interacting with CaM in a Ca^{2+}-independent manner were eventually identified [11,12]. When cellular Ca^{2+} is low, CaM exerts its effects on these proteins via the Ca^{2+}-free form or 'apocalmodulin' [13]. Apocalmodulin has been found to interact with unconventional myosins such as intestinal brush border myosin I and with neuromodulin (GAP 43/P-57) which has a lower affinity for CaM in the presence of Ca^{2+} [14].

The nature of the Ca^{2+}-independent binding sites responsible for CaM recognition has been elucidated primarily by studies of the interaction of the myosin light chains with conventional, class II myosin in conjunction with sequence analysis of unconventional myosins [15–17]. The two light-chain-binding regions of the conventional myosin neck region are highly specific. These sites bind the essential (ELC) and the regulatory (RLC) myosin light chain, respectively. The ELC and RLC belong to a large EF-hand family of proteins that includes CaM and troponin C. However, they lack most of their original divalent-cation binding [18,19]. The ELC-binding site of the conventional myosin (IQXXXRGXXXR) is referred to as the 'IQ' motif (from the first two conserved residues) [20]. The first residue of the motif is variable as seen in conventional myosin II. Generally, position 1 is ambiguous for Ile, Leu or Val, (Met, Phe and Thr may be included for certain myosins) with possibly one exception [21]; position 11 can be either Arg or Lys; and position 7 is ambiguous for several residues as seen in myosins and PEP-19. Thus, the sequence [I,L,V]QXXXRXXXX[R,K] represents a more generalized IQ motif. Most of the IQ motifs present in vertebrate myosins and neuronal proteins (i.e. neuromodulin, PEP-19) have an additionally conserved hydrophobic residue

*Corresponding author. Fax: (1)-202-8065784.
E-mail address: arhoads@howard.edu (A. Rhoads).

PII: S0014-5793(01)03239-2

at position 14 that is not predicted to participate in binding of the light chain [22], but may be essential for other functions. The IQ motif may be defined by additional residues using Profile Hidden Markov models built from motif alignments [23] that may include two or three partially conserved alanine residues located N-terminal to the primary motif. How different IQ motifs select between different members of the EF-hand family of proteins is currently not well understood. The properties of the IQ motif are summarized in Table 1.

In some instances, IQ motif-containing proteins have been shown to bind to CaM or EF-hand proteins in a Ca^{2+}-dependent manner. These include utrophin, Ras GRF1 (Ras guanine nucleotide-releasing factor), Nina C myosins, calcium-vector target protein and others. In the case of Ras GRF1, Nina C and utrophin, the IQ motif also conforms to the Ca^{2+}-dependent 1-5-8-14 motif. In others the IQ motif may function in conjunction with a Ca^{2+}-dependent motif.

Pattern and profile searches of the current protein databases for IQ motif-containing proteins, which retain the essential motif across species, yielded some previously identified proteins and additionally others that may bind CaM. These sequences are listed in Table 2 (also see Pfam Protein Families Database). They include myosins, sodium and calcium channels, EF-hand-containing protein phosphatases, an IQ-GAP protein, spindle pole or centrosomal proteins, transient receptor potential (Trp) proteins, plant cyclic nucleotide-regulated channels and ethylene-inducible proteins, and a variety of other proteins. The significance of the IQ motif in some of these proteins remains to be determined. These proteins should provide further insight into the regulatory role of CaM or other EF-hand proteins acting via the IQ motif.

1.3. IQ-motif functions in myosins

Myosins are a very large and diverse group of actin-dependent motor molecules that contribute to many forms of eukaryotic motility. They harness the energy of ATP hydrolysis to produce directed mechanical force along actin filaments. Nearly all myosins contain between one and seven IQ motifs that follow directly the conserved motor domain sequences. This domain is called the light chain-binding domain (LCBD). Often, CaM is associated with these IQ motifs, but the IQ motifs of several myosins bind CaM-related proteins, such as conventional (class II) myosin, myosin X and myosin IC from *Acanthamoeba castellanii* [24,25]. Structural studies in conventional myosins have demonstrated that the IQ motif adopts an α-helical conformation that is stabilized by the CaM-related proteins. In fact, the IQ motifs and bound light chains form a rigid structure that serves as a mechanical lever [26]. The number of IQ motifs determines the length of the lever arm and hence the step-size of the myosin motor. However, the IQ motifs and associated light chains are not merely passive mechanical devices, but also influence the chemo-mechanical properties. The ELC of smooth muscle myosin II bound to the first IQ motif has been observed to contact a loop in the motor domain that modulates ADP release [27]. The RLC, bound to the second IQ motif, is subject to regulation by phosphorylation near its N-terminal region. This phosphorylation relieves the inhibition of motor activity imposed by the dephosphorylated RLC and activates myosin function [28]. How the RLC communicates with the motor domain is currently not well understood. It might interact with the ELC or modify the IQ-motif conformation that is being transduced to the motor domain or it may modify interactions with the second myosin motor domain in this double-headed conventional myosin. In scallop muscle myosin that is activated by Ca^{2+}, the Ca^{2+} is ligated by cooperative interactions of the ELC, the RLC and the IQ-motif sequences [29]. Unconventional myosins with CaM light chains are sensitive to changes in Ca^{2+} concentration. The IQ-motif-binding affinity of CaM varies with the Ca^{2+} concentration and the particular IQ motif. CaM exhibits a low affinity N-terminal Ca^{2+}-binding lobe and a high affinity C-terminal Ca^{2+}-binding lobe. Therefore, myosin function might be differentially regulated depending on whether neither, one or both lobes have Ca^{2+} bound. Based on structural analogies of the association of ELC and RLC with the IQ motifs in conventional myosin, the C-terminal lobe of apocalmodulin is thought to interact with the IQXXXR portion of the IQ motif involving a change in lobe conformation. The second half of the motif GXXXR binds the N-terminal lobe with little effect on its conformation. In the case of an incomplete IQ motif in which the glycine and the arginine residues are replaced by two bulky hydrophobic amino acids, the N-terminal lobe will change conformation upon binding. In bovine myosin IC (formerly myosin Iβ) binding of Ca^{2+} to the C-terminal lobe has been demonstrated to inhibit in vitro motility and to elevate the basal (actin-independent) ATPase-activity [30]. Further elevation of Ca^{2+} also leads to the loss of one of the three CaMs associated with the IQ motifs in myosin IC. Although in vitro CaM can be removed from some IQ motifs by high Ca^{2+} concentrations, it is not known whether this also occurs at in vivo CaM concentrations. Inhibition of motility upon a rise in Ca^{2+} was noticed in several unconventional myosins. However, the influence on the ATPase activity was either stimulatory or inhibitory depending on the myosin studied [31,32]. The conformational changes induced in CaM upon Ca^{2+}-binding are modulatory for myosin ATPase activity and do not act as on/off switches. It is assumed that strain acting on the head domain will also influence the ATPase cycle. Strain could be altered by changing LCBD flexibility or dimensions upon Ca^{2+}-mediated conformational changes

Table 1
Properties of the IQ motif

The region is a 20–25 residues long and α-helical with no proline residues.
The region may have partial or no amphipathicity with a net positive charge (usually 2+ to 5+).
A critical hydrophobic residue occurs at position 1.
The N-terminus of CaM or the myosin light chain interacts with the C-terminus of the target protein chain.
The 14-residue motif often occurs as multiple tandem repeats separated by 9–16 residues in myosins and in some non-myosin proteins.
The motif is sometimes the target of phosphorylation by PKC or PKA (e.g. neuromodulin, neurogranin)
In some proteins that act in a Ca^{2+}-dependent manner, the IQ motif may either conform to a 1-5-8-14 motif (RasGRP1) or act to bind and retain CaM at low Ca^{2+} levels.

Table 2
IQ motifs of established and potential CaM target proteins

Neuronal		|| | |	
Neuromodulin, bovine	ATK	IQASFRGHITRKKL	KGE
Neuromodulin IGLOO, *Drosophila*	ATK	IQAVFRGHKVRETM	KHL
	ALK	IQSTFRGHLARKLV	NKD
	ATK	IQASFRGHKTRKDA	NPE
Neurogranin, bovine	AAK	IQASFRGHMARKKI	KSG
PEP 19, human	AVA	IQSQFRKFQKKKAG	SQS
Conventional Myosins (II)			
Cardiac Myosin Beta, human	ITR	IQAQSRGVLARMEY	KKL
	LLV	IQWNIRAFMGVKNW	PWM
Cardiac Myosin Alpha , rat	ITR	IQAQARGQLMRIEF	KKM
	LLV	IQMNIRAFMGVKNW	PWM
L Type and Capacitative Calcium Channels			
Alpha-1C Subunit, human	TFL	IQEYFRKFKKRKEQ	GLV
Alpha-1C Subunit, mouse	TFL	IQEYFRKFKKRKEQ	GLV
Trp4, Bovine	ERS	IQLESRTLASRGDL	NIP
Dyneins			
Dynein-like protein, rat	VKS	IQDAIRDKKQRFSF	LGE
Dynein, D. melanogaster	VKS	VQDAIRDKKDKFNF	MGE
Plant Cyclic NMP-Regulated Ion Channel			
Arabidopsis	ACF	IQAAWRRYIKKKLE	ESL
Tobacco	ACF	IQAAWRRHCRKKLE	ESL
Plant Ethylene-Inducible CaM-Binding Proteins			
CaM and DNA Binding Protein, *Arabidopsis*	AIR	IQNKFRGYKGRKDY	LIT
	IIK	IQAHVRGYQFRKNY	RKI
ER66 Protein, tomato	AVR	IQNKLRSWKGRRDF	LLI
	IIK	IQAHVRGHQVRNKY	KNI
GTPase-activating (IQGAP) protein			
CDC42/Rac1/GTPase activating protein 1, mouse	ITK	LQACCRGYLVRQEF	RSR
	ITC	IQSQWRGYKQKKAY	QDR
	VVK	IQSLARMHQARKRY	RDR
	IIK	IQAFIRANKARDDY	KTL
Ras GRF 1 and 2			
Ras GRF-1/CDC25, human	IKK	VQSFLRGWLCRRKW	KTI
Ras GRF-2, mouse	IKK	VQSFMRGWLCRRKW	KTI
Miscellaneous Proteins			
CVT, *Branchiostoma*	ATR	IQASFRMHKNRMAL	KEK
Sperm surface protein, Sp17, human	AVK	IQAAFRGHIAREEA	KKM
Utrophin, human (one of two motifs)	EDV	LQKEVRVKILKDNI	KLL
Sodium Channel, human	AVI	IQRAYRRHLLKRTV	KQA
Inversin, human	ELR	LQIIQRERRRKELF	RKK
	AAV	IQRAWRSYQLRKHL	HLR
β-Spectrin, mouse	LFT	IQSRMRANNQKVYT	PHD
Kendrin , human (one of two motifs)	KDN	LQKELRIEHSRCEA	LLA
SHA 1, mouse (two of four motifs)	TVV	VQALVRGWLVRKRV	SEQ
	VIF	IQRWFRKRLQRKRF	IEQ
SHA-like protein, human (one of sixteen motifs)	VIY	IQAIFRGKKARRHL	KMM
Phosphatase, PPEF-2, human	AAL	IQRWYRRYVARLEM	RRR
Retinal Degeneration Protein, D. melanogaster	AIF	IQKWYRRHQARREM	QRR
SF16 Pollen-Specific Protein, sunflower	ATK	IQAAYRGYTARRAF	RSL
Putative Ubiquitin ligase, S. pombe	SVA	VQSLSRGFLARRKF	KQD

in CaM. Therefore, Ca^{2+} regulation might be even more pronounced under strained conditions. Thus, Ca^{2+} via CaM interacting with IQ motifs can regulate a wide range of myosin-mediated cellular events that include membrane trafficking, cell movement and organization and signal transduction [33]. CaM may also regulate the activity of some forms of the microtubule-dependent molecular motors, dynein and kinesin [34,35].

Table 3
Dual targeting via Ca^{2+}-dependent and Ca^{2+}-independent actions of CaM

	+ Calcium	− Calcium
Growth	Ras GRF1/2 (CDC25)[a]	Neuromodulin IGLOO-L Neurogranin IQ GAP Yeast IRAs (?) PEP-19
Apoptosis	DAP kinase Plant ethylene up-regulated proteins[a]	
Channels	Voltage-gated Ca^{2+} channels[d] Ca^{2+}-Activated K^+ channel[c,d]	Sodium channels (?)
Calcium regulation	Plasma membrane Ca^{2+} pump	Capacitative calcium channel (Trp4)[d]
Contraction, motility	MLCK Troponin I (TNC)[b] NO synthase Plant kinesin-like protein Caldesmon	Conventional myosin (ELC/RLC)[b] Non-conventional myosins Dynein
Cyclic nucleotide	Adenylyl cyclases Cyclic nucleotide phosphodiesterases Olfactory/cNMP-regulated channel Plant cNMP-regulated channel[a]	
Cytoskeletal/structural	Utrophin[a] α-Fodrin Caldesmon MARCKS Nina C[a]	Brush border myosin β-Spectrin
Mitosis	NUF1/SP110p/kendrin[d] Plant kinesin-like protein	ASP (*Drosophila*) SHA1 CaM-binding protein
Phosphorylation/dephosphorylation	CaM-dependent kinases (I, II, IV) Calcineurin Plant Ca^{2+}-dependent protein kinases (CDPK, EF-hand protein)	Protein phosphatases (EF-hand containing)
Development		Sperm surface protein Inversin (?)
Miscellaneous	Calspermin p68 RNA helicase[a]	

?: Not demonstrated to be modulated by CaM or an EF-hand protein. MLCK: Myosin light chain kinase
[a]IQ motif is present in Ca^{2+}-dependent CaM-binding protein.
[b]Mediated by an EF-hand protein other than CaM.
[c]Mediated by a unique binding motif.
[d]Involves both Ca^{2+}-dependent and -independent CaM interactions.

1.4. Growth and cytoskeletal organization signaling

Neurospecific GAP-43 and neurogranin were the first non-myosin proteins to be identified as binding CaM through the IQ motif, but their exact function has remained elusive. GAP-43 is involved in outgrowth of axons and neurogranin with the budding of dendrites [36]. GAP-43-like proteins accumulate at and modulate $PI(4,5)P_2$ rafts conferring an intrinsic competence for morphogenetic activity and regulated motility [37]. GAP-43 may serve possibly to bind a localized reserve of CaM that may be subject to PKC-induced release [38] or to stimulate GDP/GTP exchange of $G_{\alpha 0}$ and $G_{\alpha i}$ for neuronal development [39]. Another growth-related target of CaM in the central nervous system is Ras GRF1, a Ca^{2+}/CaM-dependent brain-specific GTP exchange factor (GEF) that possesses an IQ motif [40,41]. This SOS-like protein contains a CDC25 homology domain, which is responsible for GEF activity and activation of small GTP-binding proteins (e.g. Ras and Rap). The protein also contains a Dbl homology domain (DH) flanked by both an IQ motif at its N-terminus and pleckstrin homology domain (PH). Mutations in the DH or PH domain prevent Ras-GRF1 activation by Ca^{2+}/CaM [42]. Other GEFs regulated by Ca^{2+} and additionally by diacylglycerol are the Ras GRPs (guanyl nucleotide releasing proteins) that include CalDAG-GEF I and II and a longer isoform of CalDAG-GEF I designated RasGRP2 [43]. Activation of the Ras GEF activity of CalDAG-GEF I by phorbol ester correlates with its translocation to the plasma membrane [43]. Although the CalDAG-GEFs do not bind CaM or have an IQ motif, they do possess one or more EF-hand domains that underscore a broader role for Ca^{2+} in the regulation of GEF activity. The IQ motif in combination with EF-hand domains occurs in a serine/threonine phosphatase of the *Drosophila*, which is also known as retinal degeneration C protein, that promotes dephosphorylation of rhodopsin and prevents light-induced retinal degeneration [44]. Homologous proteins are also present in mammals (PPEF-1 and 2) and are highly expressed in sensory neurons of cranial and dorsal root ganglia [45,46]. Although the role of the IQ motif in the regulation of the phosphatase is not known, the motif could conceivably interact with the EF-hand domain to regulate the enzyme activity. For the 800 kDa sarcomeric protein known as obscurin that is associated with myofibrillar organization, a Rho GEF domain and a non-canonical CaM-binding IQ motif have been identified in the C-terminal region [47].

The IQ motif also occurs in IQGap proteins that serve as effectors of Cdc42 and Rac, members of the Rho family of p21 small GTPases in GTP-mediated signaling pathways. IQGap1 protein contains four IQ motifs in tandem, a catalytic

Gap-related domain, a calponin homology domain (29% identity to the Ca^{2+}-binding protein), a WW domain and a region of unique repeats of 50–55 amino acids [48]. IQGap1 appears to mediate homophilic cell–cell adhesion downstream of the small GTPases, Cdc42 and Rac1, by affecting the interaction of E-cadherin and β-catenin [49]. Ca^{2+}-independent binding of CaM to IQGap1 may prevent IQGap1 from interacting with components of the cadherin–catenin complex, which would promote enhanced E-cadherin-mediated adhesion [50]. The IQGap protein has also been implicated in cytokinesis of yeast where it may be recruited to bud sites by the myosin light chain, M1c1p, to promote actin association with the actomyosin ring and contraction [51]. IQGap1 has also been shown to interact with myosin ELC emphasizing the functional similarity of the IQ motif in myosin and non-myosin proteins such as IQGap1 [52].

1.5. Specific and temporal signaling via the IQ motif

CaM signaling via the IQ motif in proteins can produce specific signaling that may be temporally both activating or inactivating depending on the intracellular Ca^{2+} levels. Recently, studies of the voltage-operated Ca^{2+} channels have begun to define the physiological effects resulting from interaction of CaM with the IQ motif. The intracellular C-terminus of the α_{1c} subunit of the L-type calcium channel in excitable cells contains an IQ motif that is responsible for strong facilitation as well as inactivation of the channel [53]. The IQ motif is believed to behave as a calcium sensor for both positive and negative modulation of the voltage-operated Ca^{2+} channel. Binding of the C-terminal lobe of CaM to the IQ motif accelerates Ca^{2+}-dependent inactivation [54]. CaM also regulates the P/Q-type Ca^{2+}-channel by promoting both facilitation and inhibition [55]. In the P/Q-type Ca^{2+}-channel, Ca^{2+} sensing, facilitation and inactivation domains and the IQ motif were all found to reside at the C-terminus of the α_{1A} subunit. More recently, the modulation of the P/Q-type channel has been attributed to an IQ-like domain at the C-terminus of the α_{1A} subunit [56]. Expression of a CaM mutant with all four of its Ca^{2+}-binding sites disrupted eliminates both forms of modulation, confirming that CaM is the calcium sensor for the channel. The IQ motif associated with the L-type voltage channel is essential for the selective nuclear signaling and specific activation of the Ras/MAPK pathway and transcription [57].

Mammalian capacitative calcium entry channels (Trp4 class) also contain one or more IQ motifs. Several studies have already reported Ca^{2+}-dependent CaM binding at one or more sites in the C-terminal region of the channel [58,59]. Also residing in the C-terminal portion of Trp4 and other Trp channels are sites that interact with IP_3 receptors [58,60]. Previously, distinct Ca^{2+}-dependent and -independent CaM-binding sites were also reported for the Trp-like channels of *Drosophila* [61]. Binding of Ca^{2+}/CaM and the IP_3 receptor may control gating of the channel.

Sperm surface protein 17 (Sp17) contains a highly conserved IQ motif in the C-terminus that binds equimolar amounts of CaM in a Ca^{2+}-independent manner [62]. During the acrosomal reaction the protein binds to the zona pellucida, and the C-terminal portion is proteolysed, possibly aiding in the release of CaM during sperm penetration and sperm–egg fusion. Another IQ motif protein, inversin, encoded by the inv gene and involved in left–right axis inversion during embryonic development contains one to two IQ motifs in addition to an ankyrin repeat region (Table 2). The mechanism by which the protein induces axis inversion is unknown [63,64]. The microtubule-based motor, left–right dynein (LRD) is also implicated in asymmetry and organ orientation [65]. Since certain dyneins have been reported to be associated and regulated by CaM and several microtubulin-associated proteins have IQ motifs, inversin may target the microtubular system and modulate regulation by CaM. Interestingly, the LRD of mouse shares 78% identity to the dynein-like protein of rat (Table 2), but lacks its conserved IQ motif, suggesting that inversin may act as a receptor for CaM regulation.

Kendrin, the human orthologue of Spc110p that links the γ-tubulin complex to the core of the yeast spindle pole body has two IQ motifs and a Ca^{2+}-dependent CaM-binding site in the C-terminus [66]. The C-terminal binding site resembles the 1-5-8-14 motif that is also found in Spc110p of *Saccharomyces cerevisiae* and in related sequences of *Schizosaccharomyces pombe, Aspergillus nidulans* and mouse. Mutation of residues within the Ca^{2+}-dependent binding site markedly decreases CaM binding to kendrin. In highly mitotic breast cancer cells, kendrin and pericentrin are limited to centrosomes of the mitotic spindle and regulate spindle microtubules. In *Drosophila*, abnormal spindle protein (ASP) or microtubule-associated protein and a structurally related mouse protein, SHA1, possess multiple IQ motifs (4 or 5) and are bound to the polar region of the mitotic spindle, suggesting a role for CaM in controlling mitotic microtubular activity [67,68]. Despite similarities in localization and CaM association, the ASP and kendrin do not share sequence homology. Further, kendrin is restricted to the centrosome whereas ASP localizes in both the centrosome and the spindle [66]. The presence of Ca^{2+}-dependent and -independent CaM-binding sites in certain mitotic proteins may permit specific and dual signaling during cell division. Several widely expressed human genes encoding SHA-like proteins containing six or more IQ motifs have been reported in GenBank, but the proteins have not been characterized. In addition to these multiple IQ motif-containing proteins, myosin V may also have a role to play in the organization and control of the mitotic spindle and centrosome [69,70].

1.6. IQ motif signaling in plants

In plants a cyclic nucleotide-regulated ion channel interacts with CaM in a Ca^{2+}-dependent manner [71,72]. The plant channel and the olfactory cyclic nucleotide-regulated channels are the only proteins so far known that possess binding sites for both CaM and cyclic nucleotide. The role of CaM binding to the plant channel may be similar to its role in binding to the cyclic nucleotide-activated olfactory channel of mammals. In the latter, Ca^{2+}/CaM decreases the affinity of the channel for cyclic AMP or cyclic GMP [73]. Based on synthetic peptide studies, the CaM-binding site of the plant channel appears to be localized on the αC-helix in a region adjacent to the IQ motif. Tryptophan residues present in a tetrapeptide sequence, WRTW, near the C-terminal end of the peptide were found to be important in the binding of CaM. The exact role of the adjacent IQ motif in the activation has not been examined, but its proximity suggests an additional binding element for CaM. In contrast, the αC-helix of the olfactory channel does not bind CaM. The CaM-binding site of the olfactory channel is located near the N-terminus and corre-

sponds to the Ca^{2+}/CaM-dependent 1-5-8-14 motif. CaM-binding motifs may be more diverse in plants because of the existence of five genes encoding CaM/CaM-like proteins differing in sequence [74]. A high level of cyclic nucleotide-regulated ion channel expression occurs in the secretory barley aleurone cell, where Ca^{2+}, cyclic GMP and CaM are major elements of gibberellic acid/abscisic acid signaling. The inhibitory response of the channel to CaM may permit these cells to respond appropriately over a wide range of cyclic nucleotide concentrations involved in plant stress signaling [71].

Another class of IQ motif-containing proteins that are induced by the plant hormone ethylene have been identified [75,76]. These proteins bind CaM and promote plant senescence and death. The association between CaM signaling and cell death is not unique. The existence of a Ca^{2+}/CaM-activated, cytoskeletal death-associated kinase (DAP kinase) that shares homology with myosin light chain kinase, and is induced in response to γ-interferon, is well established [77]. Although the specific substrates of DAP kinase have not been defined, Mills et al. [78] suggested that apoptotic membrane blebbling may be regulated by myosin light chain phosphorylation. Unlike DAP kinase, the Ca^{2+}/CaM-binding ethylene-regulated protein of *Arabidopsis* has regions that are characteristic of transcription factors and may act to induce other proteins necessary for apoptosis.

A wide diversity of biological functions that parallel the Ca^{2+}-dependent targets of CaM exists among the IQ motif-containing proteins. These proteins include a variety of neuronal growth proteins, myosins, voltage-operated channels, phosphatases, Ras exchange proteins, sperm surface proteins, a Ras Gap-like protein, spindle-associated proteins and several plant proteins (Table 3). The IQ motif can trigger specific and complex signaling as described for the L-type calcium channel and research on other target proteins should reveal additional signaling roles.

Acknowledgements: A portion of this work was supported by Grant MCB930015P from the Pittsburgh Supercomputing Center, Pittsburgh, PA, USA and the DFG (Ba 1354/6-1).

References

[1] Bonafé, N.M. and Sellers, J.R. (1998) Calmodulin-binding proteins of the cytoskeleton, in: Calmodulin and Signal Transduction (Van Eldik, L.J. and Watterson, M., Eds.), Academic Press, San Diego, CA.
[2] Sellers, J.R. (2000) Biochim. Biophys. Acta 1496, 3–22.
[3] Vogel, H.J. (1994) Biochem. Cell Biol. 72, 357–376.
[4] Cheung, W.Y. (1970) Biochem. Biophys. Res. Commun. 38, 533–538.
[5] Kakiuchi, S., Yamazaki, R. and Teshima, Y. (1971) Biochem. Biophys. Res. Commun. 42, 968–974.
[6] Persechini, A. and Kretsinger, R.H. (1988) J. Cardiovasc. Pharm. 12 (Suppl.), S1–S12.
[7] O'Neil, K.T. and DeGrado, W.F. (1990) Trends Biol. Sci. 15, 59–64.
[8] Crivici, A. and Ikura, M. (1995) Annu. Rev. Biophys. Struct. 24, 85–116.
[9] James, P., Vorherr, T. and Carafoli, E. (1995) Trends Biol. Sci. 20, 38–42.
[10] Schumacher, M.A., Rivard, A.F., Bächinger, H.P. and Adelman, J.P. (2001) Nature 410, 1120–1124.
[11] Alexander, K.A., Wakim, B.T., Doyle, G.S., Walsh, K.A. and Storm, D.R. (1988) J. Biol. Chem. 263, 7544–7549.
[12] Baudier, J., Deloulme, J.C., Dorsselaer, A.V., Black, D. and Matthes, H.W.D. (1991) J. Biol. Chem. 266, 229–237.
[13] Jurado, L.A., Chockalingam, S.P. and Jarrett, H.W. (1999) Apocalmodulin Physiol. Rev. 79, 661–682.
[14] Rhoads, A.R. and Friedberg, F. (1997) FASEB J. 11, 331–340.
[15] Mitchell, E.J., Karn, J., Brown, D.M., Newman, A., Jakes, R. and Kendrick-Jones, J. (1989) J. Mol. Biol. 208, 199–205.
[16] McNally, E.M., Bravo-Zehnder, M.M. and Leinwand, L.A. (1991) J. Cell Biol. 113, 585–590.
[17] Mercer, J.A., Seperack, P.K., Strobel, M.C., Copeland, N.G. and Jenkins, N.A. (1991) Nature 349, 709–713.
[18] Collins, J.H. (1991) J. Muscle Res. Cell Motil. 12, 3–25.
[19] Kawasaki, H., Nakayama, S. and Kretsinger, R.H. (1998) BioMetals 11, 277–295.
[20] Cheney, R.E. and Mooseker, M.S. (1992) Curr. Opin. Cell Biol. 4, 27–35.
[21] Deloulme, J.C., Prichard, L., Delattre, O. and Storm, D.R. (1997) J. Biol. Chem. 272, 27369–27377.
[22] Houdusse, A., Silver, M. and Cohen, C. (1996) Structure 4, 1475–1490.
[23] Sonnhammer, E.L., Eddy, S.R. and Durbin, R. (1997) Proteins 28, 405–420.
[24] Rogers, M.S. and Strehler, E.E. (2001) J. Biol. Chem. 276, 12182–12189.
[25] Wang, Z.Y., Sakai, J., Matsudaira, P.T., Baines, I.C., Sellers, J.R., Hammer, J.A. and Korn, E.D. (1997) J. Muscle Res. Cell Motil. 18, 395–398.
[26] Block, S.M. (1996) Cell 87, 151–157.
[27] Dominguez, R., Freyzon, Y., Trybus, K.M. and Cohen, C. (1998) Cell 94, 559–571.
[28] Trybus, K.M., Waller, G.S. and Chatman, T.A. (1994) J. Cell Biol. 124, 963–969.
[29] Houdusse, A. and Cohen, C. (1995) Proc. Natl. Acad. Sci. USA 92, 10644–10647.
[30] Zhu, T., Beckingham, K. and Ikebe, M. (1998) J. Biol. Chem. 273, 20481–20486.
[31] Wolenski, J.S. (1995) Trends Cell Biol. 5, 310–316.
[32] Stöffler, H.-E. and Bähler, M. (1998) J. Biol. Chem. 273, 14605–14611.
[33] Mermall, V., Post, P.L. and Mooseker, M.S. (1998) Science 279, 527–533.
[34] Yang, P., Diener, D.R., Rosenbaum, J.L. and Sale, W.S. (2001) J. Cell Biol. 153, 1315–1326.
[35] Reddy, V.S., Safadi, F., Zielinski, R.E. and Reddy, A.S.N. (1999) J. Biol. Chem. 274, 31727–31733.
[36] Benowitz, L.I. and Routtenberg, A. (1997) Trends Neurosci. 20, 84–91.
[37] Caroni, P. (2001) EMBO J. 20, 4332–4336.
[38] Liu, Y. and Storm, D.R. (1990) Trends Pharm. Sci. 11, 107–111.
[39] Strittmatter, S.M., Valenzuela, D., Sudo, Y., Linder, M.E. and Fishman, M.C. (1991) J. Biol. Chem. 266, 22465–22471.
[40] Martegani, E., Vanoni, M., Zippel, R., Coccetti, P., Brambilla, R., Ferrari, C., Sturani, E. and Alberghina, L. (1992) EMBO J. 11, 2151–2157.
[41] Shou, C., Farnsworth, C.I., Neel, B.G. and Feig, L.A. (1992) Nature 358, 351–354.
[42] Freshney, N.W., Goonesekera, S.D. and Feig, L.A. (1997) FEBS Lett. 407, 111–115.
[43] Clyde-Smith, J., Silins, G., Gartside, M., Grimmond, S., Etheridge, M., Apolloni, A., Hayward, N. and Hancock, J.F. (2000) J. Biol. Chem. 275, 32260–32267.
[44] Steele, F.R., Washburn, T., Rieger, R. and O'Tousa, J.E. (1992) Cell 69, 669–676.
[45] Montini, E., Rugarli, E.I., Van de Vosse, E., Andolfi, G., Mariani, M., Puca, A.A., Consalez, G.G., den Dunnen, J.T., Ballabio, A. and Franco, B. (1997) Hum. Mol. Genet. 6, 1137–1145.
[46] Sherman, P.M., Sun, H., Macke, J.P., Williams, J., Smallwood, P.M. and Nathans, J. (1997) Proc. Natl. Acad. Sci. USA 94, 11639–11644.
[47] Young, P., Ehler, E. and Gautel, M. (2001) J. Cell Biol. 154, 123–136.
[48] Weissbach, L., Settleman, J., Kalady, M.F., Snijders, A.J. and Murthy, A.E. (1994) J. Biol. Chem. 269, 20517–20521.
[49] Kuroda, S., Fukata, M., Nakagawa, M., Fujii, K., Nakamura, T., Ookubo, T., Izawa, I., Nagase, T., Nomura, N., Tani, H., Shoji, I., Matsuura, Y., Yonehara, S. and Kaibuchi, K. (1998) Science 281, 832–835.

[50] Li, Z., Kim, S.H., Higgins, J.M.G., Brenner, M.B. and Sacks, D.B. (1999) J. Biol. Chem. 274, 37885–37892.
[51] Boyne, J.R., Yosuf, H.M., Bieganowski, P., Brenner, C. and Price, C. (2000) J. Cell Sci. 113, 4533–4543.
[52] Weissbach, L., Bernards, A. and Herion, D.W. (1998) Biochem. Biophys. Res. Commun. 251, 269–276.
[53] Zuhlke, R.D., Pitt, G.S., Deisseroth, K., Tsien, R.W. and Reuter, H. (1999) Nature 399, 159–162.
[54] Pitt, G.S., Zuhlke, R.D., Hudmon, A., Schulman, H., Reuter, H. and Tsien, R.W. (2001) J. Biol. Chem. 276, 30794–30802.
[55] Lee, A., Wong, S.T., Gallagher, D., Lin, B., Storm, D.R., Scheuer, T. and Catterall, W. (1999) Nature 399, 155–159.
[56] DeMaria, C.D., Soong, T.W., Alselkhan, B.A., Alvania, R.S. and Yue, D.T. (2001) Nature 411, 484–489.
[57] Dolmetsch, R.E., Pajvani, U., Fife, K., Spotts, J.M. and Greenberg, M.E. (2001) Science 294, 333–339.
[58] Tang, J., Lin, Y., Zhang, Z., Tikunova, Z., Birnbaumer, L. and Zhu, M.X. (2001) J. Biol. Chem. 276, 213030.
[59] Troust, C., Bergs, C., Himmerkus, N. and Flockerzi, V. (2001) Biochem. J. 355, 663–670.
[60] Mery, L., Magnino, F., Schmidt, K., Krause, K.-H. and Dufour, J.-F. (2001) FEBS Lett. 487, 377–383.
[61] Warr, C.G. and Kelly, L.E. (1996) Biochem. J. 314, 497–503.
[62] Wen, Y., Richardson, R.T. and O'Rand, M.G. (1999) Dev. Biol. 206, 113–122.
[63] Morgan, D., Turnpenny, L., Goodship, J., Dai, W., Majumder, K., Matthews, L., Gardner, A., Schuster, G., Vien, L., Harrison, W., Elder, F.F., Penman-Splitt, M., Overbeek, P. and Strachan, T. (1998) Nat. Genet. 20, 149–156.
[64] Mochizuki, T., Saijoh, Y., Tsuchiya, K., Shirayoshi, Y., Takai, S., Taya, C., Yonekawa, H., Yamada, K., Nihei, H., Nakatsuji, N., Overbeek, P.A., Hamada, H. and Yokoyama, T. (1998) Nature 395, 177–181.
[65] Supp, D.M., Witte, D.P., Potter, S.S. and Brueckner, M. (1997) Nature 389, 963–966.
[66] Flory, M.R., Moser, M.J., Monnat Jr., R.J. and Davis, T.N. (2000) Proc. Natl. Acad. Sci. USA 97, 5919–5923.
[67] Craig, R. and Norbury, C. (1998) J. Cell Sci. 111, 3609–3619.
[68] Saunders, R.D., Avides, M.C., Howard, T., Gonzalez, C. and Glover, D.M. (1997) J. Cell Biol. 137, 881–890.
[69] Wu, X., Kocher, B., Wei, Q. and Hammer III, J.A. (1998) Cell Motil. Cytoskeleton 40, 286–303.
[70] Espreafico, E.M., Coling, D.E., Tsakraklides, V., Krogh, K., Wolenski, J.S., Kalinec, G. and Kachar, B. (1998) Proc. Natl. Acad. Sci. USA 95, 8636–8641.
[71] Schuurink, R.C., Shartzer, S.F., Fath, A. and Jones, R.L. (1998) Proc. Natl. Acad. Sci. USA 95, 1944–1949.
[72] Arazi, T., Kaplan, B. and Fromm, H. (2000) Plant Mol. Biol. 42, 591–601.
[73] Chen, T.-Y. and Yau, K.-W. (1994) Nature (London) 368, 545–548.
[74] Lee, S.H., Johnson, J.D., Walsh, M.P., Van Lierop, J.E., Sutherland, C., Xu, A., Snedden, W.A., Kosk-Kosicka, D., Fromm, H., Narayanan, N. and Cho, M.J. (2000) Biochem. J. 350, 299–306.
[75] Yang, T. and Poovaiah, B.W. (2000) J. Biol. Chem. 275, 38467–38473.
[76] Reddy, A.S.N., Reddy, V.S. and Golovkin, M. (2000) Biochem. Biophy. Res. Commun. 279, 762–769.
[77] Cohen, O., Feinstein, E. and Kimchi, A. (1997) EMBO J. 16, 998–1008.
[78] Mills, J.C., Stone, N.L., Erhardt, J. and Pittman, R.N. (1998) J. Cell Biol. 140, 627–636.